UNIVERSITATEA DE ȘTIINȚE AGRICOLE ȘI MEDICINĂ
VETERINARĂ "ION IONESCU DE LA BRAD" IAȘI

LUCRĂRI ȘTIINȚIFICE

VOL. 59
MEDICINĂ VETERINARĂ

PARTEA 3

EDITURA "ION IONESCU DE LA BRAD" IAȘI 2016

on -line ISSN 2393 – 4603
ISSN–L 1454 – 7406

CUPRINS

THE ZOE PROJECT: INNOVATIVE STEPS IN ZOONOSES ONLINE EDUCATION

Anca COLIBABA, Stefan COLIBABA, Cinta COLIBABA
Universitatea Gr.T.Popa Iasi, Fundatia EuroEd Iasi; Universitatea Al.I.Cuza Iasi;
Universitatea Ion Ionescu de la Brad Iasi

Abstract

The article is a study based on Zoonoses online education project (Ref project 2016-1-RO01-KA203-024732) coordinated by The University of Veterinary Medicine Iaşi in a large partnership with universities and language or ICT institutions from Italy, Croatia, Lithuania and Romania. All partners have similar mission statements and adhere to the values of the European Union. All of the universities are engaged in the process of continual renewal of their curricula and methods and the educational institutions promote the use of computerized simulation in education to prepare learners for the world of work. Therefore their collaboration in this respect have found effective and cost reasonable solutions, which fulfil the standards of the European Union and the demands set by the increased mobility. The project is in line with the 2013 Communication on Opening up Education: innovative teaching and learning for all through new Technologies and Open Educational Resources. The project supports development and availability of open educational resources in the field of veterinary medicine by developing innovative guidelines on how to develop specialized skills related to identify, monitor and control malaria and dirofilariasis and also medical communication linguistic skills, useful for the academic, professional and common public.

Key words: zoonoses, open online courses, medical communication

Introduction

The project brings together three educational sectors, all with impact on three different end beneficiaries groups:

Veterinary medicine: it aims at introducing online resources created in the current teaching and medical world; it enables students from veterinary field or nonprofessional users to develop and practise medical, language communication, ICT, autonomous learning, collaborative learning, and intercultural skills; lecturers in veterinary medicine have access to educational resources which enable them to develop their students' medical communication skills; language teachers get familiar with and can use ready-made units and support materials for teaching languages for medical purposes.

Human medicine: it aims at developing a protocol for monitoring-control of malaria and dirofilariasis from epidemiological and clinical points of view; this equips medical students and doctors/ physicians with state of the art information on the etiology, diagnosis, treatment, particularities of the epidemiological process and prevention measures. In addition, the suggested practical activities allow participants to carry out epidemiological investigations in order to come up with their own suggestions.

Pedagogy education: it aims at strengthening the professional profile of educators and students as future teachers by developing and providing them with pedagogical guidelines on health education, risks of microbes and need of vaccines; the guidelines informs teachers, pupils, parents and the general public about malaria and dirofilariasis, in terms of prevention and intervention

Material and method

Recently, a news story announced a new virus is raging for which there is no cure or vaccine; Zika virus, the latest outbreak, which could have consequences as serious as Ebola. Another story reported that the European Commission issued an alert about E. coli infection, the affected countries were Romania, Italy, France and Germany, where it is shown that the degree of risk is serious. Another piece of news says that nearly 80 people in Bucharest have

been treated for boutonneuse fever and Lyme disease, infectious diseases caused by dog parasites (The National Sanitary Veterinary and Food Safety Authority, 2011).

Zoonotic diseases represent one of the leading causes of illness and death from infectious disease. Worldwide, zoonotic diseases have a negative impact on commerce, travel, and economies. In most developing countries, zoonotic diseases are among those diseases of major public health concern. In industrialized nations, zoonotic diseases are of particular concern for risk groups such as the elderly, children, pregnant women, and immunocompromised individuals. As defined by the World Health Organization, zoonoses are "those diseases and infections that are naturally transmitted between vertebrate animals and man with or without an arthropod intermediate. (World Health Organisation, 2011)".

Zoonoses are fundamental determinants of community health. Preventing, identifying and managing these infections must be a central public health focus. Most current zoonosis research focuses on the interface of the pathogen and the clinically ill person, emphasizing microbial detection, mechanisms of pathogenicity and clinical intervention strategies, rather than examining the causes of emergence, persistence and spread of new zoonoses. There are gaps in the understanding of the animal determinants of emergence and the capacity to train highly qualified individuals; these are major obstacles to prevent new disease threats. The ability to predict the emergence of zoonoses and their resulting public health and societal impacts are hindered when insufficient effort is devoted to understanding zoonotic disease epidemiology and when zoonoses are not examined in a manner that yields fundamental insight into their origin and spread.

Infectious disease research rests on four pillars: enhanced communications across disciplinary and agency boundaries, the assessment and development of surveillance and disease detection tools, the examination of linkages between animal health determinants of human health outcomes, and finally cross-disciplinary training and research.

- the need to connect the knowledge and skills veterinary students acquire during their academic training with the world of work. The project connects students' academic knowledge and skills with real medical activity with the intent to optimize students' access to real medical situations in the world of work.

- the need to have an inter-academic cooperation with medical and veterinary doctors with a view to sensitize the medical doctors about the growing numbers of human patients suffering from diseases transmitted by animals including diseases considered extinct in some of the countries involved in the project, a fact that delays their correct diagnosis.

- the need to trained the (future) teachers and include health education in their teaching curricula, in order to increase pupils' parents' awareness about the risks and ways of prevention.

- the need for the general public to get access to immediate information about top diseases transmitted through animals of company or/and insects, prevention measures and alert symptomatology.

- the need for a unitary system of medical education across Europe, based on well documented and generally accepted educational tools. All European veterinary graduates should have equal chances to practise all over the EU.

- the need to stimulate the flow and exchange of knowledge between higher education and veterinary and medical clinics.

- the need for developing transversal skills acquisition. All the categories ICT, communication skills, foreign languages, work in multidisciplinary teams, social responsibility, problem-solving and risk management are addressed as learning targets for the involved groups.

Results and discussions

In an effort to increase the knowledge and understanding of current and probable future public health significance of zoonotic diseases, the Zoonoses Online Education project creates open digital educational resources in the field of veterinary medicine; it devises innovative guidelines on how to develop specialized skills with a view to identifying, monitoring and controlling malaria and dirofilaria. The project carries out state of art research on the national analysis of malaria and dirofilaria, taking into account literature research, interviews with specialists and identification of examples of best practices on zoonoses education within each partner country.

A team of international experts did solid research and set up as working hypothesis for the project the analysis of the two zoonoses, evaluated as top priorities by the World Organisation for Animal Health (OIE) and the Federation of Veterinarians of Europe (FVE): malaria and dirofilariasis. The diseases were selected according to the health risk they pose signalled by international organizations. Thorough research carried out by partners highlighted alarming facts, statistics and serious concern over the unprecedented upsurge of the two above mentioned diseases.

Zoonotic diseases represent one of the leading causes of illness and death from infectious disease. Worldwide, zoonotic diseases have a negative impact on commerce, travel, and economies. In most developing countries, zoonotic diseases are among those diseases of major public health significance which contribute significantly to an already overly burdened public health system. Malaria, to choose only one example of such a disease, has been reported as a newly emerging disease in Romania. This is due to the fact that although considered eradicated for the last 50 years in Romania, it has reappeared in certain areas of the country, as a result of the global warming combined with the recent presence of international students originating from countries where the populations have a different degree of resistance to malaria and bear the disease in a latent state in their blood. The mosquitoes spread it through animals or human bites. Symptomatology specific for malaria is not easily decoded by medical doctors because of their lack of information in connection to the re-appearance of the disease and time and medication are wasted on other tentative diagnoses before the malaria diagnosis is set. Similar geographical or social causes have brought about this situation first in the South of Italy, some time before it appeared in Romania. This country has already started fighting this disease with modern weapons and a coherent national approach. Research shows that Lithuania is the Northern limit of the occurrence of these diseases. Identifying the geography of spreading and the foreseeable causes of these newly emerging diseases is one of the major benefits of this project.

Based on the research a guide and an open online course guide of main infectious disease transmitted from non-human animals to humans are created. They are accompanied by videos capturing zoonoses bio-manipulation in simulation centers. The videos are processed from linguistic, cultural and communication points of view. They will be available in 6 languages.

The medical specialists are responsible for developing the scripts of the videos based on the topic of the procedures. The script includes the presentation of the procedures and manoeuvres, the patient's and the doctor's actions, the technical/scenic indications and the conversation script. Each video presents a procedure through a communicative situation. For each of the 2 proposed zoonotic diseases the videos capture the stages included in the guide developed: demographics and tracing/ movement of animals; education and communication; surveillance and infection control; risk assessment; new tools for diagnosis, prevention and therapy. When converting the videos into linguistic learning units the videos are considered in terms of the vocabulary, grammar structure, functions and cultural elements present in the

communicative situation. Each video presents a procedure through a communicative situation. For each of the 2 proposed zoonotic diseases the videos capture the stages included in the guide developed: demographics and tracing/ movement of animals; education and communication; surveillance and infection control; risk assessment; new tools for diagnosis, prevention and therapy. When converting the videos into linguistic learning units the videos are considered in terms of the vocabulary, grammar structure, functions and cultural elements present in the communicative situation. Each unit relies on the video and is accompanied by learning activities aiming at introducing, practicing and consolidating the vocabulary, structures, functions and cultural components present in each veterinary intervention. The envisaged activities cover all language skills: listening, reading, speaking and writing. The competence level of the linguistic units is negotiated in the partnership according to the specific needs of learners and other potential users of these resources.

There is a wide variety of possible activities which help learners acquire the necessary language input. The designers choose from among: filling in, matching, true or false, ordering words in a sentence or paragraphs/ lines in a dialogue, multiple choice, word master (online completion exercises/ online hangman), correcting mistakes, organizing the vocabulary under the headings given, gapped text (script from which some lines/ paragraphs have been removed and learners have to reconstruct the text by putting the lines in the gaps), word search (learners have to find the words in the box with the definitions given). The linguist teams negotiate the type of activities and exercises to be developed, taking into consideration the favourite learning styles of the learners and the input given by the technical expert. The new pedagogies that stimulate learners' empowerment through sharing communication and learners' knowledge construction are encouraged and the available new digital technologies capacitate this approach. The intercultural dimension of the cohorts of learners using this facility will give the fortuitous context in which intercultural knowledge and medical practice specific in a diversity of cultural contexts becomes relevant and shared among the users. Each unit is accompanied by a vocabulary glossary and grammar explanations.

Each unit relies on the video and is accompanied by learning activities aiming at introducing, practicing and consolidating the vocabulary, structures, functions and cultural components present in each veterinary intervention. The envisaged activities cover all language skills: listening, reading, speaking and writing. The competence level of the linguistic units is negotiated in the partnership according to the specific needs of learners and other potential users of these resources. There is a wide variety of possible activities which help learners acquire the necessary language input. The designers choose from among: filling in, matching, true or false, ordering words in a sentence or paragraphs/ lines in a dialogue, multiple choice, word master (online completion exercises/ online hangman), correcting mistakes, organizing the vocabulary under the headings given, gapped text, word search (learners have to find the words in the box with the definitions given). The linguist teams negotiate the type of activities and exercises to be developed, taking into consideration the favourite learning styles of the learners and the input given by the technical expert.

The new pedagogies that stimulate learners' empowerment through sharing communication and learners' knowledge construction are encouraged and the available new digital technologies capacitate this approach. Social media is also be taken into consideration. The intercultural dimension of the cohorts of learners using this facility will give the fortuitous context in which intercultural knowledge and medical practice specific in a diversity of cultural contexts becomes relevant and shared among the users. Each unit is accompanied by a vocabulary glossary and grammar explanations.

The assessment is available through the course. The two most common methods of assessment are machine-graded multiple-choice quizzes or tests and peer-reviewed written assignments. Peer review is often based upon sample answers (rubrics), which guide the grader on how many points to award different answers. Learners are expected to learn via grading others. To share ideas with others, learners are invited to join discussion forums to ask questions, share and gain insights into medical practice.

Concluzii

The ZOE project may have an important potential impact through its results on the involvement of its target groups at both short and long term.

Students and PhD. studying veterinary medicine develop and practise relevant transversal and work-related skills necessary and useful for the world of work.

Cohorts of medical language students get a better educational service for practising medical communication in a variety of languages.

Veterinarians get a better support through students' work in the veterinary clinics.

Veterinary clinics: better coverage of work during the holidays season when centres are understaff; economy for the veterinary centres (students are not paid) and better quality of work done by the students;

Universities: practical application linked to academic theoretical information;

Veterinary lecturers: access to educational resources to develop teaching activities for medical communication skills; learn how to create open online resources;

Language teachers: the project provides language teachers with educational support for the language subjects for medical purpose (CLIL).

References

1. The National Sanitary Veterinary and Food Safety Authority, 2011 *Activity Report, available on-line at* http://www.ansvsa.ro/documente/admin/NSVFSA%20Report%202010_18667ro.pdf
2. World Health Organisation*, Zoonoses and the Human-Animal-Ecosystems Interface, available on-line at http://www.who.int/zoonoses/en/*

THE IMPACT OF CONTAMINATION WITH *NOSEMA SSP.* SPORES ON HONEY OBTAINED BY APIS MELLIFERA CARPATHICA

Agripina ŞAPCALIU[1], Vasilică SAVU[1], Ion RADOI[2], Florentin Gheorghe MILEA[2], Petruţ TANASE[3], Victor CALIN[3], Dan BODESCU[4]

[1] Beekeeping Research and Development Institute of Bucharest, Romania
[2]University of Agronomical Sciences and Veterinary Medicine Bucharest
[3] Spiru Haret University Bucharest
[4]University of Agronomical Sciences and Veterinary Medicine Iasi
Email: drsilesavu@yahoo.com

Abstract

Nosemose disease is a parasitic disease that affects old honey bees. It is produced by ubiquitous and opportunistic germs of Nosema spp., coupled with huge losses of honey bees within colonies (by depopulation), and reducing of honey production. In Romania, nosemose disease was officially admitted as being produced by two species of Nosema (Nosema apis and Nosema ceranae). The aim of our study was to establish a possible correlation between the honey naturally infested with spores of Nosema spp. (from families diagnosed positive), and the quality of honey used for human consumption. The study was performed on 65 canola honey samples received from private apiaries, of which 40% of them were taken and analyzed (26 positive samples). Various microscopic analyzes, organoleptic and physicochemical on the properties of honey samples were made. The results showed us that there were significant changes in the honey quality correlated with the degree of its natural pollution. We were found that the honey samples with more than 5 spores of Nosema spp./experimental field have presented serious deterioration in terms of organoleptic and physical-chemical properties. We grouped honey samples (26 samples) into 3 categories, according with their physicochemical and organoleptic changes. These changes in the honey quality have a negative economic impact on the use of bee products, and on health of bee families, too.

Keywords*: Apis mellifera, honey bee quality, Nosema spp. spores*

Introduction

Nosemoses are parasitoses frequently caused by two species of microsporidia belonging to the *Nosema spp.* (*N. apis* and *N. ceranae*) usually considered to be ubiquitary and opportunistic germs. Infections become endemic in countries that have long and humid winters, when climate factors favor them, as in the case of our country (5,6). The negative effects are to be found in various forms in bee colonies: diarrhea, affected capacity for orientation and return to the colony, metabolism disorders, altered pheromones level directly affecting behavior in the colony, lower immune capacity for defense and finally depopulated bee colonies (7). The presence of parasites is to be found in the entire bee colony in the structure of bees' intestine, inside the hive, in the hive products (honey), where they may persist beyond the clinical phase (7). The paper aims for a correlation between the existence of the spores in honey, the presence of infections in the bee colonies in the initial apiary and honey quality. The correlation may be directly between the degree of spore infestation and the main organoleptic and physical-chemical characteristics of honey. Our observations belong strictly to the field of research without any statistical and epidemiological evaluation of official implication.

Material and method

The research has been conducted in cooperation with private beekeepers that called on the Pathology Laboratory to establish the diagnosis during the active season period (2014-2015). A number of 65 canola honey samples have been examined out of which 26 samples were selected that had a positive diagnosis (presence of spores in honey) as well as a positive diagnosis of disease in the bee colony. (Fig. 1) The diagnosis of nosemoses disease was

established on the samples received from the owner by use of the standard method OIE 2008 (4).

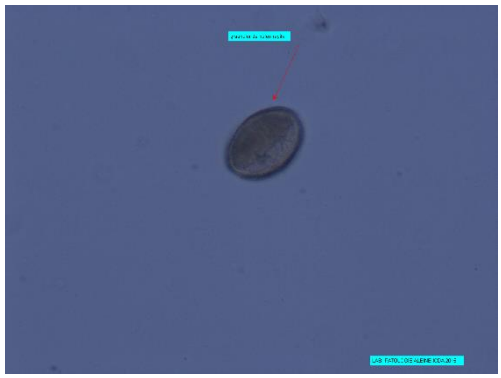


Fig. 1. Canola Honey (canola pollen in the honey)

The honey was examined to diagnose the presence of spores and to quantify their number. Samples of 10 ml were collected in depth from three different areas in the whole volume of honey. They were homogenized and examined with the x 40 optic microscope to diagnose and count spores. The tested honey lots were classified into 3 groups in correlation with the infestation degree: group 1 (1-4 spores) (Fig. 2), group 2 (5-7 spores) and group 3 (≥ 7 spores). (Fig. 3) To identify the spores, the morphological diagnosis criteria were used (hygiene quality indicator of the tested honey) (4).

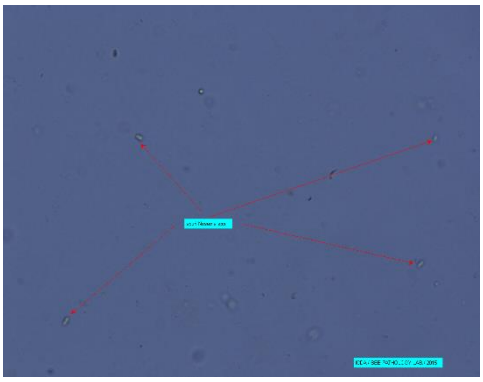


Fig. 2. Canola honey samples from group 1

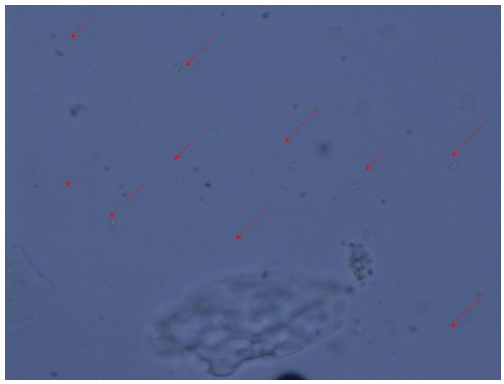


Fig. 3. Canola honey samples from group 3

For the organoleptic and physical-chemical analysis samples were sent to the Renar-accredited chemistry Laboratory and these were tested according to STAS standards and were evaluated according to criteria of organoleptic and physical-chemical quality (1, 2, 3, 9, 10). Sensory analysis as a scientific method to evaluate the organoleptic characteristics of honey has an important role in establishing its quality, being used not only to establish authenticity, classification and standardization but also to separate faulty samples and avoid contamination. Sensory analysis principles should be in conformity with the ones in STAS 784/3-1989 (1, 2, 3, 9. 10).

Fluid honey was examined organoleptically on samples as such, looking for foam and/or impurities, removing any contaminated samples with other agents that can alter honey. (Fig. 4, 5) After homogenization and filtering, parameters of aspect, consistency, color, smell and taste were also taken into account (1,2) Reference values had the following sensor

characteristics: foamless aspect, absence of foreign visible compounds, color from less to light yellow, golden-yellow, orange-yellow, dark yellow, reddish, brown yellow, dark brown, smell and taste typical of honey, more or less distinguished fragrance, sweet taste, homogenous, fluid, viscid, crystalized consistency (8).

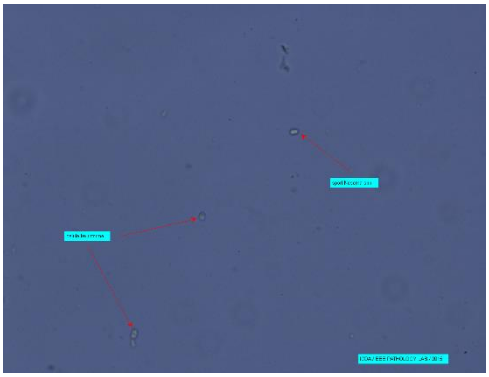


Fig. 4. Canola honey samples with *Nosema spp.* and yeast

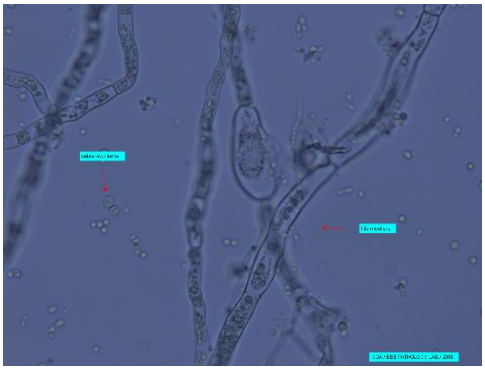


Fig. 5. Canola honey samples with *Nosema spp.* and fungal agents

Results and discussions

Microscopic method results are presented in table 1.

Table 1

Contamination degree of samples and distribution per groups

No. Sample	No. *Nosema spp.* spores /field	Group
1, 2, 3, 6, 9, 11, 12, 14,16, 18, 19,20, 21, 22, 23, 24, 26	1-4	1
4, 7, 8, 13, 15, 17, 25	5-7	2
5, 10	> 7	3

Depending on the contamination degree, the tested samples were classified into groups and it was noted that most samples belong to group 1, having a low contamination degree (1-4 spores/ microscopic field). In group 2 there are 7 samples, having a contamination degree of 5-7 spores/field, and group 3 displayed a high contamination degree only in 2 samples (≥ 7 spores/field).

The values of the witness lot were negative for the presence of *Nosema spp.* spores. Acidity did not exceed 4ºA having a water content of less than 20%, a pH of 3.5-4.5, and the HMF values under 2 mg /100 g.

The analysis of *physical-chemical* parameters for all 26 samples showed the following:

- ***Acidity*** was in the range of 1.3-4.1 ºA, the minimal excess of 4.1 ºA being noticed in samples 13 and 25, both having a medium infestation degree, as compared to the witness sample;
- ***Water content*** varied between 13-21.3 %, an excess of the admitted value (20%) being recorded in sample 13 of medium infestation degree, as compared to the witness sample;
- ***pH Value*** was of 3.8-4.2 no higher than the admitted maximum and minimum value having been found, as compared to the witness sample;
- The only altered parameter in the whole lot was that of the ***HMF (hydroximethilfurfural)*** value that registered increases to 2.07 mg/100 g and 10.7 mg/100 g, none of the samples

having the minimum accepted value for unprocessed honey of 1 mg/100 g honey, as compared to the witness sample (max 2 mg/100 g);

No correlation can be made between the groups distributed per *Nosema spp* spores contamination degrees and the changes in the physical-chemical parameters. However, 10 times higher HMF values were found in the samples contaminated with *Nosema spp* spores (2 samples).

The sensory analysis of the groups in the experiment presented low variations of the organoleptic parameters (consistency, aspect, transparency, color).

- Samples of high contamination degree (group 3) appeared opaque, having a medium or thick layer of foam, higher viscosity, humidity and mass crystallization (7,69 %);
- Samples of medium contamination (group 2) displayed mainly an altered yellow-brownish color, crystallization and opaque aspect with or without a foam layer (26,92 %);
- Organic and inorganic impurities were present in 10 cases without a direct correlation to the infestation degree (38,46 %).

Conclusions

The analysis of the *Nosema spp* spores infestation degree of honey showed in most cases a low infestation degree;

No positive correlation exists between the infestation degree of honey (hygienic quality) and the physical-chemical and organoleptic quality;

To two samples 10 times higher HMF values were found in the samples contaminated with *Nosema spp* spores;

The results that have been obtained showcase potential changes to the quality of honey in bee colonies infested with *Nosema spp.,* these being only preliminary results.

Acknowledgments

The research was supported by project ***PN 108/2012***/"*Studies on the preparation and testing of an apiphytotherapeutical product for veterinary use "NOSEMA-API" for the treatment and prophylaxis of Nosema disease in bee families*"

References

1. Banu, C. (2002), Calitatea şi controlul calităţii produselor alimentare, Editura AGIR, Bucureşti 462-465
2. Bulancea, M., (2002), Autentificarea, expertizarea şi depistarea falsificărilor produselor alimentare, Editura Academica, Galaţi, 3, 81- 98.
3. Lenca Gabriela, (2003), Caracteristicile de calitate ale mierii de albine, utilizată în procese de valorificare biotehnologică, Buletinul AGIR (3)
4. OIE (2008), Manual of diagnostic tests and vaccines for terrestrial animals, Nosemosis of Honey Bees. Office International des Epizooties; Paris, France. (Chapter 2.2.4.)
5. Sapcaliu Agripina, Mateescu Cristina, Pop Aneta, Savu Vasilică (2014), "In vitro Screening of Hydroalcoholic Plant Extracts to Control *Nosema apis* Infection", Journal of biotechnology, 185, (201409): S46, ISSN 0168-1656
6. Sapcaliu A., Mateescu C., Savu V., Radoi I., Militaru I. (2013), "Experimental infection in honeybees using honey with spores of nosema sp. (apis/ceranae)", International Apicultural Congress 29, Kyiv, Ukraine
7. Savu Vasilică, Agripina Şapcaliu, (2013), „Patologia albinelor**"**- Editura Fundaţiei România de Mâine. Bucureşti. ISBN 978-973-163-951-2. 31-38, (Chapter 7)
8. Şindilar E. (2000), Controlul igienic al produselor şi subproduselor de origine animală, Universitatea Agromomică şi de Medicină Veterinară „Ion Ionescu de la Brad", Iaşi, , p. 107 -116. 4.
9. *** Ordinul Ministerului Sănătăţii nr. 976/1998 privind „Normele igienico-sanitare pentru alimente", publicat în M.O. partea I, nr. 288/1999. (5).
10. *** STAS 784/3 – 1989

IMPORTANCE OF MICROSCOPIC TESTING OF HONEY AND POLLEN SAMPLES IN THE PROPHYLAXIS OF MAJOR BACTERIAL DISEASES IN *APIS MELLIFERA CARPATHICA* BEES

Vasilică SAVU[1], Agripina SAPCALIU[1], Petruţ TANASE[2], Victor CALIN[2], Ion RADOI[3], Cristina Ştefania PÎRVULEŢ[4]

[1]Beekeeping Research and Development Institute Bucharest, Romania
[2]Spiru Haret University Bucharest
[3]University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania
[4]Academy of Agricultural and Forestry Sciences*"Gheorghe Ionescu-Sisesti"*
E-mail address:drsilesavu@yahoo.com

Abstract

The purpose of the study was to monitor the presence of bacilli in the honey and pollen samples in correlation to the positive diagnosis of these major bacterial diseases in bees. The study took 3 years, and approximately 156 samples of honey and bee bread from reserve honeycombs and 156 live bee intestine samples were processed. To identify the bacilli in honey, bee bread (pollen) reserve and live bees intestine , we used our own method, and the confirmation of their presence was done through methodology OIE/2008. Of the total tested samples, the bacilli were found present in 63 samples from reserve honeycombs and in 67 samples from live bees' intestine. The bee colonies that did not test bacilli in the samples examined for the duration of the monitoring, did not present a disease episode and did not register mortality of pathologic nature. The mortality registered in the apiaries under study throughout the 3 year-period was 30-100 % for the apiares from which samples testing positive for bacilli had been received. The study confirms that a correlation exists between the presence of bacilli in samples of honey and bee bread from reserve honeycombs, and their presence in adult bees' intestine. The microscopic testing of honey and pollen samples, as well as of bee intestine, may constitute an important prophylactic method in the management of major bacterial diseases in bees (American and European foulbrood).

Keywords: *honeycombs, major bacterial diseases, live bee intestine*

Introduction

Major bacterial diseases (American foulbrood and European foulbrood) are infectious-contagious diseases present in almost all countries (including Romania) that exist as devastating diseases, affecting the larva stage of *Apis mellifera* and other species of *Apis* bees. (4, 5)

During the inactive period (winter), when the reserve food is insufficient to raise the brood and feed the bee families, in reserve honey and pollen in active season and in the form of solid nutrients in inactive season. Supplementary food is necessary when bees do not have sufficient reserve honey in the winter, thus avoiding losses from starvation (1, 2).

The purpose of the study was to monitor the presence of bacilli in the honey and pollen samples in correlation to the positive diagnosis of these major bacterial diseases in bees. (8)

Materials and methods

The study took 3 years from 8 apiaries, and approximately 156 samples of honey and bee bread from reserve honeycombs and 156 live bee samples were processed, compared with control lot (2 apiary). (Table 1)

Table 1

Total tested samples of reserve honeycombs (honey and bee bread) and adult live bees' intestine

Total tested samples	**Honey and bee bread from reserve honeycombs**	**Adult live bees' intestine**
312 samples	156 samples	156 samples

To identify the bacilli in honey, bee bread (pollen) and live bee intestine, we used our own method, and the confirmation of their presence was done through methodology *from* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (3, 6, 7).

Of the total tested samples, the bacilli were found present in 63 samples from reserve honeycombs, in 67 samples from live bees' intestine and 182 negative samples (absent bacilli) (Table 2) (Fig. 1).

Table 2

Total tested positive and samples negative from honey, pollen and live bees' intestine

Honey and pollen from reserve honeycombs (present bacilli)	**Honey and pollen from reserve honeycombs (absent bacilli)**	**Live bees' intestine (present bacilli)**	**Live bees' intestine (absent bacilli)**
63 positive samples (40.4 %)	93 negative samples (59.6 %)	67 positive samples (42.9 %)	89 negative samples (57.1 %)

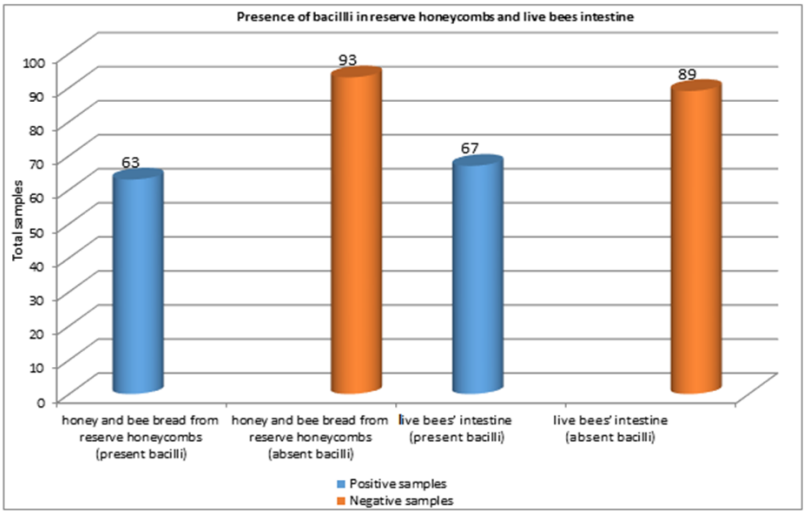


Fig. 1 Total tested positive and samples negative from honey, pollen and live bees' intestine

Distribution of samples and hives mortality percentages in the experimental lot is presented in table no. 3

Table 3

Distribution of samples and hives mortality percentages in the experimental lot

	Apiary no. 1	**Apiary no. 2**	**Apiary no. 3**	**Apiary no.4**	**Apiary no.5**	**Apiary no. 6**	**Apiary no. 7**	**Apiary no. 8**
Experimental lot (no. samples / no. colony)	13	27	17	10	32	24	15	18
Mortality in colonies (%)	13 (100%)	9 (35%)	10 (60%)	3 (30%)	16 (50%)	10 (40%)	5 (30%)	18 (100%)

According to Table no. 3, the distribution of samples in the control lot in the 8 apiaries studied for three years show that 10- 32 samples (pollen, honey, live bees' intestine) were collected for laboratory tests. Mortality percentages in the studied apiaries oscillated between 30-100% depending on the seriousness of the bacterial infestation and colony size. There is no correlation between colony size and mortality percentage in the beehives belonging to the control lot.

Mortality percentage in the control lot is presented in table no. 4

Table 4

Distribution of bee colonies and hives mortality percentages in the control lot

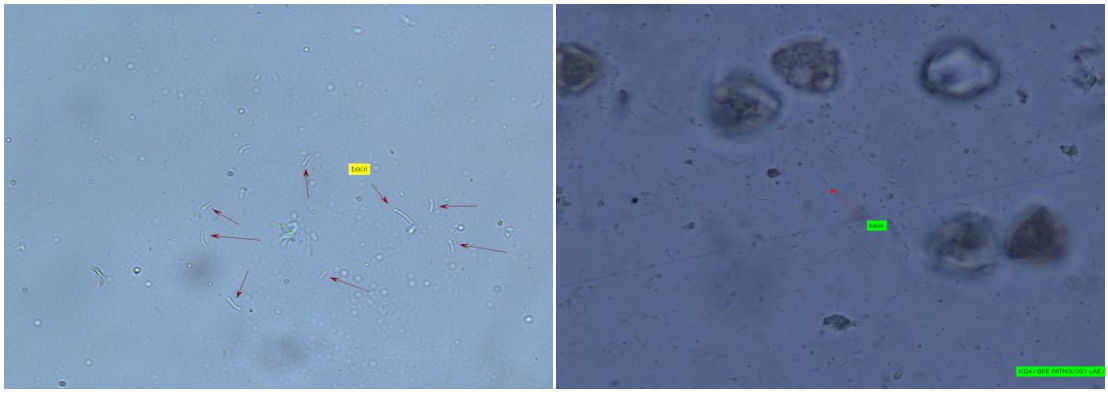
	Apiary lot no. 1	Apiary lot no. 2
No. beehives (control lot)	35	20
Mortality in colonies (%)	0	0

Bee colonies in the witness lot were distributed in the 2 apiaries as follows: 35 colonies in apiary no. 1 and 20 bee colonies in apiary no. 2, the mortality percentage being zero in both apiaries.

Results and discussion

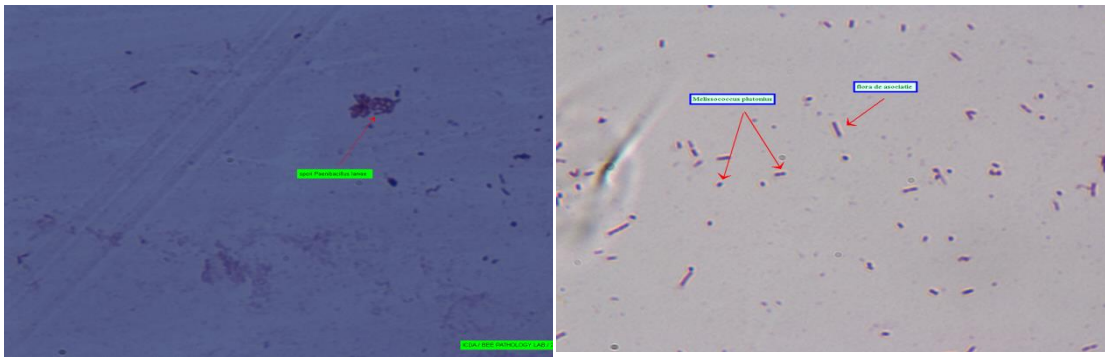
Direct microscopic investigations made on the honey and live bees' intestine samples showed the presence of vegetative forms of etiological agents suspected of major bacterial diseases (Fig. 2 a, b).

The presence of bacilli in reserve honeycombs and in intestine (Fig. 3 a, b) was correlated to the bacterioscopic identification of etiological agents for the American foulbrood and for the European foulbrood (Fig 4, a, b), and subsequently with the evolution of disease episodes clinically manifested, of high morbidity and mortality in adult bees.



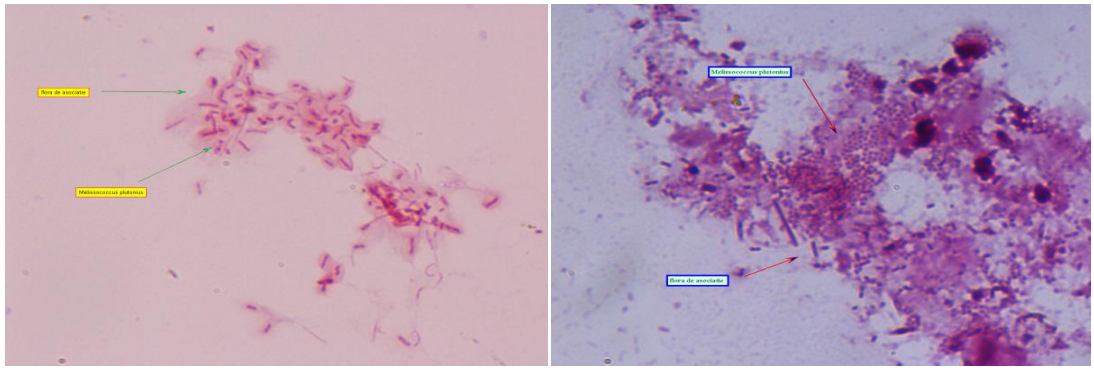
a b

Fig. 2 Vegetative forms of etiological agents suspected of European and /or American foulbrood from honeycombs (a) and live bees' intestine (b) (microscopic test directly from the intestine) x 400



a b

Fig. 3 Highlighting the etiological agent suspected of American foulbrood (a) and European foulbrood from *live bees' intestine* (b) (Gram colored smear) x 1000

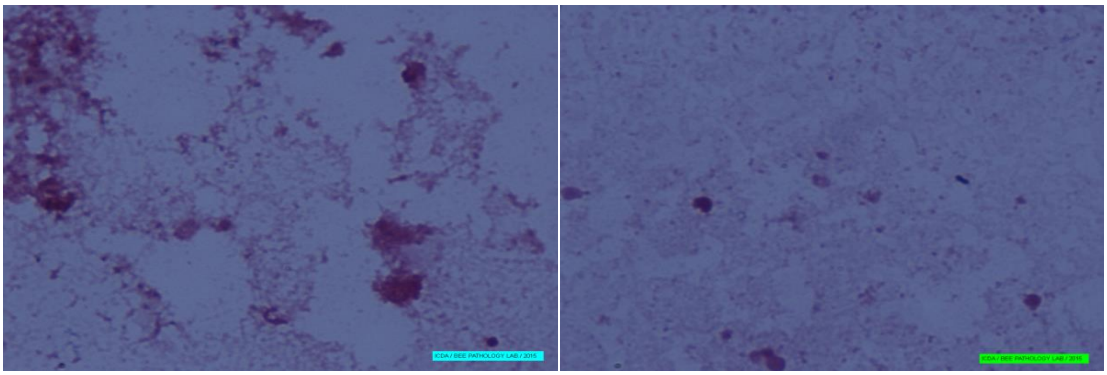


a b

Fig. 4 Highlighting the etiological agent suspected of American foulbrood (a) and European foulbrood from *honeycomb*s (b) (Gram colored smear) x 1000

The identification of etiological agents suspected of major bacterial diseases by bacterioascopic examination, after Gram colouring, of reserve honey and live bees' intestine samples constituted the base for the confirmation diagnosis.

Confirmation examination on the witness lot were negative for all tested samples, correlated to the zero mortality percentage in these lots (Fig. 5 a, b).



a b

Fig. 5 Negative samples of reserve *honey combs* (a) and *live bees' intestine* (absent bacilli) (b) (Gram colored smear) x 1000

The bee colonies that did not diagnostic tested bacilli in the samples examined for the duration of the monitoring, did not present a disease episode and did not register mortality of pathologic nature. The mortality registered in the apiaries under study throughout the 3 year-period was 30-100 % for the apiaries from which samples testing positive for bacilli had been received.

Conclusion

The study confirms that a correlation exists between the presence of bacilli in samples of honey and bee bread from reserve honeycombs, and their presence in adult bees' intestine.

The microscopic testing of honey and pollen samples, as well as of bee intestine, may constitute *an important prophylactic method in the management of major bacterial diseases* in bees (American and European foulbrood).

Acknowledgements

„This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI–UEFISCDI, project number ***PN 157/2014***"

References

1. ACA (2012). Manualul cursantului, Ed. I, Editura LVS Crepuscul, 144-146
2. ACA (2011). Ghid de bune practici în apicultură, Editura LVS Crepuscul, 114-115
3. Bailey, L. (1983). *Melissococcus pluton*, the cause of European foulbrood of honey bes (Apis spp.). Journal of Applied Bacteriology 55: 65-69.
4. Dirk C de Graaf et al., **(**2013). Standard methods for American foulbrood research. Journal of Apicultural Research 52(1): DOI 10.3896/IBRA.1.52.1.11
5. Eva Forsgren et al., (2013). Standard methods for European foulbrood research. JJournal of Apicultural Research 52(1): DOI 10.3896/IBRA.1.52.1.12
6. O.I.E. (World Organisation for Animal Health) (2008) - American foulbrood of honey bees. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees), vol.1, 6 ed.: 395-404
7. O.I.E. (World Organisation for Animal Health) (2008) - European foulbrood of honey bees. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees), vol.1, 6 ed.: 405-409
8. Savu Vasilică, Agripina Şapcaliu (2013) - „Patologia albinelor"- Editura Fundaţiei România de Mâine. Bucureşti. ISBN 978-973-163-951-2. 31-38

ASPECTS REGARDING THE ACTIVITY OF THE PHAGOCYTIC MECHANISMS IN IMMUNITY REGULATION OF THE NEW-BORN CALVES

Rita GOLBAN
[1]The State Agrarian University of Moldova, Chișinău
golbanrita@gmail.com

Abstract

The scientific investigations revealed in this research present the objectives of studying some immunological aspects of the phagocytic activity in immunity regulation in the neonatal period of the new-born calves at the age of 5, 10, 20 and 30 days. As a result of the immunological investigations were revealed various indexes which characterize the activity and intensity of the phagocytic activity, which varies at different age periods. The dynamics of these indexes demonstrates that in the neonatal period, at the calves aged of 5 days, the phagocytic activity constituted 52.33 ± 0.60, compared to the age of 10 days, which constituted 41.67 ± 0.65, which shows a decrease expressed by various aspects of external factors, which acts on the newborn animal in the first days of life. The values of the phagocytic activity at the age of 20 and 30 days constituted 38,56 ± 0,56 and 35,44 ± 0,47, which confirms the decrease of the phagocytic processes at the animals. From the obtained results, it is noticeable that the phagocytic intensity at the neonatal animals at the age of 5 and 10 days determined significant values which constituted 2,32 ± 0,02 and 1,83 ± 0,01, compared to the animals at the age of 20 and 30 days, where these values constituted 1,78 ± 0,01 and 1,57 ± 0,01. The results of the research demonstrated that at the neonatal animals the defense mechanisms are not sufficiently triggered in order to protect against the microorganisms, viruses and other pathogenic agents aggression.

Key words: *Phagocytosis, Phagocytic activity, Phagocytic intensity, Macrophages.*

Introduction

According to the classic conception, is considered that the activity of the immune system has exclusively a beneficial, protective effect on the organism. From this point of view, the immune system is tolerant to its own substances, because it has learnt to recognize them during embryonic life, but it has the property to recognize and differentiate promptly the foreign substances, towards which it activates and removes them from the organism.

The cellular base of the cellular immune response is represented by the phagocytic mechanisms, immunocompetent cells, etc. In this context, the cellular immune response protects the organisms against the aggression of fungi, parasites, viruses and bacteria with intracellular localization. Therefore, they are responsible for the cellular immunity, they express receptors that recognize only short peptide sequences from the protein antigens [1], [3], [4].

The elements of the immune system - macrophages, lymphocytes - T - B with populations, determine the immunological profile or the immune status and results from the complex analysis process of the elements that take part from the immune system. These immunocompetent cells are developing till the animal is born, after which they begin intensively to function, favoring the development of the immune response from the first days of life of the newborn animal.

In its concept, the immune system is one of the most complex from the organism. Its complexity derives from the complicated network structure of communication, cellular and molecular, maintaining permanently the supervision of organisms. They recognize almost limitless variety of foreign cells and molecules, distinguishing them from those of the organism itself. Therefore, they "remember" each infection and at a second exposure to the same pathogen agent, they react more efficiently.

The immune system is so cleverly designed that, if it would be in a perfect functioning condition, it may oppose to any type of aggression. It is consisted from cellular and humoral

components, capable to specifically recognize the antigenic determinants and to bind by them through efficient structures. The antigen (the non-self structure) penetrated in the organism, induces the clonal selection of lymphocytes through a mechanism of the lymphocytes selection, which possess specific receptors, for the non-self structure, after that appearing the cell division, through the"clonal expansion", followed by the increasing number of lymphocytes [2], [5], [6].

The mechanisms through which it operate are external and internal mechanisms. The external mechanisms are represented by skin, mucous membranes and body fluids. These constitute natural barriers that prevent the penetration of pathogens in tissues. When these mechanisms are defeated, it is trying the removal of the pathogen antigens through the internal mechanisms of the nonspecific immunity: additional physiological factors, phagocytosis and inflammation, constituting the immune defense mechanisms, which acts on the defense mechanisms of the host organism.

The study of the immune system components and their intervention in ensuring the organisms homeostasis exposed to harmful external and/or internal environmental factors influence is currently a significant desiderate.

Structural integrity of immunocompetent organs, as of the immunologic postagresional ways of response constitute a 'sine qua non' survival condition of individuals and species [7], [8], [9].

Therefore, the main objectives of these researches constitute the study of the phagocytic mechanisms in regulation of the immunity at the newborn calves.

Material and method

The scientific investigations were performed at the Laboratory of Microbiology from the Faculty of Veterinary Science of the State Agrarian University of Moldova. For realization of the investigations were used samples of blood from the new-born calves aged up to 30 days, in order to determine the immunological blood indixes. The blood samples were collected from the jugular vein with heparin, based on the 0,3 ml of heparin and 10,0 ml of blood, for the purpose of anticoagulation.

Blood samples were collected from the jugular vein with heparin in the calculation of 0.3 ml to 10.0 ml heparin blood, for the purpose of anticoagulation.

Samples of blood were used for the opsono-phagocytic test constituted from the cellular mechanism of the phagocytosis and the involved cells in this process.

For performing the opsono-phagocytic test was used 1,0 ml of blood stabilized with heparin and 0.1 ml suspension of E. coli from 1.0 ml physiological solution with 500 mln. microbial cells. The tubes were stirred, then incubated in the thermostat during 30 minutes at T-37 ° C and centrifuged at 1500 rot./ min. The supernatant was removed using the Pasteur pipette. Were performed smears colored according to the Romanowsky – Giemsa method, fixed with methanol and colored during 30 minutes. The samples of blood were viewed under a microscope immersion 90.

The test determined the number of microorganisms phagocytosed per 100 neutrophils. The index of activity and intensity of the phagocytosis of the cells was determined as the percentage of neutrophils cells that participate in the process of phagocytosis.

At the same time the phagocytic intensity was determined by the number of the microorganisms incorporated by one neutrophil. The calculation was represented by the report between the sum of the phagocytosed microorganisms devided to the number of the neutrophils which participate at the reaction.

Results and discussion

As a result of the immunological investigation, regarding the study of the immune phagocytic mechanisms in the neonatal period at the new-born calves revealed different indices characteristic to the phagocytic activity and intensity which varies in different age periods.

The indices of the phagocytic activity at the neonatal calves determined significant values at different age periods (Fig. 1). These data reveal that the animals possess resistance to infectious germs. Therefore an important factor of the protecting organism cellular system is represented by the opsono – phagocytic reaction of the leukocytes.

The dynamics of these indexes demonstrates the fact that in the neonatal period at the calves aged 5 days, the phagocytic activity was 52.33 ± 0.60, compared to age 10 days, which constituted 41.67 ± 0.65, which reveals a decrease expressed by various aspects of external factors acting on the newborn animal in the first days of life. Analyzing the dynamic of the indices of phagocytic activity at the age of 20 and 30 days it was found that the values constituted 38.56 ± 0.56 and 35.44 ± 0.47, fact which confirms the decrease of the phagocytic processes at these animals.

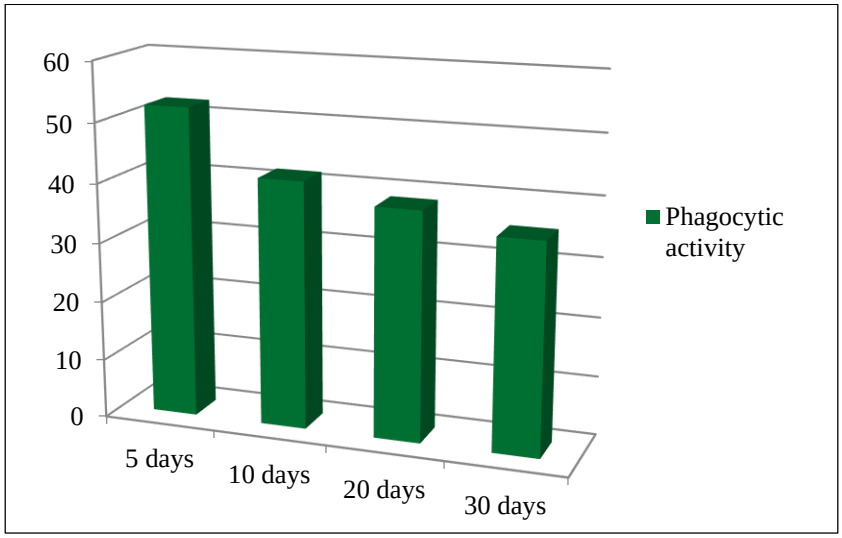


Fig.1. The indices of the phagocytic activity at the new-born calves depending on age

The dynamics of these indexes demonstrates the fact that in the neonatal period at the new-born calves aged 5 days the phagocytic activity constituted,33 ± 0,60, compared to the age of 10 days, which constituted 41,67 ± 0,65, which reveals a decrease expressed by various aspects of the external factors, acting on the newborn animal in the first days of life. Analyzing the dynamics of the phagocytic activity indexes at the age of 20 and 30 days was stated, that the values were 38,56 ± 0,56 şi 35,44 ± 0,47, which confirms the decrease of the phagocytic processes at these animals.

In the immunological aspect, one can see that the phagocytic activity in this period of life of neonatal calves is attributed to the fore neutrophils, the rest being made by the macrophages. Therefore, the phagocytic mechanisms induce phenomena which can be realized in two ways, depending on the bacteria resistance: first way, without opsonizing using the direct interaction between the phagocytic cell and the antigen; and the second way, with the opsonizing which constitute the interaction for which is necessary an additional molecule,

opsonine, which plays an adaptor role between the bacteria and the leukocyte. In this context, the phagocytosis is continuing with the adhesion, after that with the fase when the pseudopodia surround the bacteria. The final faze of distruction offers the final digestion of the bacteria.

The mechanism of the immune response regulation is based on the immune reaction, controled by the regulation systems. In this context in the situation of blocking the regulation mechanisms, the clonal proliferation or the syntheis of the immunoglobulins cannot be limited, which leads to the profound alteration of the immune response, accompanied by installation and evolution of diseases that usually have a fatal end.

Therefore, the cellular activity processess are initiated through the mechanisms and the complexes characterized by the initiation and realization of functions of cells implicated in the immumne response. According to these reserches we can affirm that the immune system cells pass the steps of the cellular cicle.

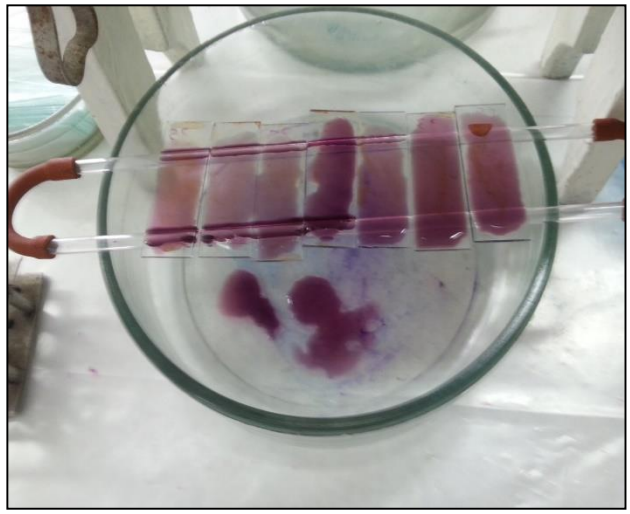


Fig. 2. Romanowsky – Giemsa coloration method.

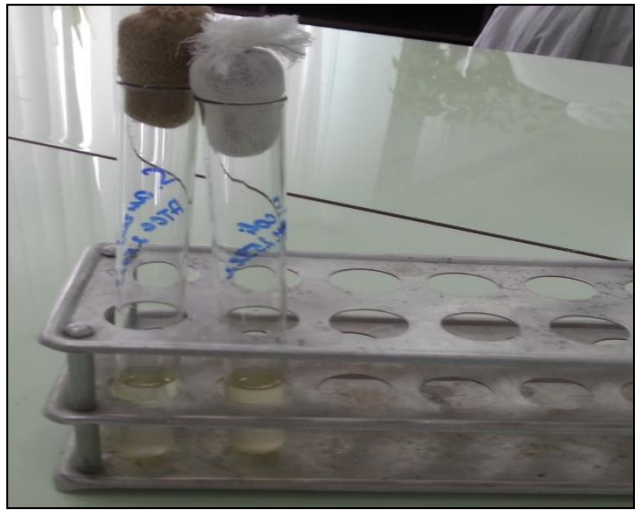


Fig. 3. Microbial cultures.

Important data were registered related to the phagocyticic intensity at the neonatal animals in diverse age periods (Fig. 4)

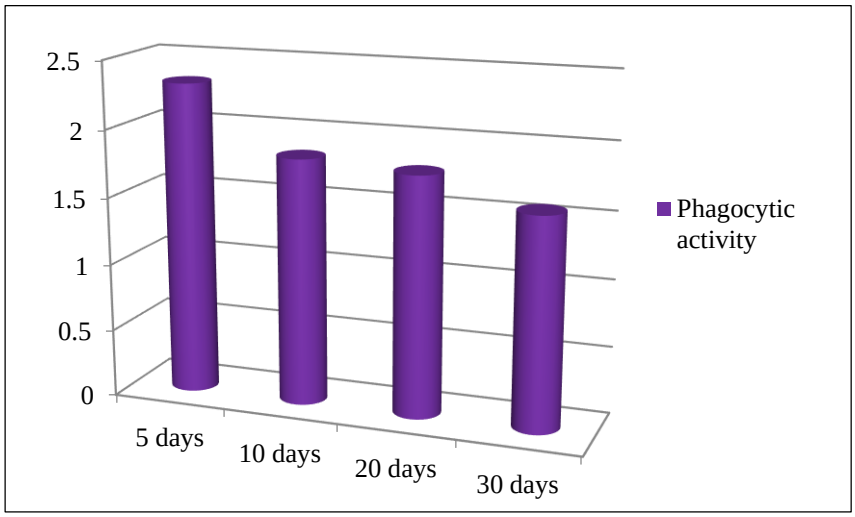


Fig.4. The indices of the phagocytic intensity at the new-born calves depending on age

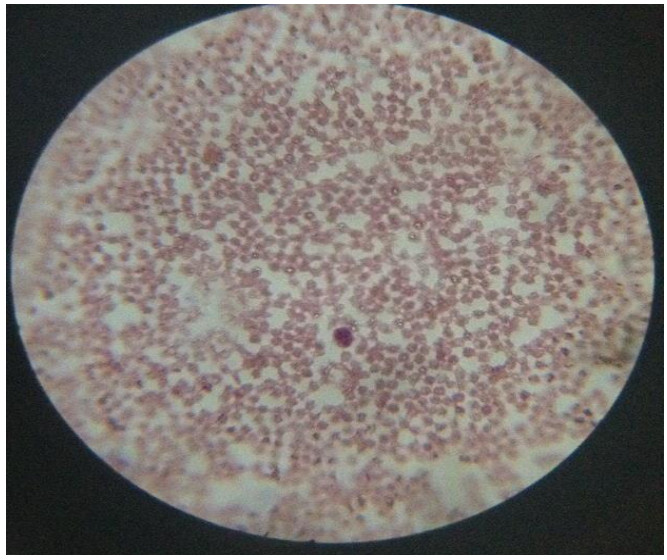


Fig. 5. Opsono-phagocytic test

According to the obtained results, it can be stated that the phagocytic intensity at the neonatal animals with the age of 5 and 10 days determined significant values 2,32 ± 0,02 şi 1,83 ± 0,01, compared to the animals with the age of 20 and 30 days, where these values constituted 1,78 ± 0,01 and 1,57 ± 0,01. Therefore, at the neonatal animals the defense mechanisms are not sufficiently triggered for the protectiom against the aggression of the microorganisms, viruses and other pathogens.

At the same time, these data reveal that the cellular activation processes are initiated through mechanisms characterised by the initiation and realization of the cellular functions

implicated in the immune response. According to these researches we can affirm the fact that the immune system cell, pass the steps of the cellular cycle. From these point of view, the activation of the immune system cells are realized through the signals of the antigen and of a costimulatory molecule represented by the cytokine IL-1. In this aspect are realised other activations of the immune processess as a result of the recognition of the antigen by the molecule BCR.

According to the specialty studies the neutrophils, monocytes and macrophages implicated in this process, constitute the first line of defence against the pathogenic organisms. The elimitation of the bacterial infections through the process of phagocytosis implicates the recruitment of the neutrofils at the level of the blood circluation and from the level of bone marrow by chemotaxis to the place of infection. Thus the neutrophil phagocytic activity is potentiated by the complement system and by antibodies.

The development of immunity and tolerance is supposed to some mechanisms of fine regulation because the immune response against the self antigen or the toleration of a pathogen potential can have unfavorable consequences for life. The regulation of the humoral or cellular immune response is a complex process of modulation where intervene a number of ways through which is mentained the specific defence of the organism at a certain level an with a certain duration, in order to realize the homeostasis and to mentain the health.

Conclusions

1. The nonspecific immunity is considered the first means of defense in the immune response. The mechanisms through which it operates are external and internal, which constitute the main natural barriers, preventing the penetration of the pathogenic antigens in the tissues.
2. The results, regarding the phagocytic activity in this period of life at the neonatal calves is attributed first of all to neutrofils, the rest being performed by macrophages.
3. The phagocytic mechanisms induce phenomena that can be achieved in two ways, they depend on the resistance of bacteria which is assumed to the direct interaction between the phagocytic cell and the antigen and the adapter process between the bacteria and the leukocyte.
4. Phagocytic intensity values at neonatal animals in different age periods determined significant values, fact which confirms that the defense mechanisms are not sufficient to protect against the aggression of microorganisms, viruses and other pathogens.

References

1. Andrieş L., Barba D., Cerneţchi O., Stratan V., 2014: Imunologie clinică. Chişinău: Editura „Tipografia Centrală". 556 p: ISBN 978-9975-53-383-6.
2. Andrieş L., Olinescu A., 1992: Compendiu de imunologie fundamentală. Chişinău: Editura „Ştiinţa". 476 p.
3. Brokaw A., 2013: Immunology: Use Howard Hughes Medical Institute Resurces to Teach. Ohio. 37 p. Disponibil: http: // www.hhmi.org/ biointeractive/ teacher-guide-immunologzy.
4. Cristea V., Crişan M.,2011: Curs de imunologie pentru studenţii facultăţii de medicină. Cluj-Napoca.255 p.
5. Gâjâilă G., 2002 : Imunologie analitică. Aspecte fundamentale şi metodologice. Bucureşti: Editura „ Printech". 224p: ISBN 973-652-583-X.
6. Gâjâilă G., 2003 : Sistemul imunitar la suine. Bucureşti: Editura „Cartea Universitară". 131 p. ISBN 973-86231-7-0.
7. Rosen R Hugo., 2008: Transplantation Immunology: Whatthe Clinician Needs to Know for Immunotherapy Gastroenterology. 134 : 1789-180.
8. Siloşi I., 2014 : Imunologie. Craiova: Editura „SITECH". 266P. ISBN 978-606-11-3717-6.
9. Taşbac A., 2014: Îndrumar pentru laboratorul de imunologie veterinară. Bucureşti: Editura „Larisa", Câmpulung Muscel, 170p. ISBN 978-606-715-271-5.

INVESTIGATIONS ON LYMPHOCYTES BLASTIZATION MECHANISM AS A RESULT OF STIMULATION WITH A SPECIFIC ANTIGEN

Rita GOLBAN[1], Mihai CARP-CĂRARE[2], Cristina RÎMBU[2], Eleonora GUGUIANU[2], Cătălin CARP-CĂRARE[2], Cristina HORHOGEA[2] , Andreea-Paula COZMA[2]

[1]The State Agrarian University of Moldova, Chișinău

USAMV Iași[2] Faculty of Veterinary Medicine, 8 Mihail Sadoveanu Alley

golbanrita@gmail.com

Abstract

In this scientific paper are reflected the results of the investigation on the lymphocytes blastization mechanism aspects as result of stimulation with anti-anthrax antigen and protein purified derivative. The aim of the scientific research was to study the lymphocytes blastization mechanism as a result of stimulation with a specific antigen in order to determine the immunity of the vaccinated and unvaccinated animals. The study was made on twenty bovines using the method of lymphoblastic transformation test. The evaluations take part of a preliminary study in order to identify the transformation process of lymphocytes in macrophages cells determined by nucleus and cytoplasm cells increasing. The obtained results reveal that the majority percentage of the macrophages at this stage of cultivation is determined by the reduced phagocytic activity. As a result of visualization the preparations, there were observed macrophages young cells without vacuoles, with basophilia in the cytoplasm and well-defined contours of nucleus. The lymphocytic stimulation intensity was evaluated by the percentage determination of the blasts from the population, procedure known as morphological method of evaluation the blasts transformation. The active transformations of the macrophages after revaccination of young animals constituted 15,5% of blasts, compared to control group, where the index of stimulation constituted 3,5%.

Key words: *lymphoblasts, immune response, mitogenic activity, specific antigen.*

Introduction

The lymphocytic blastogenesis mechanism represents the researches regarding the immunological aspects in realization of the complex metabolic phenomena, based on the lymphocytes, which are in the pending stage, and which change morphologically and behaviorally, fact, which gives possibility to be engaged in the process of the immune response. From this point of view, from the initial population of the lymphocytes, only a part of them pass through blast transformation, these lymphocytes being characterized by membrane receptors corresponding to the antigenic epitopes, which causes the immune reaction.

The consequence of the blastization mechanism constitutes the proliferation of the stimulated lymphocytes, but their mitosis ensures a sufficient number of the immunologic implicated lymphocytes, practically determining a cell population numerically and functionally appropriate for a high quality immune response (Siloşi, 2014; Rosen, 2008; Olinescu et al, 2004; Parham, 2005; Zeanea, 1990).

According to the immunologic studies, the cellular and humoral components are capable to recognise in a specific way the antigenic determinants and to link by them through efficient structures - the lymphocytes membrane receptors. In the same time, the entered in the body antigen, reveals the induction of clonal selection of lymphocytes, by a mechanism of selecting of the lymphocytes, which has specific receptors for the non-self structure, then triggering cell division by "clonal expansion" followed by increasing the number of lymphocytes.(Andrieş, 2014; Răpuntean et al., 2008; Vior et al., 2005; Tizard, 2014; Carp Cărare et al., 2002; Găjăilă, 2002).

In the last years, the LTT has known a significant improvement, due to new techniques of cellular cultures and analytical methods, becoming a high sensitive test, reproductible both for medical research and for routine diagnosis. The LTT principle is based on the fact, that the

lymphocytes which were previously sensitized towards a certain antigen proliferates and are transforming in blasts when are exposed again to the same antigen.

The lymphoblastic transformation test (LTT) is frequently used with applications on the immunity determination at the intact and vaccinated animals, at the infectious agents diagnostic, drug allergies, food, etc. The test is based on the lymphocytic cellular division induced by the specific antigen contact. The blast transformation of lymphocytes takes place under the action of phytohemagglutinin and concanavalin A, which are mitogenic substances. The transformation is given by the increasing by 2-3 times of the lymphocytes dimensions, with nucleus modifications and appearance of a large number of cytoplasmic organelles with the secretory function. Thus the lymphocytes are capable to proliferate while contacting with some specific antigens (memory cells) in the animals blood. LTT offers useful information regarding the functional capacity of the T lymphocytes, which can be obtained by "in vitro" proliferative responses evaluation of these cells to different incentives.

From this point of view, the reason of the researches was to study the lymphocytes blastization mechanism, as result of stimulation with a specific antigen, in order to determine the immunity of the intact and vaccinated animals.

Material and method

In the scientific research were evaluated 20 intact and anti anthrax vaccinated bovines. The immunological investigation were performed in the Microbiology and Immunology Laboratory from the Veterinary Medicine Faculty of the Moldavian State Agrarian University. Lymphocytes proliferative response was evaluated by the blast transformation test as proof of the determination of the cell's reactivity. This test intends to highlight the capacity of reaction of the isolated lymphocytes or of the leukocites from the whole blood towards different antigens.

The objective of the research consists of the lymphoblast transformation index determination, using immersion microscopy. The lymphoblastic transformation test was performed with the PPD (protein purified derivative) antigen extracted from Mycobacterium tuberculosis. The lymphoblastic transformation was determined in cellular lymphocytic cultures, cultivated in thermostat at 37 °C.

For this purpose, on heparin from the jugular vein (10-15 ml) of bovines, were collected samples of blood. The samples of blood were left 5-6 hours at the room temperature in an inclined position. Subsequently the blood plasma was separated, after which were separated the lymphocytes, with the form of a white color ring, located at the erythrocytes and blood plasma boundary layer of the sample tube.

The plasma with lymphocytes was carefully drawn of with pasterovskhy pipette in a sterile sample tubes, after which being centrifuged for 5 minutes at 1000 rpm (rev/min). The supernatant layer was removed. The precipitate was filled with Medium 199, making resuspension of lymphocytes 3 times. The lymphocytic suspension, obtained in volume of 3,0 ml, was distributed in 1,0 ml centrifuge tubes with M-199, being filled with antibiotic suspensions: penicillin, streptomycin, nystatin, pH = 7.2-7.4.

The content of the tubes constituted from samples with anti-anthrax antigen, PPD antigen and control. Simultaneously the tubes were thermostated at the temperature 37°C, 120 hours, after which were centrifuged during 5 minutes at 1000 rpm. From sediment were made smears, fixed with methanol and colored using the Romanovsky-Gimsa method.

After counting 200 lymphocytes cells and macrophages, was expressed in percentages the number of the blast cells and untransformed lymphocytes. The counting of the cells was

visualized on 3 fields of microscopy, where the cells lay isolated and their structure was clearly seen allowing to determine their species.

Results and discussions

The functional investigations results of lymphocytes represented by the lymphoblastic transformation test gave the possibility to appreciate the capacity of these cells to react promptly and intensely under the influence of some mitogenic – antigenic or non antigenic.

In aspect of the process of lymphoblastic transformation was stated the differentiation of lymphocytes by increasing the cell's dimensions, the number of mitochondria, ribosomes, lysosomes etc. The differentiation forms manifested through increased dimensions of blast cells, was characterized by the presence of the nucleus and the cytoplasm. The blasts were represented by round cells with central nucleus and basophilia cytoplasm. Therefore in the process of lymphocytes blastogenesis were determined biochemical processes, which favors the proteins, ARN, ADN synthesis and mitogenic division of cell. Simultaneously a blastic cell favored formation of a clone composed of 16-32 immunocompetent cells

At the same time was stated lymphoblastic transformations induced by specific and non specific stimulators (table 1). The lymphocytic non specific stimulators were distinguished by the characteristic structures of the origin and chemical activity. Therefore, substances with mitogen characters are characteristic for bacteria, for products and structures of their activities.

Table 1.

The vegetal and animal origin bacterial products with mitogenic lymphocytic activity

Mitogenic stimulus	Species	Lymphocytic
Protein A	Staphylococcus aureus	T- and–B-limphocytes
The filtrate of culture in the broth	Streptococcus pyogenes	T-limphocytes
Con A	Enterobacteriaceae	B-limphocytes
Tuberculin	Mycobacterium tuberculosis	B-limphocytes
Antiimunoglobulin	Heterologous immune serum	B-limphocytes

The immunologic researches reveal that numerous species of bacteria posess mitogenic activity, determined by the components of the capsular substance, flagella, cell wall membrane, cytoplasmic ribosomal fractions, bacterial metabolic products etc.

There were stated important bacterial mitogens from the composition of the protein A at staphylococcus, Gram- bacterial lipopolysaccharide, tuberculin and filtrates of cultures from streptococci broth medium. According to some authors the property to perform lymphoblastic transformations at animals possess various products of animal origin (immunoglobulins, identified in heterologous immune sera) and vegetal origin (CoA etc.).

It is remarkable that the lymphoblastic transformation test is based on the immunocytobiologycal investigations, which has the purpose to characterize the status of the cellular components of the immune system of animals. These in vitro evaluations are difficult, but at the same time, very important and necessary in order to explain several immune dysfunctions.

The performed investigations, regarding the blastization mechanism, gives us the possibility to appreciate the importance of the stimulated lymphocytes mechanism and of their proliferation, but their mitosis ensures a sufficiently big number of immunologically implicated lymphocytes or practically a cellular population, suitable for a well-defined immune response.

As a result of the performed investigations, on the coloured preparation were determined the macrophages transformations of lymphocytes. Under the action of the PPD specific agent, was determined the stimulation of lymphocytes from the presensitized animals which determines the selective stimulation mechanism of a certain clone of lymphocytes, which specifically recognizes the antigen and after that the allogenic lymphocytes in a mixed culture of lymphocytes.

The lymphocytes stimulation intensity was evaluated by the percentage determination of the blasts from population, procedure known under the name of the morphological evaluation method of blast transformation.

The macrophages transformation dynamics determined values of 13,21±0,52 in the experimental group compared to the control group which constituted 3,24±0,42 and 14,32±0,16 compared to 3,15±1,13 during 7-15 days (figure 1).

In the same time during 30 and 60 days the macrophages transformations constituted 15,22±1,25 and 3,50±0,62 compared to the control group which constituted 15,50±1,25 and 3,50±0,62.

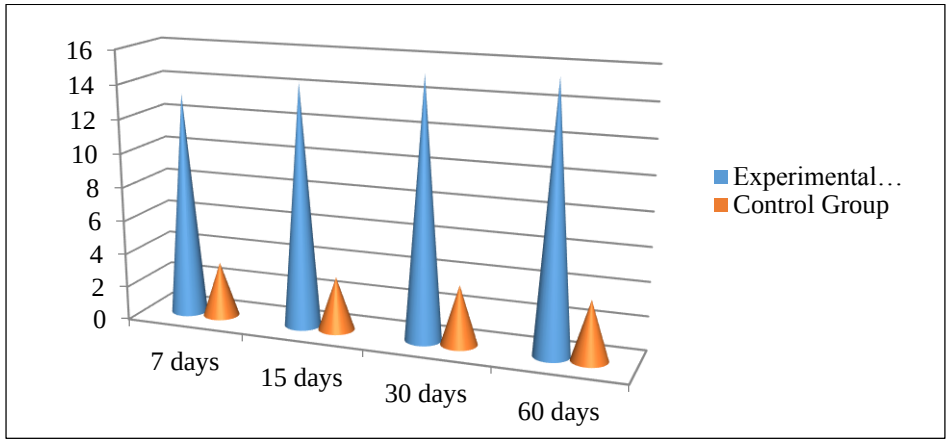


Fig.1. Active macrophages transformations values at the animals

Therefore at the investigated animals, were determined active macrophages transformations after the revaccination, which constituted 15,5% of blasts, compared to control group, where the stimulation index constituted 3,5%. The stimulation index was considered positive, its value constituted - 3. These data reveal the changes of the unspecific blastization index and represent a significant evidence for the appreciation of the functional capacity of the lymphocytes.

Thus the decreasing of the stimulation index can be considered as an indicia of the cellular immunodeficiency, suggesting the reducing of the response capacity. Therefore, high indexes can be interpreted by the existence of a reactive process which is in evolution, from the side of some clones of lymphocytes with normal functionality.

In this aspect the cellular immunity testing has a high importance in the diagnosis of the immune deficiencies associated with severe infections at the level of the respiratory tract, intestinal tract, skin, etc. The most frequent infections are induced by microorganisms with intracellular tropism, especially viruses, fungi, bacteria.

As a result of the lymphoblastic transformation test, the lymphocytes are activated and stimulated for proliferation at the contact with the specific antigen. In this context, the

lymphocytes after receiving signals from the antigens for which they have membrane receptors, enter in the cellular division cycle or "blast transformation". As a result, immediately after receiving the stimulator signal at the membrane level, take place modifications of electric charges, being followed by the activation of the metabolic processes and the increasing of the cellular density. Gradually the cell increases its volume and becomes "blast", with big nucleus, rich in chromatin, with one or more nucleoids, intensely basophil cytoplasm, rich in polyribosomes.

The table 2 and 3 reveal different possibilities of cultivation of lymphocytes in vitro. Therefore there is a correlation between the response of the mitogenic stimulated lymphocytes and the immune status of the animal. In case of our investigations, the lymphocytes of the investigated animals reacted in a positive way to the intradermoreaction with PPD and transformed blastic in vitro in presence of PPD.

Table 2.

In vitro cultivation of lymphocytes

Cellular population	Other populations	Cellular and molecular populations presented in the culture dish
The separation by centrifution in gradient of density	Without glass accession With glass accession	Lymphocytes, monocytes Lymphocytes
Not separated in density gradient (total blood)	Without washing With washing	All blood elements, inclusively plasma All blood elements, inclusively plasma

Table 3.

The correlation between the stimulus and the duration of cultivation the lymphocytes in vitro

Duration of cultivation	Antigenic stimulus
2-3 days	Mitogenic (lectins)
5-6 days	Antigenic, allogenic
7-8 days	IL-2

During the immunologic study of the mechanism of the cellular activation, there were initiated complex processes, characterized by initiation and realization of cells functions implicated in the immune response. Therefore, the cells passed through the stages of cellular cycle. The activation of the T cell was realized by the signals of the antigen and of a costimulatory molecule represented by a cytokine (IL - 1). At the same time were triggered activations of B cells as a result of antigen recognition by the BCR molecules. After the activation of the B cells, the proliferation and synthesis of antibodies was realized.

The process of lymphocytes transformation in macrophages cells was determined by increasing of nucleus and cells of cytoplasm. The majority percentage of macrophages at this stage of cultivation determined a reduced phagocytic activity. Therefore, after visualization the preparations, was observed young macrophages cells without vacuoles with basophilia in the cytoplasm and well defined contours of the nucleus.

In this context, the organism is permanently requested by factors of the external medium which entering in contact with its protection forces, generates reactions against non-self elements.

Thus, the immune response is considered as a protection mechanism, by which the organism recognizes the non-self elements.

The recognition of self-elements by the distinction of non-self is exact and proper to each organism. Among mechanisms which generate diseases or favours chronicization a special role is taken by the immune response regulation.

Given the aforementioned, it can be stated that the macrophages are essential cells for the protection function. They constitute a protection line before activation of the T and B cells. The macrophages are activated by the lymphokines issued by the T cells. The lymphokines stimulate the immune function.

As a result of the conducted investigations, was stated that the lymphoblastic transformation was determined at the intact and immunized animals, with the purpose to determine the intensity of the cellular immunologic process.

The lymphoblastic transformation in the blood of the anti anthrax immunized animals increased by 3 times, compared to control group, which reveals a high level of antibodies synthesizing. The investigation of the older animals under the action of PPD, determined the lymphoblastic transformation in their blood and formation of an insignificant percent of blasts.

Conclusions

The investigations on the lymphoblasitc transformation reveals that the lymphocytes are activated and stimulated for proliferation while contacting with the specific antigen. The blastization mechanism of lymphocytes determines morphological and behavioral changes, allowing them to engage in realization of the immune response.

The lymphoblastic transformation index at the immunized animals, compared to intact animals demonstrated high level synthesis of antibodies.

The non specific blastization index represent an evident significance for appreciation of the functional capacity of the lymphocytes. In this context the active macrophages transformations after the revaccination of the young animals constituted 15,5% of blasts, compared to the control group, where the stimulation index constituted 3,5%.

References

1. Andrieş L., 2014. Imunologie clinică. Tipografia Centrală, Chişinău, 556p. ISBN 978-9975-53-383-6.
2. Carp Cărare M., Timofte D.2002. Imunologie şi Imunopatologie. Editura Venus, Iaşi, 290p.
3. Găjăilă G., 2002. Imunologie analitică. Aspecte fundamentale şi metodologice..Printech, Bucureşti, 224p. ISBN 973-652-583-X.
4. Olinescu A., Panait M., 2004. Introducere în imunologie. Editura Medicală, Bucureşti, 542p.
5. Parham P., 2005. The Immune System (Second Edition)- Garland Science Publishing, New York and London.
6. Răpuntean Gh., Răpuntean S., Nicodin F., 2008. Imunologie veterinară. Editura Academic Pres, Cluj-Napoca, 344p. ISBN 978-973-744-120-1.
7. Rosen R., 2008. Transplantation Immunology: Whatthe Cllinician Needs to Know for Immunotherapy Gastroenterology. 134:1789-180.
8. Siloşi I., 2014. Imunologie. SITECH, Craiova, 266p. ISBN 978-606-11-3717-6.
9. Tizard R., 2004. Veterinary Immunology: An Introduction –Seventh edition, Sunders, An Imprimant of Elsevier.
10. Vior C., Răducănescu H. 2005. Imunopatologie. Editura Brumar, Timişoara, 452p.
11. Zeanea C. 1990. Imunologie clinică. Editura Medicală, Bucureşti, 423p.

CHARACTERSTICS OF THE E.COLI STRAINS ISOLATED FROM CHICKENS

Hasan Majid H. HASAN*, Doina DANES, Ana Virgilia POPA***, Costin STOICA******
* PhD student, Dep. Infectious Diseases and Preventive Medicine (DIDPM), Veterinary Medicine Faculty, University of Agronomic Sciences and Veterinary Medicine. Bucharest.
majidhasan1971@gmail.com
** Prof. Univ. Dr., Head of DIDPM, The scientific sponsor. UASVM, Bucharest
*** Prof. Dr., National Institute for Veterinary Medicine "Pasteur", Rom.
**** DVM, Romvac Company, Bucharest.

Abstract

In chickens, colibacillosis refers to any local or systemic infection produced by Escherichia coli (APEC) and it's economically worldwide impact is measured in the morbidity and mortality rates of affected birds. This is an acute and often systemic disease. Colibacillosis is a complex syndrome, starting from the respiratory mucous characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis being most typical. The E.coli infection is generally associated with other factors, as mycoplasmas, viruses and/or environmental factors. However, management approaches based only on protecting poultry against the predisposing conditions have limited effect for the disease control. Antimicrobial drugs remain important in reducing both incidence and mortality associated with this disease, but there is increasing evidence that (APEC) is becoming more resistant to antimicrobial agents. A vaccine-based approach for the control remain highly desirable. Currently available vaccines are not totally effective, mainly because of the diversity of APEC strains. Currently E. Coli serotypes O1:K1, O2:K1 and O78:K80 are recognized as the most prevalent, however the number of serotypes is increasing. The APEC isolates vary profoundly in virulence. Virulent factors are encoded by at least 12 virulence genes, located either in the bacterial genome or in plasmids. Many studies demonstrated that virulence factors are rarely all present in the same isolate, their frequencies in clinical isolates is varying. This all indicates that APEC strains are a heterogeneous group. The virulence genes can be transmitted among APEC strains and can be detected using the PCR multiplex technique fastening the diagnosis on bacterial colonies or on samples from organs. For the development of effective vaccines, virulence genes studies are also important. These genes code for adhesins (F1-, P-fimbriae), iron acquisition systems (aerobactin, yersiniabactin), hemolysins (hemolysinE, temperature sensitive hemagglutinin), resistance to the bactericidal effects of serum and phagocytosis (outer membrane protein, iss protein, lipopolysaccharide, K/1), capsule and colilcin production) as well as toxins and cytotoxins (heat stable toxin, cyto-/verotoxin and flagella toxin). The limited knowledges on pathogenicity and immunogenicity of APEC infections urges further experimental studies. APEC share specific virulence factors with humans E.coli, their zoonotic potential is under consideration.

***Key words**: E.coli, diagnosis, colibacillosis, PCR, virulent*

Introduction

Escherichia coli was identified in 1885 by the German pediatrician Theodor Escherich [1] belongs to the Enterobacteriaceae family, rod-shaped, Gram-negative, facultatively anaerobic and live on a many different kinds of substrates, it uses the mixed-acid fermentation, producing lactate, ethanol, succinate, acetate and carbon dioxide, also the optimal growth of most E. coli strains occurs aerobically at 37°C and some exceptional strains can grow at temperatures up to 49°C. Some of strains are motile due to having of flagella [2], highly distributed in humans and animals intestine as a normal inhabitant to maintain the physiology of their host newborns protecting them against enterotoxigenic E. coli and Salmonella spp [1], [53]. Two kinds of E. coli strains exist, the commensal and the pathogenic one, the latter one is subdivided into intestinal pathogenic strains and extra-intestinal pathogenic E.coli (ExPEC) [3]. ExPEC strains are not able to cause enteritis. However, they can colonize and predominate to the other strains in 20% of healthy hosts, and entry by such group to extra-intestinal sites like urinary tract is an essential prerequisite for infection occurrence, in contrary to commensal E. coli strains. In intestinal lumen the E. coli strains may cause infectious diseases in cases of immunosuppressed or when the gastrointestinal barriers are damaged, so the predisposing

factors are necessary. The avian primary respiratory condition is due to certain pathogenic agents such as mycoplasma, infectious bronchitis virus (IB) or Newcastle disease (ND) virus usually is accompanied by the E. coli infection as a secondary infection [4]-[8]. Also ND vaccine and IB vaccine may induce colibacilosis occurrence [4], [9], due to host defense impairment [10]. The most serotypes of E. coli that find in the avian intestine are nonpathogenic, and a limited number produce extra-intestinal infections. There is a high diversity of serogroups among APEC isolates that result in high number of untypeable. APEC. It is a concern at all ages of poultry production, can distribute through vertical and horizontal transmission, and results in a variety of manifestations of infection [11], [12].

APEC are difficult to defend against because of the high diversity of strains and acquired resistances to the host defenses as commensal bacteria, due to plasmid transferring from gut bacteria to those located in the respiratory tract [13]. Although APEC strains clearly belong to the phylogenetic group of extra-intestinal pathogenic E. coli, it has a wide serological diversity [14], [15] also they belong predominantly to the three serogroups, O1, O2, and O78 [16]. Several vimerulence genes have been demonstrated to be implicated in avian colibacillosis, those encoding for adhesins (F1, P, and Stg fimbriae, curli, and EA/I), iron acquisition systems (aerobactin, Iro proteins, yersiniabactin), anti-host defense factors (OmpA, Iss, lipopolysaccharide, and K1), and the Sit iron acquisition locus), auto-transporters, Vat, and AatA), the phosphate transport system, sugar metabolism, and the IbeA protein [17]-[23]. However, these virulence factors (VFs) are rarely all present in the same APIC isolate, demonstrating that APEC strains constitute a heterogeneous group. This diversity currently impedes clear characterization or identification of an avian E. coli isolate as a pathogenic or nonpathogenic strain. Thirteen virulence genes were more frequently exist in APEC isolates than in nonpathogenic isolates but, individually, none of this genes could allow the identification of an isolate as an APEC strain, and it has been reported that virulence factors (VFs) could be used as markers for detection of APEC [24]. Certain virulence factors have been considered to be positively linked with the pathogenicity of APIC including, Adhesions, Iron acquisition systems, Temperature-sensitive hemagglutinin, Colicins, Capsule, Serum resistance, Toxins, Other virulence factors found among APEC strains include pathogenicity islands [25]-[27] and the locus of enterocyte effacement (LEE) [28]-[30] also the Congo red linkage capacity in agar medium which was observed among APEC strains was considered as a virulence marker to APEC strains [31], [32].

APEC cause localized and systemic infections, such as respiratory colibacillosis that commonly happen in the first several weeks old broiler chickens followed by a systemic infection with distinctive fibrinous lesions,and an acute colisepticemia [33]. Colibacillosis is the major cause of morbidity and mortality in poultry and is responsible for significant economic losses worldwide through decline in production and performance and recurring costs associated with antibiotic prophylaxis and therapy so it is important economically [11], [10], [34], [35], [33]. Study and deep knowledge of the APEC virulent genes is very important to understand how APEC is able to avoid conventional host immune responses against infection, and what can we do to induce the best defensive immunity against APEC strains [36]. The therapeutic treatment of E. coli infections is threatened due to the prevalence of the multi-resistant ability against antibiotics and some of disinfectants, among commensal host gut E. coli strains and respiratory tract E. coli [13], and this induces a threat to the public health and the agricultural industries [36]. At slaughter, the gut resistant strains readily soil the carcasses of chickens and as a result chickens meats will be often contaminated with multiresistant E. coli [37]-[43], also eggs will be contaminated during laying [44]. Hence, resistant faecal E. coli of chickens can infect human directly and by food. These resistant strains may colonize the

intestine of human and may also distribute the resistance genes to human endogenous flora [45]. Two major problems currently make it difficult to control poultry colibacillosis, namely, the lack of a dependable method to identify the pathogenic strains of E. coli and the not totally effective available vaccines, also vaccines against E. coli are not widely employed, and this may be due to the large variety of serogroups involved in field outbreaks.

The control of colibacillosis has been largely based on upon using of autologous bacterins vaccines [46] but these gave ineffective short-lived serotype-specific protection due to the high diversity of E. coli capable of infecting poultry so it's necessary to extend the cross-serotype efficacy stimulated by the vaccines. The killed and attenuated strains vaccines induced sufficient protection against homologous strains infections, but less efficient against heterologous strains infections [11], [12].

The aim of study was to perform a systematic review of the more important characters of the worldwide isolated chicken E. coli and the diagnostic methods used for detection and characterization or identification of the isolated human and animal E. coli bacteria to control e coli infections.

Materials and Methods

Sixty three articles were selected from hundreds of articles analyzed in PubMed, Google Academic databases, ncbi, research gate, vet research. Biomed central, and science direct. The research method followed three main steps:

1- Scientific databases research of the relevant articles concerning E. coli surveillance and diagnostic.
2- Analysis and selection of the relevant data
3- Extraction and summarization of the results

Results and conclusions

Most of studied articles described the risky pathogenic nature of the E. coli strains isolated from infected ,not infected poultry and from the environment, and the virulent factors induced high pathogenicity and the genetic diversity, also they described the more famous identification techniques that are depended for E. coli classifying and diagnosis, which they are:

a) The conventional Identification (morphological and routine biochemical tests) [47]

b) Serotyping analysis for depending on their surface antigens O, H, and K antigens (more than 700 serotypes of E. coli are existed), this technique is more specific but it has no ability to provide us with clear information about E. coli pathogenic strains also it can't classify all of these strains and it's s not an effective diagnostic tool, particularly since serotype does not reflect the virulence trait [48], [49].

c) Molecular Identification (for screening of certain virulence genes and ColV plasmids which concern with the E. coli strains pathogenicity [50], [48] by using of multiplex PCR. A study described a multiplex PCR protocol to detect the existence of iss, tsh, iucC, and cvi [51]. Another study characterized at least a number of genes to identify an APEC strain, namely, iutA,iroN, hlyF, iss,, and ompT [52]. In another study a multiplex PCR protocol was depended to detect the existence of eight virulence genes (toxin genes astA and vat, the iron acquisition genes irp2 and iucD, increased serum survival protein gene iss, adhesin genes papC and tsh, and the ColV plasmid operon genes cva-cvi) [50]. Schouler et al., 2012, determined a validated PCR diagnostic assay that depended on four different associations of virulent factors that enable the characterization of 70.2% of APEC with a 4.3% error margin during identifying of pathogenic strains and the four different associations had been serogrouped using all

available O (O1 to O181) antisera virulence genotyping (multiplex PCR), detection of cytotoxic activity of bacterial supernatants and lysates (Detection of toxins using phenotypic assays) and Virulence for chicks .

d) Other acceptable techniques, like molecular typing methods including consensus-PCR typing [54], multi-locus enzyme electrophoresis [55], Selective capture of transcribed sequence studies [47], random amplification of polymorphic DNA [56], genomic suppression subtractive hybridization [57]. The prediction of the pathogenicity of E. coli strains isolated from diseased animals is crucial [24]. It was clear that the use of prevalent pathogenic serotypes, in each country, is advisable to prepare at first the suitable vaccines that should work against all gram negative bacteria determined by LPS, O-polysaccharide side chain [58] and be heterogenic and second preparing of suitable the vaccination strategies [59]. Use of multiple antibiotics has been shown to control extent of pathology after challenging with several resistant APEC strains [13]. Furthermore, using of antibiotic drugs in the future will tend to be reduced and restricted in commercial farms so E. coli vaccines are an alternative way [60]. The E. coli strains isolates from human UTI and avian colibacilosis have a substantial overlap in terms of phylogenetic groups, serogroups, and virulence genotypes, including plasmid-DNA-related sequences, adhesion, iron uptake, protectins and toxins-related sequences [61]. APEC is genetically closely related to extra-intestinal pathogenic E. coli of human origin such as uropathogenic E. coli (UPEC) and neonatal meningitis-causing E. coli [62]-[65].

Currently, E. coli is considered to be the most significant example of gram-negative bacterium related with diverse diseases because of the different pathogenicity mechanisms and diseases that E. coli able to cause so it's important to

- Check and study of the genetic construction of isolated E. coli strains continuously to know and adapt of the suitable programs for preventing and treating to avoid infections occurrence.
- Use of Multiplex PCR as the most effective specific diagnostic tool for the high biological diversity APEC strains.
- It’s important to apply an ongoing investigation for diagnose of the prevalent E. coli strains in the country to prepare the suitable vaccines (heterogeneous bacterins & work against all gram negative bacteria determined by LPS, O-polysaccharide side chain).
- Use of multiple antimicrobials for controlling extent of pathology.
- Birds also need to be protected against the pathogens that induce infections with APEC. This is possible by using of mycoplasma-free birds, protecting the birds against mycoplasma infection and viral diseases by vaccinations, housing climate must be kept optimal for birds like bird’s density, humidity, ventilation, dust ammonia and disinfection etc to prevent stress factors and reduce the probability to get infection by E. coli.

References

1. P. Feng, S. Weagent and M. Grant, *Bacteriological Analytical manual*, 2002. [Online] Available: www.lib.ncsu.edu/pubweb/www/ETDdb/web_root/collection/available/et d-04102005- 213953/ unrestricted/ etd.pdf
2. U. Fotadar. P. Zaveloff and L. Terracio, “Growth of Escherichia coli at elevated temperatures,” *Journal of Basic Microbiology,* vol. 45, 2005, pp. 403-4
3. T. Russo et al., “Identification of a new iron-regulated virulence gene, ireA, in an extraintestinal pathogenic isolate of Escherichia coli,” *Infect. Immun.,* vol. 69, 2001, p. 6209–6216
4. K. Nakamura, H. Ueda, T. Tanimura, K. Noguchi, “Effect of mixed live vaccine (Newcastle disease and infectious bronchitis) and Mycoplasma gallisepticum on the chicken respiratory tract and on E.coli infection,” *J. Comp. Pathol.,* vol. 11, 1994, pp. 33-42

5. H.J. Barnes, W.B. Gross and J.P. Vaillantcourt "Colibacillosis," in *Diseases of poultry,* vol. 11, 2003, p. 631– 652
6. M. Dho-Moulin and J. Fairbrother, "Avian pathogenic Escherichia coli (APEC)," *Vet. Res.,* vol. 30, 1999, pp. 299-316
7. W. Gross, "Diseases due to Escherichia coli in poultry," *Escherichia coli in domestic animals and man, 1994,* pp. 237-259
8. N. F. Cheville et al., "Comparative pathologic findings of Escherichia coli infection in birds," in *Journal of the American Veterinary Medical Association,* vol. II, 1978, pp. 584-587.
9. J. B. Kaper, J.P. Nataro and H.L. Mobley, "Pathogenic Escherichia coli," in *Nat. Rev. Microbiol.,* vol. 2, 2004, pp.123–140.
10. K. Dhama, S.Chakraborty, R. Barathidasan, R. Tiwari, S. Rajagunalan and S.D. Singh, "Escherichia coli, an economically important avian pathogen, its disease manifestations, diagnosis and control and public health significance," *Anim. Vet. Sci,* vol. 3, 2013, pp. 179-194
11. H.J. Barnes, L.K. Nolan and J.P. Vaillantcourt., "Colibacillosis," *Diseases of Poultry,* vol. 12, 2008, pp. 691-732
12. S. M. Lutful Kabir, "Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns," *Int. J. Environ. Res. Public Health,* 2010, pp. 89–114
13. Dheilly, A. Bouder, L. L. Devendec, G. Hellard and I. Kempf, "Clinical and microbial efficacy of antimicrobial treatments of experimental avian colibacillosis," *Vet. Microbiol.,* 2011, pp. 422-429.
14. M. Moulin-Schouleur, M. Répérant, S. Laurent, A. Brée, S. Mignon-Grasteau, P. Germon, D. Rasschaert and C. Schouler,"Extraintestinal pathogenic Escherichia coli strains of avian and human origin: link between phylogenetic relationships and common virulence patterns," *J. Clin. Microbiol.,* 2007*,* pp. 3366-3376
15. J. E. Blanco et al.,"Serotypes of Escherichia coli isolated from septicemic chickens in Galicia (northwest Spain)." in *Vet. Microbiol.,*1998, pp. 229 –235
16. M. Mellata, M. Dho-Moulin, C. M. Dozois, R. Curtiss, P. K. Brown; P. Arné, A. Breé, C. Desautels, and J. M. Fairbrother, "Role of virulence factor in resistance of avian pathogenic Escherichia coli to serum and in pathogenicity," in *Infect. Immun.,* (2003a), pp.536-540
17. E. M. Antao, "Signature-tagged mutagenesis in a chicken infection model leads to the identification of a novel avian pathogenic Escherichia coli fimbrial adhesin," in *PLoS One,* 2009, 4:e7796
18. Chouikha et al.,"A selC-associated genomic island of the extraint-estinal avian pathogenic Escherichia coli strain BEN2908 is involved in carbohydrate uptake and virulence," *J. Bacteriol.,* 2006, pp. 977–987
19. M. Cortes et al., "Inactivation of ibeA and ibeT results in decreased expression of type 1 fimbriae in extraintestinal pathogenic Escherichia coli strain BEN2908," *Infect. Immun.,* 2008*,* pp. 4129 – 4136
20. F. Dziva and M.P.Stevens,"Colibacillosis in poultry: unraveling the molecular basis of virulence of avian pathogenic Escherichia coli in the natural hosts," *Avian Pathol.,* 2008*,* pp.355–366.
21. S.Kariyawasam and L.K. Nolan,"Pap mutant of avian pathogenic Escherichia coli O1, an O1:K1:H7 strain, is attenuated in vivo," *Avian Dis.,* 2009*,* pp. 255–260
22. G. Li et al., "AatA is a novel auto-transporter and virulence factor of avian pathogenic Escherichia coli.," *Infect. Immun.,* 2010*,* pp. 898 –906.
23. Rouquet, G. et al., "A metabolic operon in extraintestinal pathogenic Escherichia coli promotes fitness under stressful conditions and invasion of eukaryotic cells," *J. Bacteriol.,* 2009, pp. 4427–4440.
24. C. Schouler, B. Schaeffer, A. Brée, A. Mora, G. Dahbi, F. Biet and M. Moulin-Schouleur, "Diagnostic Strategy for Identifying Avian Pathogenic Escherichia coli Based on Four Patterns of Virulence Genes," *Journal of Clinical Microbiology, 2012,* pp.1673–1678
25. V. R. Parreira and C.L. Gyles, "A novel pathogenicity island integrated adjacent to the thrW tRNA gene of avian pathogenic Escherichia coli encodes a vacuolating autotransporter toxin," *Infect. Immun.,* 2003*,* pp.3118- 3126
26. S. Kariyawasam, T.J. Johnson and L.K. Nolan, "The pap operon of avian pathogenic Escherichia coli strain O1:K1 is located on a novel pathogenicity island," *Infect. Immun.,* 2006*,* pp.744-749
27. S. Kariyawasam, T.J. Johnson and L.K. Nolan, "Construction and characterization of type I fimbriae fimH deletion mutant from avian pathogenic Escherichia coli," *Infect. Immun.,* 2008, pp.744-749.

28. G. Foster, H.M. Ross, T.W. Pennycott, G.F.Hopkins and I.M. McLaren, “Isolation of Escherichia coli O86:K61 producing cyto-lethal distending toxin from wild birds of the finch family,” *Lett. Appl. Microbiol.,* 1998, pp.395-398.
29. T.W. Pennycott, H.M. Ross, I.M. McLaren, A. Park, G.F. Hopkins and G. Foster, "Causes of death of wild-birds of the family Fringillidae in Britain," *Vet. Rec.,* 1998, pp. 155-158.
30. R. M. La Ragione et al., "Efficacy of a Live Attenuated Escherichia coli O78:K80 Vaccine in Chickens and Turkeys," *Avian Diseases,* vol. 57, no. 2, June, 2013, pp. 273-279.
31. H. A. Berkhoff and A.C. Vinal, “Congo red medium to distinguish between invasive and non-invasive Escherichia coli for poultry,” *Avian Dis.,* no. 30, 1986, pp. 117-121.
32. W. T. Corbett, H.A. Berkhoff and A.C. Vinal,“Epidemiological study of the relationship between Congo red binding Escherichia coli and avian colisepticemia,” *Can. J. Vet. Res.,* no. 15, 1987, pp. 312-315
33. G. R. Huff, W. E. Huff, N. C. Rath and G. Tellez, “Limited treatment with β-1,3/1,6- glucan improves production values of broiler chickens challenged with Escherichia coli,” *Poult. Sci. ,* no. 85, 2006, pp. 613–618
34. K. Kemmett, T. Humphrey, S. Rushton, A. Close, P. Wigley and N.J. Williams, ”A longitudinal study simultaneously exploring the carriage of APEC virulence associated gene and the molecular epidemiology of faecal and systemic E. coli in commercial broiler chickens,” *PLoS One, 2013,* 8:e67749
35. Derakhshandeh, T. Zahraei Salehi, H.Tadjbakhsh and V. Karimi,“Identification, cloning and sequencing of Escherichia coli strain chi1378 (O78: K80) iss gene isolated from poultry colibacillosis in Iran,” in *Lett Appl Microbiol.,* no. 49, 2009, pp. 403-404
36. T.J. Johnson, S. Kariyawasam, Y. Wannamuehler, P. Mangiamele, S. J. Johnson, C. Doetkott, J. A. Skyberg, A. M. Lynne, J. R. Johnson and L. K. Nolan, “The genome sequence of APIC O1:K1:H7 shares strong similarities with human extra-intestinal pathogenic E. coli genomes,” *J. Bacteriol,* 2007, pp. 3228-3236
37. J. Bensink and F. Botham, "Antibiotic resistant coliform bacilli, isolated from freshly slaughtered poultry and from chilled poultry at retail outlets," *Aust. Vet. J. ,* vol. 60, 1983, pp. 80-83
38. S. D. Caudry and V.A. Stanisich, "Incidence of antibiotic resistant E.coli associated with frozen chicken carcasses and characterization of conjugative R-plasmids derived from such strains. Antimicrob," *Agents Chemother,* no. 16, 1979, pp. 701-709.
39. E. Chaslus Dancla and J. Lafont, "IncH plasmids in Escherichia coli strains isolated from broiler chicken carcasses," *Appl. Environ. Microbiol.,* vol. 49, 1985, pp. 1016-1018
40. Jayaratne, et al., " Occurrence of aminoglycoside phosphotransferase subclass I and II structural genes among Enterobacteriaceae spp. isolated from meat samples," *Appl. Microbiol. Biotechnol.,* no. 33, 1990, pp. 547-552
41. H. Linton, K. Howe, C.L. Hartley, H.M. Clements, M.H. Richmond, A.D Osborne, "Antibiotic resistance among Escherichia coli O-serotypes from the gut and carcasses of commercially slaughtered broiler chickens: a potential public health hazard," *J. Appl. Bacteriol.,* no. 42, 1977, pp. 365-378
42. H. Nazer, "Transmissible drug resistance in Escherichia coli isolated from poultry and their carcasses in Iran," *Cornell. Vet. 1980,* no. 70, 1980, pp. 365-371
43. G. C.Turtura, S. Massa and H. Chazvinizadeh, "Antibiotic resistance among coliform bacteria isolated from carcasses of commercially slaughtered chickens," *Int. J. Food Microbiol.,* no. 11, 1990, pp. 351-354
44. R. L. Lakhotia and J.F. Stephens, "Drug resistance and R factors among enterobacteria isolated from eggs," *Poult. Sci. ,* no. 52, 1973, pp. 1955-1962.
45. E. Van den Bogaard, N. London, C. Driessen and E.E. Stobberingh, "Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers," *J. Antimicrob. Chemother,* no. 43, 2001, pp. 763-771
46. D.W. Trampel and R.W. Griffith, ""Efficacy of aluminium hydroxide- adjuvanted Escherichia coli bacterin in turkey poults”, *Avian Dis.,* 1997, p. 263– 268
47. C.M. Dozois, F. Daigle and R. Curtiss, "Identification of pathogen specific free conserved gene expressed in Vigo by an avian pathogenic E.coli strain," *Proc. Natl Acad. Sci, 2003,* pp. 247-252.
48. Ewers, T. Janssen, S. Kiessling, H. C. Philipp and L.H. Wieler, “Molecular epidemiology of avian pathogenic Escherichia coli (APEC) isolated from colisepticemia in poultry,” *Vet. Microbiol.,* 2004, pp.91–101

49. M. Ozawa, K. Harada, A. Kojima and T. and S. T. Asai, "Antimicrobial susceptibilities, serogroups, and molecular characterization of avian pathogenic Escherichia coli isolates in Japan," *Avian Dis.,* no. 52, 2008, pp. 392-7
50. Ewers, T. Janssen, S. Kiessling, H. C. Philipp and L.H. Wieler, "Rapid detection of virulence-associated genes in avian pathogenic E. coli by multiplex polymerase chain reaction," *Avian Dis,* no. 49, 2005, pp. 269-73
51. J. A. Skyberg et al.,"Characterizing avian Escherichia coli isolates with multiplex polymerase chain reaction," *Avian Dis.,* vol. 47, 2003, p.1441–1447
52. T.J. Johnson, Y. Wannemuehler, S.J. Johnson, A.L. Stell, C. Doetkott, J.R. Johnson, K.S. Kim, L. Spanjaard and L.K. Nolan, "Comparison of extra-intestinal pathogenic E. coli from human and avian sources reveals a mixed subsetrepresenting potential zoonotic pathogens," *Environ. Microbiol.,* 2008. Epub ahead of print
53. S. Hudault, J. Guignot and A.L. Servin, "Escherichia coli strains colonising the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection." Gut, 49, 2001, pp. 47–55
54. A.C. Carvalho de Moura, K. Irino, M.C. Vidotto, "Genetic variability of avian E.coli strains evaluated by enterobacterial repetitive intergenic consensus and repetitive extragenic palindromic polymerase chain reaction," *Avian Dis,* no. 45, 2001, pp. 173-181.
55. D.G. White, M. Dho-Moulin, R. A. Wilson and T. Whittam, "Clonal relationships and variation in virulence among Escherichia coli strains of avian origin," *Micro. Pathog.,*1993, pp.99-409.
56. J.J. Maurer, T.P. Brown., W.L. Steffens. and S.G. Thayer "The occurrence of ambient temperature-regulated adhesions, curli, and the temperature- sensitive hemagglutinin Tsh among avian Escheri-chia coli," *Avian Dis.,* no. 42, 1998, pp.106-118.
57. Mokady, U. Gophna, E.Z. Ron, "Extensive gene diversity in septicemic Escherichia coli strains," *J Clin Microbiol,* no. 43, 2005, pp. 66–73
58. S. Schubert, A. Rakin, H. Karch, E. Carniel, and J. Heesemann, "Prevalence of the "high pathogenicity island of Yersinia species among Escherichia coli strains that are pathogenic to humans," *Infect. Immun.,* 1998, pp.480–485
59. T. Zahraei Salehi, Z., Rajabi, M. Modirsanei "Evaluation of the immunogenicity of endemic Escherichia coli serotypes isolated from poultry in Iran," *J. Vet. Res. University of Tehran,* 2004*,* pp. 189-195
60. X.M. Wang, X.P. Liao, W.J. Zhang, W.J. Wang, H.X.Jiang, J. Sun, X.F. He, D.X. Lao, Y.H. Liu, "Prevalence of serogroups, virulence genotypes, antimicrobial resistance, and phylogenetic background of avian pathogenic Escherichia coli in south of China," *Foodborne Pathog Dis,* no. 7, 2010, pp.1099–1106
61. K.E. Rodriguez-Siek, C.W. Giddings, C. Doetkott, "Characterizing the APEC pathotype",*Vet. Res.,* 2005, pp. 241–256
62. Moulin-Schouleur, C. Schouler, P. Tailliez, M. R. Kao, A. Bree, P.Germon, E.Oswald, J. Mainil, M. Blanco, and J. Blanco, "Common virulence factors and genetic relationships between O18:K1:H7 Escherichia coli isolates of human and avian origin," *J. Clin. Microbiol.,* 2006, pp. 3484–3492
63. Z. Ron, "Host specificity of septicemic Escherichia coli: human and avian pathogens," *Curr. Opin. Microbiol.,* no. 9, 2006. pp. 28–32
64. D.G. Moriel et al., "Identification of protective and broadly conserved vaccine antigens from the genome of extraintestinal pathogenic Escherichia coli.", *Proc. Natl. Acad. Sci. U. S. A.* 2010*.,* pp. 9072–9077
65. O.M. Weinack, G.H. Snoeyenbo, C.F. Smyser, A.S. Soerjadi."Competitive exclusion of intestinal colonization of E.coli in chicks," *Avian Dis.,* 1981, pp. 696-705

COMPARATIVE EVALUATION OF THREE TESTING METHODS FOR DETECTION OF MEDIATED RESISTANCE MBL IN *PSEUDOMONAS AERUGINOSA* ISOLATES

Oana – Alexandra CIOCAN (MOŢCO)[1]*, Alper CIFTCI[2], Mihai CARP – CĂRARE[1], Mihai MAREŞ[1], Mariana GRECU[1], Cătălin CARP – CĂRARE[1], Carmen – Valentina PANZARU[3]

[1]Faculty of Veterinary Medicine, Iasi

[2]University of Ondokuz Mayıs, Faculty of Veterinary Medicine, Turkey

[3]"Grigore T. Popa" University of Medicine and Pharmacy Iasi

Corresponding author*, e-mail: veterinarians_phd@yahoo.com

Abstract

Metallo – β – lactamase (MBL) producing Pseudomonas aeruginosa have been reported to be important cause of nosocomial infections. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial therapy. The present study was undertaken to determine which method is better to use in laboratory for detecting MBL producing P. aeruginosa. A total of 182isolates of P. aeruginosa from human and animals, 125 from human and 57 from animals, (burns, pus, urine, blood cultures, etc.), collected between 2013 and 2015 were subjected to susceptibility testing against various antibiotics by disc diffusion test according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2015. Imipenem resistant isolates were selected for the detection of MBL production by E-test strips for screening MBL, double disc method (IPM and IPM+EDTA) and EDTA solution application on microcaps IPM. The positive results have been based on inhibition zone around imipenem discs impregnated with EDTA as compared to those without EDTA confirmed MBL production and for E-test strips the strains were positive those that have developed around area with EDTA solution. The double disc method (IPM and IPM+EDTA is most effective way to use in laboratory to determine early producer P. aeruginosa MBL, having 2 advantage: first is the low cost for materials (MH agar and microcaps) and the technique is very easy to be applied.

Keywords: *resistance, MBL, Pseudomonas aeruginosa*

Introduction

The global spread of MBL on GN bacillus is a reason to worry. Metallo-β-lactamases hydrolyze carbapenems and β-lactam with most broad spectrum (Nordmann et al., 2002; Sarkar e al., 2006). Early detection of MBL-producing strains is essential for infection control and prevent dissemination of these microorganisms (*Bhattacharjee et al., 2008*). The fact that the activity of MBL is inhibited by chelators, such as EDTA, 2 metacarptopropionic acid, dipicolinic acid has led to the development of various methods of screening for microorganisms that produce MBL. In 2002, there was proposed three such methods for detection of MBL with good results for *Pseudomonas spp*. strains: Test EDTA and o-phenanthroline (*Migliavacca et al., 2002*), the test disc combined IMP-IMP + EDTA and E-test MBL and modified Hodge test disc IMP + ZnSO4, (*Yong et al, 2002*). Strains positive for at least one phenotypic test must be confirmed by PCR.

The data available we believe that MBL exist in our country for over 10 years.

Materials and methods

A total of 182 isolates of *P. aeruginosa* from human and animals, 125 from human and 57 from animals, (burns, pus, urine, blood cultures, etc.), collected between 2013 and 2015 were subjected to susceptibility testing against various antibiotics by disc diffusion test according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2015. Imipenem resistant isolates were selected for the detection of MBL production by E-test strips for screening MBL, double disc method (IPM and IPM+EDTA) and EDTA solution application on microcaps IPM.

E-test MBL

E-test-MBL consists of strips of plastic or paper impregnated with a gradient of concentrations of imipenem (MPI) at one end and imipenem + EDTA (MP) to the other. The test is based on inhibition of MBL by EDTA zinc chelator. The test is considered positive if the proportion of MPI / MP is ≥ 8 or if a so-called ghost area, by diffusion from the MPI for MP EDTA (fig.1).

Fig. 1. E-test MBL, *Pseudomonas aeruginosa*

Double Disc Synergy Test (IPM and IPM+EDTA)

I placed two discs at a convenient distance first imipenem with a concentration of 10 µg and second imipenem + 750 µg EDTA.

After incubation at 37 ℃ we have measured and compared the diameters of the zones of inhibition occurred around the 2 discs. Positivity strains were: widening zone of inhibition in the presence of EDTA microcaps with at least 5 mm on the imipenem simple (fig.2).

Fig.2. Double Disc Synergy Test for MBL detection

EDTA solution application on microcaps IPM

I placed two imipenem discs (Oxoid, UK) at a convenient distance, on one of them I added 5 µl of EDTA solution (Merck), (fig. 3).

Fig. 3. EDTA solution application on microcaps IPM

After incubation at 37 ℃ we have measured and compared the diameters of the zones of inhibition occurred around the 2 discs. The criteria of positivity were following: increasing the diameter of inhibition zone in the presence of EDTA, at least 5 mm, (fig. 4).

Fig.4. Diameter of inhibition zone in the presence of EDTA

Results and discussions

- 52 strains human origin and 37 strains animal origin were resistance to imipenem (IMP) and tested to see if they are probable proceeds MBL enzyme (fig. 5).

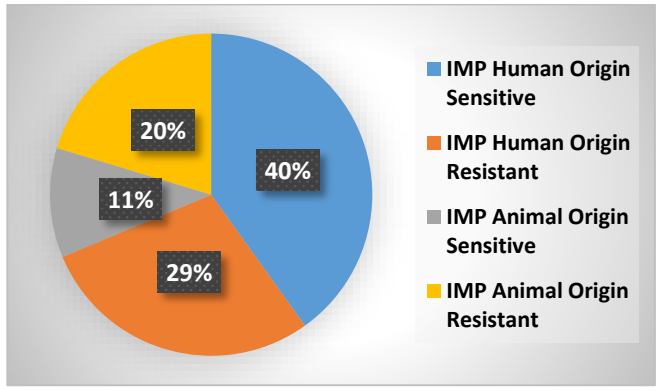


Fig. 5. IMP results

- All of strains that were resistant to the imipenem were tested to see if they produce enzymes type MBL and they give positive response to action of EDTA in all 3 tests (fig. 6).

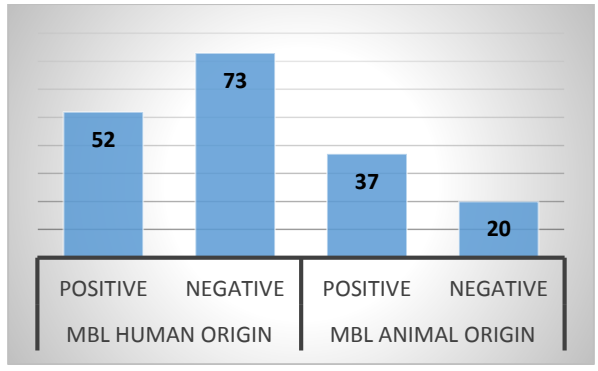


Fig.6. MBL results

- The most important problem with MBLs is there unsurpassed broad spectrum resistance profile.
- In spite of the precision of MBL E-test detection, it is costly compared to the antibiotic disks to be used by health care institutions or clinical laboratories for routine MBL screening procedure. DDST (Double Disc Synergy Test), which exhibited up to 96.6% specificity, is perhaps a more suitable routine screening procedure to be considered for early detection of MBL-producing bacteria. Still, in order to minimize false positivity, isolates positive by DDST can be supplementary confirmed by MBL E-test.

Pseudomonas aeruginosa is a nosocomial pathogen germ commonly being involved in a wide range of infections, sometimes difficult to control.

Major necessity of the use of specific methods diagnosis and identification of bacteria involved in the pathology we motivated us to compare different methods of detection MBL with the aim to highlight the effectiveness, speed and that each of them.

Early detection of MBL-producing strains it is essential for infection control and to prevent dissemination of these microorganisms. That activity MBL is inhibited by chelators like EDTA, thiol compounds, dipicolinic acid has led to the development of various methods for screening for MBL producing microorganisms. In 2002, three such methods were proposed with good results in strains of *Pseudomonas spp*.: Test EPI, EDTA and o-phenanthroline (Migliavacca et al., 2002), the test disc combined EPI-IPM + EDTA (Yong et al., 2002) and Etest MBL (Walsh et al., 2002).

Establishing a protocol for detecting MBL producing strains has major importance both in establishing treatment guidelines and the prompt recognition of outbreaks of nosocomial infections and taking measures to control the infection.

Conclusions

- In the evaluation of three selected MBL phenotypic assays, all three methods were shown to have a sensitivity nearly to 100%.
- The double disc method IPM and IPM+EDTA is an effective way to use in laboratory to determine early producer *P. aeruginosa* MBL, having 2 advantage: first is the low cost for materials (MH agar and microcaps) and the technique is very easy to be applied.
- We recommend that all IPM non-susceptible *Pseudomonas aeruginosa* isolates be routinely screened for MBL production and that PCR confirmation be performed at a laboratory.

References

1. Bhattacharjee A, Sen MR, Prakash P, Gaur A, Anupurba S. Increased prevalence of extended spectrum beta lactamase producers in neonatal septicemic cases at a tertiary referral hospital. Indian J Med Microbiol. 2008;26:356–60.
2. Migliavacca R, Docquier JD, Mognaioli C, Amicosante G, Daturi R, Lee K. Simple microdilution test for detecting metallo-beta-lactamase production in *Pseudomonas aeruginosa*. J. Clin. Microbiol., 40: 4388-4390
3. Nordmann P, Poirel L. Emerging carbapenemases in gram negative aerobes. Clin. Microbiol. Infect., 2002, 8: 321-333
4. Sarkar B, Biswas D, Prasad R. A clinic microbiological study on the importance of Pseudomonas in nosocomially infected ICU patients with special reference to metallo-β-lactamase production. Indian J Pathol Microbiol. 2006;49:44–6
5. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y (2002). Imipenem-EDTA disk method for differentiation of metallo-ß-lactamase producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J. Clin. Microbiol., 40: 3798-3801.

THE FIRST CASE OF ISOLATION AND IDENTIFICATION OF *ACINETOBACTER RADIORESISTENS* IN INFANT FROM IASI COUNTY (case report)

Oana – Alexandra CIOCAN (MOŢCO)[1]*, Alper CIFTCI[2], Mihai CARP – CĂRARE[1], Mihai MAREŞ[1], Mariana GRECU[1], Cătălin CARP – CĂRARE[1] , Carmen – Valentina PANZARU[3]

[1]Faculty of Veterinary Medicine, Iasi

[2]University of Ondokuz Mayıs, Faculty of Veterinary Medicine, Turkey

[3]"Grigore T. Popa" University of Medicine and Pharmacy Iasi

Corresponding author*, e-mail: veterinarians_phd@yahoo.com

Abstract

Members of the genus Acinetobacter are described as gram-negative, strictly aerobic diplococcoid rods that are oxidase negative and catalase positive. The genus includes at least 19 genomic species, defined on the basis of DNA relatedness criteria, which are ubiquitous in nature and have become increasingly responsible for a range of systemic infections in critically ill and immunocompromised patients. In most clinical microbiology laboratories, identification of Acinetobacter cannot routinely be achieved at the genospecies level because commercial identification systems are substantially deficient and poorly discriminatory in distinguishing these organisms. This implies that local data on the prevalence of individual species in human infections should be interpreted cautiously. In this article we describe a case study of community acquired Acinetobacter radioresistens. Bacteriae have been isolated from blood culture was isolate from one child, 9 month age, hospitalized in oncology sections, with the usual method of identification, the medium was seeded with blood cultures, it not developed in McK agar, but grows very well on blood agar at 37ºC for 24 h. Biochemical confirmation was achieved using galleries API 20 NF. In conclusion, it is very important to identify Acinetobacter radioresistens accurately because of being a silent source of carbapenem resistance for Acinetobacter spp.

Keywords: *isolation, oncology, Acinetobacter radioresistens*

Introduction

The germs of the genus Acinetobacter have been long time regarded as opportunistic infections possibly involved lacking gravity. In the past 20 years but was recorded an increase in incidence and severity their products nosocomial infections Acinetobacter spp. Triggering signal alarm in the medical world due to the severity their development and lack of therapeutic means effective against multi-resistant to antibiotic.

Acinetobacter radioresistens is a species of radiation-resistant bacteria. It is Gram-negative, oxidase-negative, not spore-forming, nonmotile, non-fermentative, aerobic, pleomorphic, and coccobacilli-shaped. (1)

In terms of nutrition, are chemoorganotrophic and multiply by division cross, like most bacteria. In terms of frequency, Acinetobacter sp. are currently in place strictly aerobic microorganisms second among involved in the etiology of nosocomial infections severe. Are encountered especially in wards surgery and intensive therapy, especially in immunocompromised patients. (3)

Material and method

Identification of Acinetobacter species is complicated by lack of standard identification techniques. Initially, identification was based on phenotypic characteristics such as growth temperature, colony morphology, growth medium, carbon sources, gelatin hydrolysis, glucose fermentation.

Bacteria have been isolated from blood cultures, with the usual method of identification, the medium was seeded with blood cultures, it not developed in MacConkey agar, but grows very well on blood agar at 37^0C for 24 h.

Blood culture methods is one of the most important and delicate to the microbiology laboratory. Blood, is normally sterile isolation and identification of bacteria in blood culture has a considerable diagnostic significance. (2)

Sampling is collected before antimicrobial therapy, according to predictive evolutionary curve febrile or when issuing the patient shiver. If this is not possible, the blood will be taken immediately before administering a new dose of antibiotic.

The sample will be incubated at 37ºC. The duration of incubation in thermostat and tracking of proof is on average 7 days. It requires longer incubation prolonged in patients with subacute endocarditis, the antibacterial samples (up to 14 days) or follow when demanding microorganisms. (4)

Antimicrobial susceptibility of *Acinetobacter radioresistens* were verified with Kirby-Bauer Disk Susceptibility on Mueller-Hinton agar plates and incubated 24 h at 37°C. The Kirby-Bauer (K-B) test utilizes small filter disks impregnated with a known concentration of antibiotic. The disks are placed on a Mueller-Hinton agar plate that is inoculated with the test microorganism. Upon incubation, antibiotic diffuses from the disk into the surrounding agar. If susceptible to the antibiotic, the test organism will be unable to grow in the area immediately surrounding the disk, displaying a zone of inhibition. The size of this zone is dependent on a number of factors, including the sensitivity of the microbe to the antibiotic, the rate of diffusion of the antibiotic through the agar, and the depth of the agar. Microorganisms that are resistant to an antibiotic will not show a zone of inhibition (growing right up to the disk itself) or display a relatively small zone.

Results and discussions

Acinetobacter radioresistens is a commensal species of the skin of healthy individuals and hospitalized patients. The virulence role of that bacterium may be limited, since only few cases of bacteremia has been reported so far and occurred in a human immunodeficiency and immunoexpressed patients.

A. radioresistens may be more prevalent than expected in the hospital environment, since it has been identified as the most common *Acinetobacter* species in hospital environmental samples. The identification of same plasmid types in *A. radioresistens* and *A. baumannii* further strengthens the possibility of gene exchange between those two species.

This sample was isolate from one child, 9 month age, hospitalized in oncology section. After a period of hospitalization, the patient developed a severe form of respiratory infection, followed by a kidney infection, manifested by increasing the temperature to 41.2^0C.

The culture medium inoculated with increased blood very well on blood agar at 37^0C for 24 hours (fig. 1).

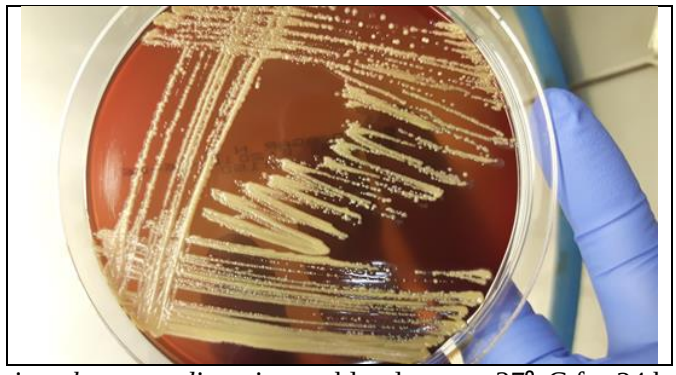


Fig. 1. *Acinetobacter radioresistens*, blood agar at 37^o C for 24 h

The bacteria belonging to different species of Acinetobacter have been isolated from numerous authors who have framed in different genres. Within the genre recognized 32 genospecies 17 of which could not be differentiated on the basis of biochemical characteristics.

In our study, biochemical confirmation was performed using API 20 NE galleries (fig. 2).

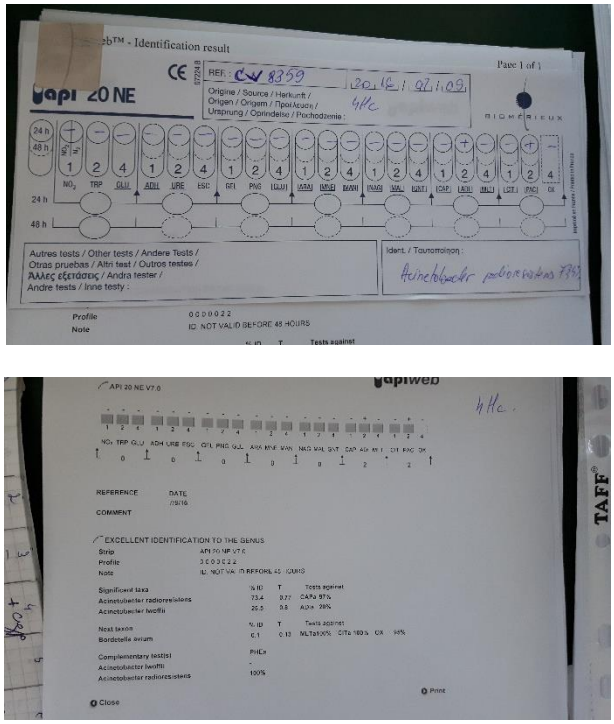


Fig. 2. Biochemical confirmation of *Acinetobacter radioresistens*

Regarding of frequency, Acinetobacter sp. it is currently on the second place among strictly aerobic microorganisms implicated in the aetiology of severe nosocomial infections.

Acinetobacter is difficult to eliminate in the hospital because of antibiotic resistance and resistance in the external environment - can survive on dry surfaces up to 5 months. Also, Acinetobacter strains have been identified which are resistant to all known antibiotics.

Antimicrobial susceptibility results:

Sensible: SXT, SXT-25 - Trimethoprim+Sulphamethoxazole
CIP - Ciprofloxacin
TPZ - Piperacillin + Tazobactam
TOB - Tobramycin
AK - Amikacin
IMP - Imipenem
MEM - Meropenem
TE - Tetracycline

Resistant: CAZ Ceftazidime
CRO Ceftriaxone

This study identified the source of an acquired and clinically relevant resistance gene. The unambiguous identification of the reservoir (origin) of an acquired resistance gene has very rarely been reported. Our findings further emphasize the possible role of the hospital environment as a reservoir of antibiotic resistance genes and a place where gene exchange may

occur. The future control of multidrug resistance may necessitate identification of not only the multidrug-resistant isolates but also their reservoirs by molecular-based techniques.

The bacteriological diagnosis is performed in order to isolate and identify microorganisms and for testing the behavior towards the antibiotics. Preliminary identification is based on morphological, biochemical and culture. Final identification is possible using API 20 NE galleries or automated systems (5).

Conclusions

It is important to go to the level of gene identification of this bacteria, because some studies have shown that *Acinetobacter radioresistens* is the progenitor of the blaOXA-23-like genes currently emerging as the sources of carbapenem resistance in *Acinetobacter baumannii* worldwide (3)

References

1. Nishimura, Y.; Ino, T.; Iizuka, H. (1988). "*Acinetobacter radioresistens* sp. nov. Isolated from Cotton and Soil". International Journal of Systematic Bacteriology. 38 (2): 209–211. doi:10.1099/00207713-38-2-209. ISSN 0020-7713.
2. Visca P, Petrucca A, De Mori P, Festa A, Boumis E, Antinori A, Petrosillo N (2001). Community-Aquired Acinetobacter radioresistens Bacteremia in an HIV-Positive Patient. Emerg Infec Dis 7(6):1032-5
3. Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P (2008). *Acinetobacter radioresistens* as a Silent Source of Carbapenem Resistance for Acinetobacter spp.. Antimicrob Agents and Chemotherapy 52(4):1252-1256.
4. Dumitru Buiuc. Hemocultura in diagnosticul infectiei. In Tratat de Microbiologie Clinica. Dumitru Buiuc, Marian Negut, Editura Medicala, Romania, 2 ed. 2008, 165-182.
5. Infectio.ro Nr. 38 (2/2014) by Versa Media - issuu. https://issuu.com/versamedia/docs/infectio-2-2014

SEROLOGICAL INVESTIGATIONS REGARDING THE EFFECT OF INFECTION WITH SWINE INFLUENZA VIRUS H1N1 ON THE EVOLUTION OF ENZOOTIC PNEUMONIA IN FATTENED PIGS FROM WESTERN ROMANIAN FARMS

Fini Corina GREMA., Corina PASCU., Viorel HERMAN
Faculty of Veterinary Medicine Timișoara, Calea Aradului 119
viorelagrema@msn.com

Abstract

It is well-kown that to minimize the impact of respiratory diseases in pig farms, modern production systems have been developed. Despite these trends toward high health levels, Mycoplasma hyopneumoniae and swine influenza virus (SIV) remain significant pathogens in the pig industry. Therefore, in recent years, the occurrence and the relevance of these pathogens in growing and fattening pigs has been examined in several studies. However, none of these studies included a detailed analysis regarding the effect of infection with swine influenza virus H1N1 on the evolution of enzootic pneumonia in fattened pigs. For this reason, the aim of the present study was to compare the seroprevalence of infection caused by Mycoplasma hyopneumoniae in fattened pigs with that determined by swine influenza virus subtype H1N. To achieve this goal, detection of antibodies against Mycoplasma hyopneumoniae and swine influenza virus H1N1 was performed by ELISA technique in pig fattening farms located in Western Romania. The value of seroprevalence of infection with Mycoplasma hyopneumoniae within the infected farms ranged between 5.88% and 92.85%. Mixed infections of swine influenza virus subtype H1N1 and Mycoplasma hyopneumoniae had been identified in four out of the six farms studied for both types of infection. By comparing the seroprevalence of infection caused by swine influenza virus subtype H1N1 with that determined by Mycoplasma hyopneumoniae, it was concluded that in farms (AR2, AR3, TM3 and TM4), where the value of seroprevalence of swine influenza virus subtype H1N1 ranged between 5.88% and 25%, the seroprevalence of infection caused by Mycoplasma hyopneumoniae had increased from 5.88% (in farm TM4) to 92.85% (in farm AR3). From a statistical point of view, no correlation between the two infections was found in this study. Knowing and understanding this aspect is important for both the pig industry and a public health point of view. Taking into account the multifactorial character of enzootic pneumonia and swine influenza virus infection, the results of this study emphasize that a comprehensive herd specific prevention program is a prerequisite to reduce transmission of both diseases in pig fattening farms.

Key words*: Mycoplasma hyopneumoniae, seroprevalence, infection, ELISA*

Introduction

Enzootic pneumonia is a chronic, respiratory disease of swine characterized by its ability to produce a persistent dry cough, slow onset and reduced feed conversion efficency. The disease usually results from the combination of infection caused by *Mycoplasma hyopneumoniae* with one or more bacterial agents such as: *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Mycoplasma hyorhinis*, *Streptococcus suis*, *Haemophilus parasuis*, *Bordetella bronchiseptica* și *Trueperella pyogenes*. The presence of species *Mycoplasma hyopneumoniae* in swine populations represents a risk factor for the occurrence of porcine respiratory disease complex (PRDC). *Mycoplasma hyopneumoniae* acts synergistically with the bacterial agents mentioned above, and also with the viral pathogens (12).

In the intensive pig farming in which domestic pigs are raised up to slaughter weight, enzootic pneumonia in pigs is considered a major cause of economic loss due to morbidity, mortality, reduced weight gain, reduced efficiency of feed utilization and also due to the expenses of preventing and combating the disease (6).

The present researches were aimed to investigate the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* in pigs in fattening units located in Western Romania, in relation to the seroprevalence of infection caused by swine influenza virus subtype H1N1.

Materials and methods

To determine the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* in swine, investigations were performed in six swine fattening farms located in three western counties (three farms in Timiș County; two farms in Arad County; and one farm in Bihor County). Also, in ten swine fattening farms, investigations were conducted to assess the seroprevalence of infection caused by swine influenza virus subtype H1N1. To perform serological screening, blood samples were collected from two age groups (91 days to 140 days and 141 days to 180 days, respectively) from growing-to-fattening farms, from the three counties. The processing of serum samples was performed by ELISA method using the CIVTEST SUIS MHYO kit in the Laboratory of the Department of Infectious Diseases and Preventive Medicine of the Faculty of Veterinary Medicine Timișoara.

Results and discussions

Based on the results from the investigations carried out by ELISA immunoassay technique, it was concluded that five of the six farms monitored had infections caused by *Mycoplasma hyopneumoniae*, which means that in 83.33% of pig farms, infections with *Mycoplasma hyopneumoniae* were present. Seroconversion at the farm level, based on the number of samples examined, was situated between 5.88% and 92.85%, which highlights the significant differences among the investigated farms (figure 1).

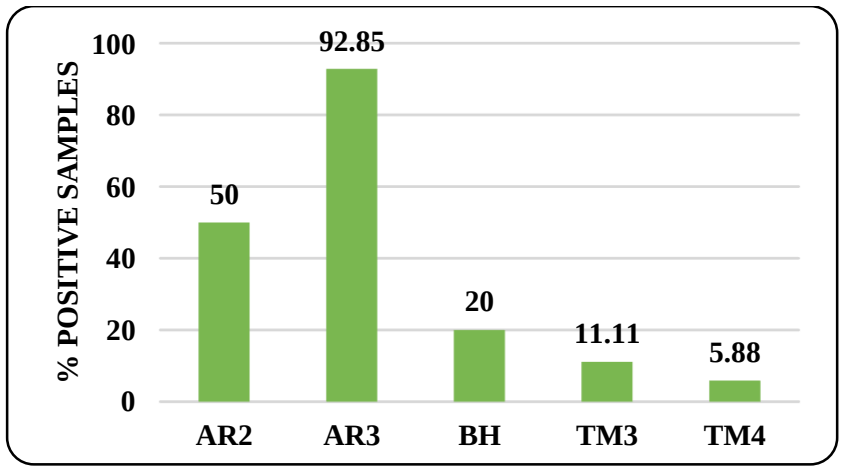


Figure 1. Seroprevalence of *Mycoplasma hyopneumoniae* infection in infected farms

The results obtained from the serological examination highlight the fact that the highest value of seroprevalence of infection with *Mycoplasma hyopneumoniae* was recorded in farm AR3 from Arad County, at 92.85%, based on the number of samples investigated in this farm. The lowest value of the seroprevalence of infection recorded in the present study was reported in farm TM4 from Timiș County, which was 5.88%, based on the number of samples investigated in this farm.

The seroepidemiological studies conducted so far have shown that infection with *Mycoplasma hyopneumoniae* has a global distribution. Therefore, the seroprevalence of mycoplasma infection at farm level has been studied in several countries from Europe and throughout the world. The values that were obtained from these studies showed wide variations among countries depending on several factors included in the study.

The results obtained in our study are comparable to the results obtained in similar studies from neighboring countries within Europe. Thus, in Ukraine, the seroprevalence of mycoplasma infection ranged between 5.70% and 57%. In Poland, the value of seroprevalence of infection caused by *Mycoplasma hyopneumoniae* was 91.30%, and in Germany, the value of seroprevalence of mycoplasma infection recorded was 65% (4, 5, 8).

In Sweden, a study done by Mattson et al., in 1995, has showed that antibodies against *Mycoplasma hyopneumoniae* were detected in 90% of the pigs examined by ELISA immunoassay technique (12).

In a study conducted in Tibet-China by Nian-Zhang et al., serum samples obtained from 423 pigs were tested. The value of seroprevalence of mycoplasma infection was 58.86% (10).

Figure 2 shows the results obtained from the serological screening carried out by ELISA method, on the distribution of antibodies against *Mycoplasma hyopneumonie* in the three counties studied.

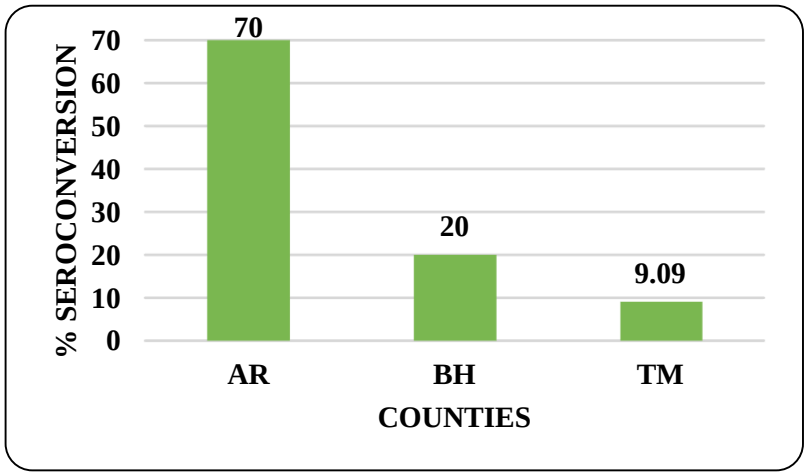


Figure 2. Distribution by counties of seroconversion of *Mycoplasma hyopneumoniae* infection

By analyzing the seroprevalence of mycoplasma infections at the county level, it appears that there are significant differences among the counties analyzed. Therefore, the highest level of seropositivity was 70%, which was recorded in Arad County, reported to the number of samples examined in the two farms within Arad County. The lowest value of seroprevalence of mycoplasma infections recorded was in Timiș County, which was 9.09%, reported to the number of samples tested in the two farms within Timiș County.

Analysis of the results obtained from the serological investigation revealed that mycoplasma infection was present in both farms monitored within Arad County (100%). In Bihor County, the infection was notified in the farm monitored (100%). In Timiș County, the infection caused by *Mycoplasma hyopneumoniae* was detected in two of the three farms monitored (66.66%), which highlights the significant differences among the investigated counties.

Similar to the results obtained in our study, in Slovakia, a study conducted by Prokes et al., in 2012, demonstrated that infection with *Mycoplasma hyopneumoniae* was detected in all four monitored farms. Seroconversion at the farm level was 42.10% (12).

Another serological study conducted in 12 farms in China has revealed that infection caused by *Mycoplasma hyopneumoniae* was present in 10 farms, and the value of seroprevalence of infection was 45.70% (6).

In 2007, in a study carried out in 13 pig farms in Bulgaria, infection induced by *Mycoplasma hyopneumoniae* was found in all studied farms (100%), and the value of seroprevalence of infection within the investigated farms ranged between 4.50% and 100% (1).

In the current study, conducted on the population of pigs from several fattening farms located in Arad County, Bihor County and Timiș County, pigs studied were divided by age categories, so the interpretation of the results obtained from the ELISA test was done separately according to the age of the pigs from which blood samples were collected. It has been noted that by comparing the results from farms identified positive for two age categories (91-140 days and 141-180 days), it was determined that among pigs in the contrasting age group were significant differences. Thus, the increased level of seropositivity for the first category (91-140 days) was 87.50% recorded in farm AR3, while category (141-180 days) had the highest level of seropositivity, at 100%, recorded in the same farm.

Overall, based on the results acheived in the present study and those available in specialized literature, mycoplasma infections have a high seroprevalence and cause significant economic losses in swine during the fattening phase. In this period, the seroprevalence of infection may be between 75% and 100%, an aspect that was also confirmed in our study indicating that almost all pigs are infected in that specific period.

In a study conducted by Maes et al., the sero-epidemiological aspects of *Mycoplasma hyopneumoniae*, influenza H1N1 and H3N2 viruses and Aujeszky disease virus in fattening pig farms were investigated. The results of the study have shown that antibodies to *Mycoplasma hyopneumoniae* were highly prevalent, thus the value of seroprevalence of infection was 76% (9).

A sero-epidemiological study conducted by Yagihashi et al., in 42 fattening pig farms has revealed that the highest seroconversion occurred when pigs were four months of age, reaching the maximum at six months by the end of the fattening phase, an aspect that was remarked in our study (15).

In a serological study performed by ELISA in Finland, 1.773 samples were analyzed for antibodies to *Mycoplasma hyopneumoniae*. The value of seroprevalence of infection was somewhat lower than the apparent prevalence of 39% (13).

A serological study conducted by Okada et al., has revealed that 80% of pigs at the end of the fattening phase presented antibodies against *Mycoplasma hyopneumoniae* (11).

According to the data from the medical literature, deterioration of the repiratory epithelium as a result of infection with swine influenza virus increases the rate of infection with *Mycoplasma hyopneumoniae*. Analyzing the results obtained in our study by comparing the seroprevalence of infection caused by swine influenza virus subtype H1N1 with that determined by *Mycoplasma hyopneumoniae*, it was concluded that swine influenza H1N1 virus infection increases the susceptibility to infections caused by *Mycoplasma hyopneumoniae* (14).

By analyzing data shown in figure 3, it was revealed that mixed infections of swine influenza virus subtype H1N1 and *Mycoplasma hyopneumoniae* had been identified in four of six farms studied. More specifically, in farms AR2, AR3, TM3, and TM4, where the viral infection seropositivity values were between 5.88% and 25%, the value of seroprevalence of infection caused by *Mycoplasma hyopneumoniae* had increased progresively from 5.88% in farm TM4 (Timiș County) to 92.85% in farm AR3 (Arad County).

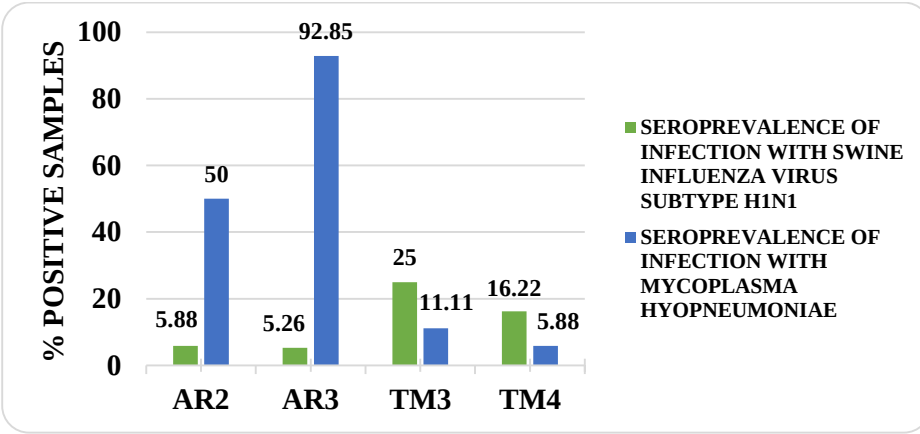


Figure 3. Comparative results of seroprevalence of swine influenza virus subtype H1N1 and *Mycoplasma hyopneumoniae*

Analysis of the results obtained in the study, shown in figure 4, revealed the fact that in farm BH, which was identified seronegative for swine influenza virus infection, the value of seroprevalence of infection induced by *Mycoplasma hyopneumoniae* was relatively high (20%).

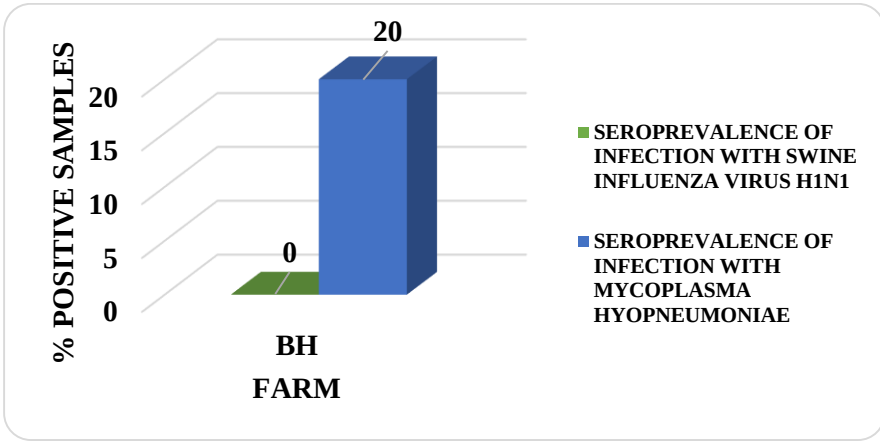


Figure 4. Seroprevalence values of Mycoplasma infections in the farm identified seronegative for swine influenza virus H1N1

Also, analysis of the data presented in figure 5 showed that although farm TM2 was identified seronegative for mycoplasma infection, infection caused by swine influenza virus subtype H1N1 was present. All things considered, the results obtained in the present study revealed that these two pathogens may act synergistically or independently.

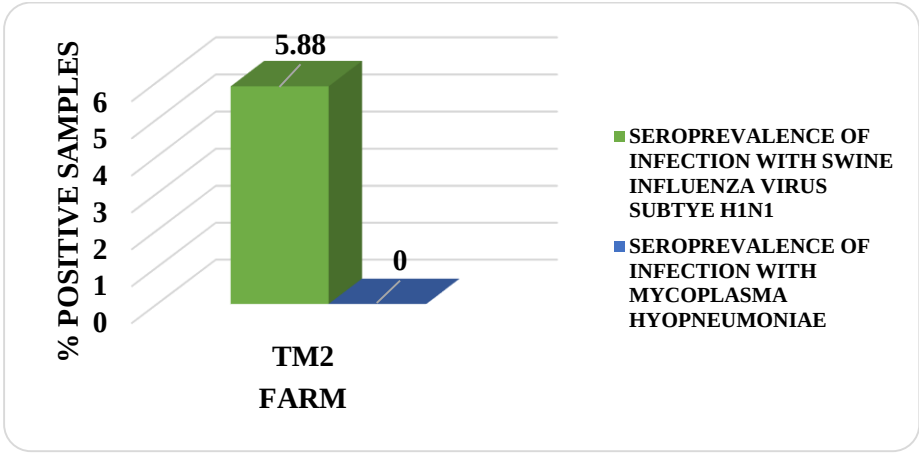


Figure 5. Seroprevalence values of Mycoplasma hyopneumoniae infections in the farm identified seropositive for swine influenza virus H1N1

The interaction between *Mycoplasma hyopneumoniae* and swine influenza virus (SIV) to induce pneumonia in susceptile pigs was investigated by using an experimental respiratory model. In the study, the pigs were inoculated with *Mycoplasma hyopneumoniae* 21 days prior to inoculation with SIV. Clinical observations revealed that pigs infected with the bacterial and viral pathogen coughed significantly more than pigs inoculated with a single agent, and the level of pneumonia in dual-infected pigs was less severe. The results of the study demonstrated that combined effect of dual infection with *Mycoplasma hyopneumoniae* and SIV is transitory in nature, and that the interaction between the two pathogens is minimal. However, under field conditions, disruption of airway epithelium by these two pathogens would significantly increase the risk of appearance of secondary infections, which contribute to increased severity of pneumonia (14).

According to data published in medical literature, the severity of swine influenza is highly variable and can be exacerbated by many factors, such as infection of pigs with *Mycoplasma hyopnemoniae* and other pathogens. Thus, in an experimental study, the oxidative stress determined by *Mycoplasma hyopneumoniae* and the impact of the stress on the evolution of infection with swine influenza virus subtype H1N1 was investigated. The results of the study suggested that the severity of infection caused by influenza virus may vary depending on the level of oxidative stress induced by infection with *Mycoplasma hyopneumoniae* (3).

In another experimental study, were compared the clinical and pathological effects of dual infection with swine influenza virus and *Mycoplasma hyopneumoniae* with those determined only by *Mycoplasma hyopneumoniae*. The results obtained in the study revealed that the lung lesions of pigs inoculated with both pathogens is more severe than that of pigs inoculated only with *Mycoplasma hyopneumoniae* (16).

The investigations done in an experimental model of coinfection (SIV and *Mycoplasma hyopneumoniae*) lead to the assumption that the two pathogens act synergistically. More specifically, the results of the study suggest that SIV associated with *Mycoplasma hyopneumoniae* are a risk factor for the severity of respiratory diseases (2).

In an experimental study, the impact of feed restriction on the ability of pigs to resist and be tolerant to a coinfection with *Mycoplasma hyopneumoniae* and swine influenza virus subtype H1N1 was investigated. After inoculation of pigs with swine influenza virus subtype H1N1, pigs that were previously infected with *Mycoplasma hyopneumoniae* and were fed at

libitum presented coughing, hyperthermia and weight loss due to reduced consumption of food. On the other hand, feed restricted pigs that had H1N1 infection and mycoplasma infection as compared to pigs fed ad libitum, did not decrease their feed consumption and did not lose weight. The results of the study had shown that feed restriction in coinfected pigs had no impact on severity of pneumonia lesions, but reduced the severity of clinical signs caused by the two pathogens, having a beneficial effect on the productive performance of these animals (7).

All things considered, it is appropriate to permanently monitor the prevalence of infection with *Mycoplasma hyopneumonie* and associated diseases in order to obtain useful information necessary for implementation of prevention and control programs of such diseases in the investigated area. More specifically, it is important to adopt special measures for epidemiological surveillance that combine several diagnostic methods, since the use of a single method (culture, serology, PCR) is not sufficient to bring under control and to liquidate the epidemiological process, due to drawbacks posed by each technique.

Additionally, taking into account the increased seroprevalence of mycoplasma infection in studied pig farms and considering the fact that the efficiency of the immunoprophylaxis measures applied in fattening pig farms is affected by infection with *Mycoplasma hyopneumoniae* and other associated pathogens, it is absolutely necessary that each farm adopt a methodology for vaccination according to the existing epidemiological situation achieved after performing the serological tests.

Acknowledgements

This research work was carried out with the support of the project „Dezvoltarea infrastructurii de cercetare, educaţie şi servicii în domeniile medicinei veterinare şi tehnologiilor inovative pentru RO 05, cod SMIS-CSNR 2669".

Conclusions

- In the present study, the investigations made by ELISA technique showed that infection with *Mycoplasma hyopneumoniae* had been identified in five of the six farms studied. The infected farms were located in Arad, Bihor, and Timiș Counties, which have a high density of pig farms. The seroprevalence of infection within the infected farms ranged between 5.88% and 92.85%.
- The value of seroprevalence of mycoplasma infection within the counties studied ranged between 9.09% and 70%, reported to the total number of samples tested in the farms identified positive.
- The seroprevalence of infection in positive farms at the age of 91-140 days presents variations ranging between 0% and 87.50%. At the age of 141-180 days, the pigs from infected farms had the highest seroprevalence of 100%, and the lowest seroprevalence of 0%.
- Coinfection between swine influenza virus subtype H1N1 and mycoplasma hyopneumoniae has been identified in four out of the six farms studied; from a statistical point of view, an existence of a correlation between the two infections had not been proven.

Reference

1. Bochev, I., 2007 - Porcine Respiratory Disease Complex (PRDC), Bulgarian Journal of Veterinary Medicine, 10(3):131-146;
2. Deblanc, C., Gorin, S., Queguiner, S., Gautier-Bouchardon, A. V., Ferre, S., Amenna, N., Cariolet, R., Simon, G., 2012 – Pre-infection of pigs with Mycoplasma hyopneumoniae modifies

outcomes of infection with European swine influenza virus , but not H1N2, subtype, Veterinary Microbiology, 157(1-2):96-105;
3. Deblanc, C., Robert, F., Pinard, T., Gorin, S., Queguiner, S., Gautier-Bouchardon, A. V., Ferre, S., Garraud, J. M., Cariolet, R., Brack, M., Simon, G 2013 – Pre-infection of pigs with Mycoplasma hyopneumoniae induces oxidative that influences outcomes of a susequent infection with a swine influenza virus of H1N1 subtype, Veterinary Microbiology, 162(2-4):643-51;
4. Dors, A., Czyzewska, E., Kwit, K., Pejsak, Z., 2012 – Seroprevalence of important swine pathogens in Polish farms, Proceedings of the 22th IPVS Congress, Jeju, Korea, 362;
5. Grosse-Beilage, E., Rohde, N., Krieter, J., 2009 – Seroprevalence and risk factors associated with seropositivity in sows from 67 herds in north-west Germany infected with Mycoplasma hyopneumoniae, Preventive Veterinary Medicine, 88(4):255-63;
6. He, Y., Xu, M. J., Zhou, D. H., Zou, F. C., Lin, R. Q., Yin, C. C., He, X. H., Liang, R., Liang, M., Zhu, X. Q., 2012 – Seroprevalence of Mycoplasma hyopneumoniae in pigs in subtropical southern China, Tropical Animal Health and Production, 43(3):695-698;
7. Le Floc´H, N., Deblanc, C., Cariolet, R., Gautier-Bouchardon, A. V., Merlot, E., Simion, G., 2014 – Effect of feed restriction on performance and postprandial nutrient metabolism in pigs co-infected with Mycoplasma hyopneumoniae and swine influenza virus, PLOS ONE, doi:10.1371/journal.pone. 0104605;
8. Lysenko, N. V., Milanko, A. Ya., Dubov, N. S., Zabora, N. P., 1980 – Serological survey for porcine enzootic pneumonia in the Ukraine, Veterinariya , 51:18-22;
9. Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B., Kruif, A. D., 2000 – Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds, Vet. Res, 313-327;
10. Nian-Zhang, Z., Dong-Hui, Z., Xin-Chun, S., Si-Yang, H., Min-Jun, X., Danba, C., Ga, G., Hui-Qun, S., 2013 – First report of Mycoplasma hyopneumonie seroprevalence in Tibetan pigs in Tibet, China, African Journal of Microbiology Reseach, 7(17):1735-1738;
11. Okada, M., Asai, T., Futo, S., Mori, Y., Mukai, T., Yazawa, S., Uto, T., Shibata, I., Sato, S., 2005 – Serological diagnosis of enzootic pneumonia of swine by a double –sandwich-enzyme-linked immunosorbent assay using a monoclonal antibody and recombinant antigen (P46) of Mycoplasma hyopneumoniae, Veterinary Microbiology, 105(3-4):251-9;
12. Prokes, M., Zendulkova, D., Rosenbergova, K., Treml, F., Ondrejkova, A., Benisek, Z., Ondrejka, R., Korytar, L., Slepecka, E., Suli, J., Haladova, E., Mazensky, D., 2012 – Detection of Mycoplasma hyopneumoniae by ELISA and nested PCR from blood samples and nasal swabs from pigs in Slovakia, Acta Veterinaria, 81:327-331;
13. Rautiainen, E., 1998 – The prevalence of Mycoplasma hyopneumoniae in pig herds in western Finland based on the demonstration of antibodies in colostrum by ELISA, Acta Vet Scand, 39(3):325-30;
14. Thacker, E. L., Thacker, B. J., Janke, B. H., 2001 – Interaction between Mycoplasma hyopneumoniae and Swine Influenza Virus, Journal of Clinical Microbiology, 39(7):2525-2530;
15. Yagihashi, T., Kazama, S., Tajima, M., 1993 – Seroepidemiology of mycoplasmal pneumonia of swine in Japan as surveyed by an enzyme-linked immunosorbent assay, Veterinary Microbiology, 34(2):155-166;
16. Yazawa, S., Okada, M., Ono, M., Fujii, S., Okuda, Y., Shibata, I., Kida, H., 2004 – Experimental dual infection of pigs with an H1N1 swine influenza virus (A/Sw/Hok/2/81) and Mycoplasma hyopneumoniae, Veterinary Microbiology, 98(3-4):221-8;

SEROLOGICAL INVESTIGATIONS REGARDING THE EFFECT OF INFECTION WITH SWINE INFLUENZA VIRUS H1N1 ON THE EVOLUTION OF ENZOOTIC PNEUMONIA IN WILD BOARS FROM WESTERN ROMANIA

Fini Corina GREMA., Corina PASCU., Viorel HERMAN
Faculty of Veterinary Medicine Timișoara, Calea Aradului 119
viorelagrema@msn.com

Abstract

The transmission of infectious diseases between wild animals and domestic animals is becoming a global issue of growing interest for the pig producing industry and human public health. In medical literature, that are several reports that wild boars may act as a reservoir for economically important infectious diseases that endemically affect domestic pigs, such as enzootic pneumonia and swine influenza. Although the risk of transmission of these diseases between wild boars and domestic pigs is likely to increase in Western Romania, there is very few data on the seroprevalence of Mycoplasma hyopneumoniae and swine influenza virus in wild boar populations. Therefore, the aim of the present study was to determine the seroprevalence of infection caused by Mycoplasma hyopneumoniae in wild boar populations from Western Romania in order to contribute to the information necessary for the control of the disease. Also, in order to determine the effect of infection with swine influenza virus H1N1 on the evolution of enzootic pneumonia, the value of seroprevalence for both types of infections was compared. The seroprevalence of infection with Mycoplasma hyopneumoniae, reported to the total number of samples tested on nine hunting grounds (which belong to Caraș-Severin County, Timiș County, and Bihor County) was 66.67%. The seroprevalence of swine influenza virus infection, reported to the total number of samples tested on 25 hunting grounds (which belong to Caraș-Severin County, Timiș County, and Bihor County) was 11.80%. Mixed infections with Mycoplasma hyopneumoniae and swine influenza virus subtype H1N1 were detected in two out of the three counties included in the study (Timiș County and Bihor County), with a substantial increase in Mycoplasma hyopneumoniae seropositivity. The results obtained in this study provide information on the disease exposure and health status of wild boars suggesting that Mycoplasma hyopneumoniae and swine influenza virus are widespread in wild boar populations from Western Romania and that these pathogens represent a source of infection for domestic pigs, as well as humans.

Key words: *enzootic pneumonia, wild boars, pathogens, hunting grounds, disease exposure*

Introduction

Mycoplasma hyopnemoniae is recognized as the causative agent of enzootic pneumonia. The organism is the smallest bacteria and is ubiquitous within swine herds throughout the world. Recent serological studies demonstrated that *Mycoplasma hyopneumoniae* can also infect wild boars (7). According to data from medical literature, transmission of *Mycoplasma hyopneumoniae* between domestic pigs and wild boars is possible in both directions (4).

Therefore, this study had 2 objectives: 1) to determine the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* in wild boar populations with the use of an enzyme-linked immunosorbent assay (ELISA), and 2) to compare the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* with that induced by swine influenza virus subtype H1N1 in wild boar populations from western Romania.

Materials and methods

In order to detect and quantify the antibodies against *Mycoplasma hyopneumoniae* in wild boar populations from western Romania, a total of 45 thoracic fluid samples were harvested and tested. The samples were collected and sent for investigation by the County Sanitary Veterinary Divisions, and samples were harvested in the 2013-2014 hunting three western counties (Caraș-Severin, Timiș, and Bihor).

The processing of samples was performed by ELISA method using the kit CIVTEST SUIS MHYO in the Laboratory of the Department of Infectious Diseases and Preventive Medicine of the Faculty of Veterinary Medicine Timişoara.

Results and discussions

The results obtained after performing ELISA test on the samples collected from wild boars from nine hunting grounds in the three counties (Caraş-Severin County, Timiș County and Bihor County) were systematized and presented in table 1.

Table 1

Positive samples identified from hunting funds of Caraș-Severin, Timiș, and Bihor Counties

County	Hunting grounds	Number.positive samples	Total number positive samples/number analyzed samples (% infection)
Caraș-Severin	Bigăr	2	5/8 (62.50)
	Bocșa	1	
	Moldova Nouă	2	
	Slatina Timiș	0	
Timiș	Surduc	2	14/21 (66.67)
	Pișchia	7	
	Luncani	4	
	Traian Vuia	1	
Bihor		11	11/16 (68.75)
Total number positive samples		30	30/45 (66.67)

The results obtained from the serological examinations performed by ELISA method and presented in table 1 show that infection with *Mycoplasma hyopneumoniae* was reported in all three counties monitored (Caraș-Severin, Timiș, and Bihor). Thus, the seroprevalence of infection in the investigated areas was 100%.

In Caraș-Severin County, positive specimens were identified in three out of the four hunting grounds taken into the study; the seroprevalence of mycoplasma infection reported to the total number of hunting grounds monitored in the county was 75%. In the case of infection with swine influenza virus subtype H1N1, negative samples were identified in all four hunting grounds investigated. Thus, the value of seroprevalence of viral infection was 0%. The analyzed results regarding infection with *Mycoplasma hyopneumoniae* had shown that in the four hunting grounds of Caraș-Severin County, five positive samples were identified out of the eight samples taken into the study. Thus, the seroprevalence of infection with *Mycoplasma hyopneumoniae* was 62.50%.

In Timiș County, positive samples were identified in all four hunting grounds investigated; the seroprevalence of infection reported to the number of hunting grounds monitored in the county was 100%, which is higher compared to that reported for infection with swine influenza virus subtype H1N1, which was 30%. In the four hunting grounds of

Timiș County, there were 14 positive samples identified out of the 21 samples taken into the study; the seroprevalence of infection with *Mycoplasma hyopneumoniae* reported to the number of samples monitored within the county was 66.67%, which is higher than the seroprevalence of infection with swine influenza virus subtype H1N1, at 22.40%, reported to the total number of samples monitored within the hunting grounds in Timiș County.

Bihor County was represented by only one hunting ground from which 16 samples were harvested and tested. After analyzing the samples using ELISA method, 11 positive samples were identified; the seroprevalence of infection reported to the total number of samples monitored within Bihor County was 68.75%. In the case of infection with swine influenza virus subtype H1N1, the seroprevalence was 5.80%, reported to the total number of samples tested within the hunting ground.

Overall, the seroprevalence of infection induced by *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the hunting grounds, was 62.50% in Caraș-Severin County, 66.67% in Timiș County, and 68.75% in Bihor County. Average values of seroprevalence of infection with *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the nine hunting grounds in Caraș-Severin County, Timiș County, and Bihor County, was 66.67% (30/45). The seroprevalence of infection caused by swine influenza virus subtype H1N1 on the hunting grounds investigated was 0% in Caraș-Severin County, 22.40% in Timiș County, and 5.80% in Bihor County. Average values of seroprevalence of infection with swine influenza virus subtype H1N1, reported to the total number of samples tested on the 25 hunting grounds in Caraș-Severin County, Timiș County, and Bihor County, was 11.80% (Figure 1).

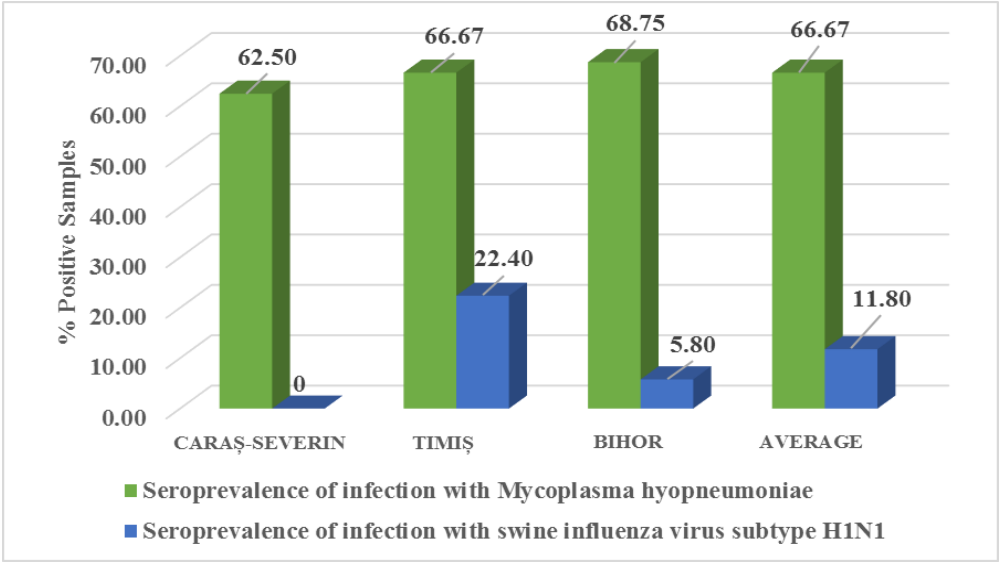


Figure 1. Comparative results of seroprevalence of *Mycoplasma hyopneumoniae* and swine influenza virus infection H1N1 within hunting grounds from monitored counties

The seroprevalence of infection with *Mycoplasma hyopneumoniae* in wild boar populations was reported in several countries in Europe and throughout the world, varying from one country to another. In this context, the value of seroprevalence of mycoplasma infection of 66.67% that was obtained in the present study was higher than that achieved in Slovenia

(15.80%), France (58%), and in United States, where the seroprevalence of infection was 32% (8).

A comparative analysis of results obtained in our study to data published by other authors in other countries from Europe had shown that the value of seroprevalence of mycoplasma infection in these countries were lower. Thus, a study conducted by Sibila et al., in 2010, demonstrated that the seroprevalence of infection induced by *Mycoplasma hyopneumoniae* in wild boar populations was 21% (7).

In a serological ELISA testing performed by Chiari et al., in 2013, antibodies against *Mycoplasma hyopneumoniae* were detected in 655 out of the 2.177 analyzed wild boars, so the value of seroprevalence of mycoplasma infection was 30% (2).

In another study conducted by Vengust et al., blood samples were taken from wild pigs and subjected to an examination by ELISA for the detection and quantification of antibodies against several pathogens, including *Mycoplasma hyopneumoniae*. The obtained results concluded that the seroprevalence of infection induced by *Mycoplasma hyopneumoniae* was 21% (9).

In Russia, a study done by Kukushkin et al., in 7 regions of the country, showed the presence of antibodies to *Mycoplasma hyopneumoniae* in 52% of samples (4).

Serological studies conducted worldwide have revealed that *Mycoplasma hyopneumoniae* may persist in infected pigs more than 28 months' post infection. In this context, the results of the current study demonstrated that mycoplasma infection occurred in wild boars monitored many months prior to collection of blood samples (10).

Currently, serological studies conducted worldwide have emphasized that there is an increasing tendency of infection with *Mycoplasma hyopneumoniae* in wild boar populations. Given this context, in the present study the relatively high value of seroprevalence of infection with *Mycoplasma hyopneumoniae* (66.67%), recorded in wild boar populations in the investigated area, shows that the infection is prevalent in the sylvatic environments. However, the danger of transmission of disease from wild animals to domestic animals is not sufficiently known. Therefore, a thorough investigation is needed to explore the genetic linkage that exists between infection caused by *Mycoplasma hyopneumoniae* detected in wild boar populations and mycoplasma infection reported in domestic swine herds. It is important to note that this investigation is of fundamental importance to further establish whether wild boars can serve as a reservoir of the disease for domestic pigs in that location (10).

From the literature reviewed, it is well-known that age class is involved in transmission of mycoplasma infection in wild boars. Thus, the value of seroprevalence of mycoplasma infection is higher in young and adult wild boars (3).

It is well-understood that nowadays, globalization, climate change, increased animal movements, and trade have led to an increasing number of emerging diseases. Given this context, in order to understand the role of environmental change in disease emergence and transmission, the expertise of specialists working in several areas such as ecology, zoology, microbiology, human medicine, and veterinary medicine is required. Meanwhile, veterinarians and other public health professionals have to become an integral part of the research teams involved in control of mycoplasma infection in wild boar populations. More specifically, the major role of these professionals is to understand and prevent the transmission of disease from wild boars to domestic animals and humans (6).

The probability of transmission of infection induced by *Mycoplasma hyopneumoniae* from wild boars to domestic pigs is affected by prevalence and mode of transmission of the agent. Determining the likelihood of transmission of mycoplasma infection from wild boars to domestic pigs plays a special role in the implementation of biosecurity measures in order to

decrease or even to eliminate the risk of exposure of domestic pigs to mycoplasma infection transmitted by wild boars (5).

Given this context, close and continue collaboration among biologists, ecologists, veterinarians, and epidemiologists is crucial for successful prevention interventions concerning mycoplasma infection and other respiratory diseases that are prevalent in wild boar populations. Additionally, dispersion ability of wild boars should be considered in developing surveillance programs. Knowledge of host dispersal rates is considered vital in understanding the spread of diseases in wild animals. The combination of higher dispersal ability of wild boars and the fact that wild pigs are known to be an important reservoir for many zoonotic pathogens can facilitate the transmission of diseases that affect wild boars to domestic pigs and humans (1).

All things considered, taking into account the higher rate of prevalence of *Mycoplasma hyopneumoniae* infections in wild boar populations, a combination of serological tests are required to establish an accurate diagnosis of mycoplasmosis in order to implement successful prevention interventions in the future (10)

Acknowledgements

This research work was carried out with the support of the project „Dezvoltarea infrastructurii de cercetare, educaţie şi servicii în domeniile medicinei veterinare şi tehnologiilor inovative pentru RO 05, cod SMIS-CSNR 2669".

Conclusions

- Infection with *Mycoplasma hyopneumoniae* in wild boar populations was reported in all three counties monitored (Caraş-Severin, Timiş, and Bihor); the seroprevalence of infection in the investigated area was 100%.
- The seroprevalence of infection induced by *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the hunting grounds investigated, was 62.50% in Caraş-Severin County, 66.67% in Timiş County, and 68.75% in Bihor County.
- The seroprevalence of infection with *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the nine hunting grounds in Caraş-Severin County, Timiş County, and Bihor County, was 66.67%.
- The seroprevalence of infection with swine influenza virus subtype H1N1, reported to the total number of samples tested on the 25 hunting grounds in Caraş-Severin County, Tmiş County, and Bihor County, was 11.80%.

References

1. Casas-Diaz, E., Closa-Sebastia, F., Peris, A., Nino, A., Torrento, J., Casanovas, R., Marco, I., Lavin, S., Fernandez-Llario, P., Serrano, E., 2013 – Recorded dispersal of wild boar (Sus scrofa) in northeast Spain: implications for disease monitoring programs, Wildl. Biol. Pract., 9(3):19-26;
2. Chiari, M., Ferrari, N., Zanoni, M., Alborali, L., 2014 – Mycoplasma hyopneumoniae temporal trends of infection and pathological effects in wild boar populations, European Journal of Wildlife Research, 60(2):187-192;
3. Closa-Sebastia, F., Casas-Diaz, E., Cuenca, R., Lavin, S., Mentaberre, G., Marco, I., 2011- Antibodies to selected pathogens in wild boar (*Sus scrofa)* from Catalonia (NE Spain), European Journal of Wildlife Research, 57(4):977-981;
4. Kukushkin, S., Baborenko, E., Baybikov, T., Mikhalishinv., Domskiy, I., 2009 – Seroprevalence of antibodies to main porcine infectious pathogens in wild boar in some regions of Russia, Acta Silvatica et Lignaria Hungarica, 5:147

5. Pearson, H. E., 2012 - Understanding and mitigating the risk of pathogen transmission from wild animals to domestic pigs in Australia- Teza de Doctorat;
6. Pierre, M., Degiorgis, R., 2013 – Wildlife health investigations: needs, challenges and recommendations, BMC Vet. Res., 9:223;
7. Sibila, M., Mentaberre, G., Boadella, M., Huerta, E., Casas-Diaz, E., Vicente, J., Gortazar, C., Marco, I., Lavin, S., Segales, J., 2010 – Serological, pathological and polymerase chain reaction studies on Mycoplasma hyopneumoniae infection in the wild boar, Veterinary Microbiology, 144(1-2):214-8;
8. Stukelj, M., Toplak, I., Vengust, G., 2014 – Prevalence of antibodies against selected pathogens in wild boars (Sus scrofa) in Slovenia, Slov Vet Res, 51(1):21-8;
9. Vengust, G., Valencak, Z., Bidovec, A., 2006- A serological survey of selected pathogens in wild boar in Slovenia, Journal of Veterinary Medicine B, Infectious Diseases and Veterinary Public Health, 53(1):24-7;
10. Wiratsudakul, A., Prompiram, P., Pholtep, K., Tantawet, S., Suraruangchai, D., Sedwisai, P., Sangkachai, N., Ratanakorn, P., 2013 – A cross-sectional study of porcine reproductive and respiratory syndrome virus and mycoplasma hyopneumoniae in wild boars reared in different types of captive setting in Thailand, J. Veterinar. Sci.Technolo, 4:146;

FREQUENCY OF INFECTIOUS DISEASES ASSOCIATED WITH REOVIROSIS IN BROILERS

Oana MATEIU-PETREC, Alina GHIȘE, A. STANCU, N. CĂTANA
Banat's University of Agricultural Science and Veterinary Medicine, "King Mihai I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului, no. 119, Timisoara , oana_petrec@yahoo.com

Abstract
Reovirosis has an endemic progress, in broilers farms, alone or in combination with other infectious diseases due to immunosuppression induced by the avian reovirus. The researches was carried out in a flock of 10,000 broilers, where avian reovirosis evolved from the first week of life, moving with reovirosis characteristic syndromes. Following necropsy examinations, performed biweekly, were identified characteristic lesions for reovirosis and for following infectious diseases: colisepticemia, mycoplasmosis, pseudomonosa and Marek's disease. Through this examination were identified pathological dystrophic lesions, whose etiology was confirmed more difficult. Immunosuppression occurred consecutively to avian virus multiplication in lymphoid organs, especially in the Fabricius bursa, favored the emergence of associated infectious diseases which are frequently signaled during the last years in broilers.
Keywords: *reovirosis, broilers, immunosuppresssion;*

During the time, the degree of resistance of birds has decreased due to: the selection and improvement, intensive technology and stress, global trade with poultry material has contributed to the spread of pathogens infectious, viral and bacterial pathogens that can trigger endemic disease, regarded as infections farm. These diseases are difficult to control and evolves in effective as associated infections (2,3,4,5)

Avian reovirosis has detached by frequency and economic importance being very well studied because immunosuppression induced by avian reovirosis promotes the development of other viruses (2,3,5)

The research has pursued, in broiler farms, the presence of viruses that cause infectious diseases associated with avian reovirosis.

Materials and methods

The research was carried out in a flock of 10,000 broilers, COBB 500 hybrid, increased ground. The flock was monitored by epidemiologic, clinic and pathologic examinations (macroscopic and microscopic exams), performed beweekly during the growth, until the age of 41 days.

The results were processed and shown in tables and graphs.

Results and discussions

In the monitored flock of broiler have evolved viral and bacterial diseases, which started at different ages of chicks, being diagnosed based on clinical signs, macroscopic anatomopatological and histopathological lesions and based on laboratory tests.

Necropsy examination performed biweekly, have revealed characteristic macroscopic lesions, which were noted and shown in the table and graphs, based on their being suspected more associated diseases. Necropsy results are presented based on controls, in Table 1 and the incidence of pathological lesions is presented as several graphs.

Macroscopic anatomopathological lesions of diseases associated of reovirosis

Leziuni	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Peritonitis, Omphalitis	26,66 %	16,66 %	9,09%	8,33%	0%	0%	25%	0%	6,66%	0%
Rinitis, Tracheitis	46,66 %	8,33%	33,33 %	27,27 %	14,28 %	12,50 %	12,50 %	0%	13,33 %	0%
Congestion, pulmonary edema, bronchopneum o nia	13,33 %	41,66 %	50%	36,36 %	100%	62,50 %	50%	42,85 %	33,33 %	57,14 %
Airsaculitis fibrinous	0%	0%	0%	0%	0%	12,50 %	12,50 %	0%	20%	42,85 %
Hyperplastic spleen	0%	0%	0%	18,18 %	0%	12,50 %	12,50 %	14,28 %	20%	0%
Fibrinous polyserositis	6,66%	0%	0%	9,09%	9,09%	12,50 %	12,50 %	14,28 %	6,66%	14,28 %
Dystrophy of liver, heart, kidney, ureteral gout	33,33 %	8,33%	16,66 %	9,09%	0%	0%	25,00 %	0%	6,66%	0%
Marek disease	0%	0%	0%	0%	14,28 %	12,50 %	12,50 %	14,28 %	20%	28,57 %

Colibacillosis has evolved with gross pathological lesions characteristic of both localized infections and septicemia, these infections are based on the age of chickens. Localized infections were reported in the first days of life and the septicemic lesions have been reported after the age of 12 day. At 28 days, colibacillosis had maximum frequency. Colibacillosis is frequently reported in broiler chicken flocks like disease associated with reovirosis, evolutionary forms being correlated with chickens age (3,4).

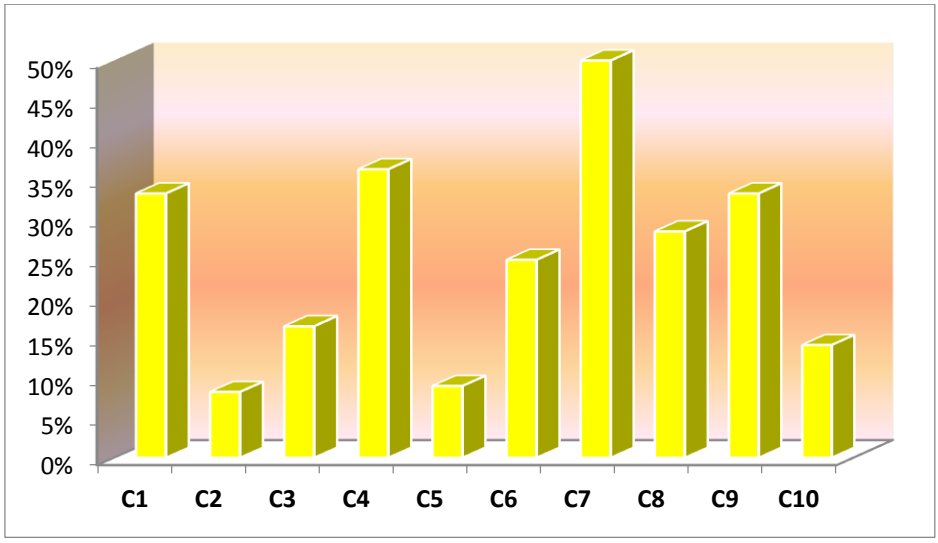


Figure 1. The frequency of colibacilosis

Mycoplasmosis has evolved with several syndromes, depending on age of chickens and duration of evolution. It started with rhinitis and tracheitis with a frequency of between 8.33% and 46.66%, followed by congestion, pulmonary edema and bronchopneumonia with a

frequency of between 13.33% and 100%. Airsaculitis fibrinous appeared at the age of 26 days and was kept constant until the liquidation of flock.

Mycoplasmosis is, also frequently mentioned as infectious disease associated with reovirosis and the frequency, clinical forms and mortality is variable depending on many factors (1,3,4).

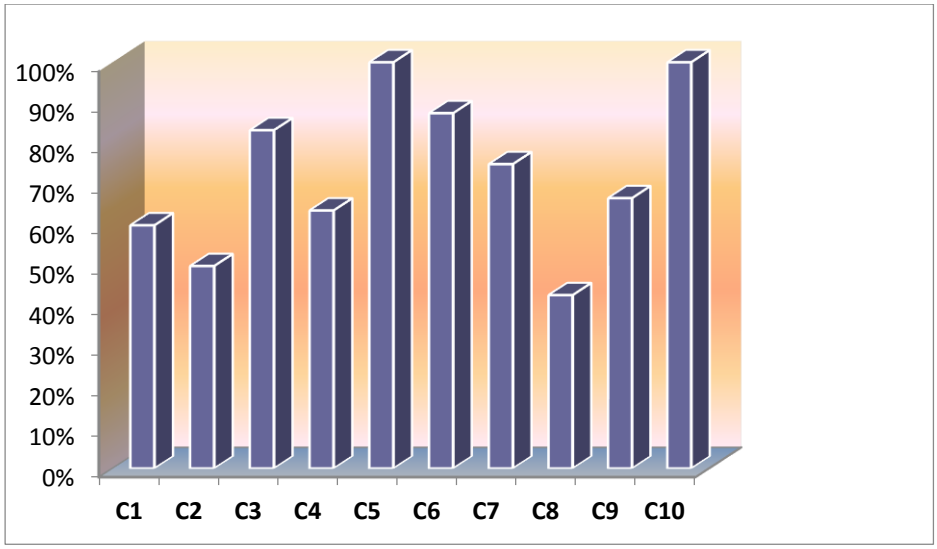


Figure 2. The frequency of mycoplasmosis

Pseudomonas infection was diagnosed only autopsy examinations based on a small number of corpses after 3 weeks of age. This disease is reported less frequently, being regarded as a consequence of immunosuppression status produced by some viruses, including the avian reovirus (2,3,4).

Marek's disease has appeared at the age of 21 days. At necropsy of corpses were found macroscopic lesions represented by splenomegaly and hepatomegaly. The appearance of Marek disease in broiler flocks, where reovirosis has evolved, has been reported in recent years, quite often.

This disease has evolved as an associated infectious disease, consecutive to immunosuppression status induced by natural infection with reovirus or vaccines that contain live attenuated strains of avian reovirus. The cited authors have found that status immunosuppression has favored the increasing prevalence of Marek's disease and lowering of the age-old chicks which were first cases (3,6).

The observations, concerning to Marek disease occurrence, confirmed the existing data in the literature and explained the disease from the age of 21 days (2,3,6).

Conclusions

1. Necropsic exams, carried out biweekly, showed macroscopic pathological lesions characteristic for avian reovirosis and for diseases associated with reovirosis.
2. Infectious diseases associated with reovirosis which have emerged are: colibacillosis, mycoplasmosis, pseudomonas infection and Marek's disease.
3. Immunosuppression induced by avian reovirus has favored the emergence and evolution of viral infectious diseases with endemic evolution.

Bibliography

1. Al-Afaleq A.I., Bradbury J.M., Jones R.C., Metwali A.M., (1989) – *Mixed infection of turkeys with Mycoplasma synoviae and reovirus: field and experimental observations,* Avian Pathology, Vol.18, 441-453.
2. Cătana N., Ionica Fodor, Daniela Botuș, Virgilia Popa, (2008) – *Infecţiile cu reovirusuri la păsări,* Magazin Avicol, Anul 5, Nr.5 (21), 24 – 25.
3. Davis J.F., Kulkarni A., Fletcher O., (2013) – *Reovirus infections in young broiler chickens,* Avian Diseases, Vol.57 (2), 321-325.
4. Jones R.C., (2013) - *Other reovirus infections*, În *Diseases of Poultry,* 13th Edition, Editor-in-Chief David E. Swaine, Ed. Wiley Blackwell, State Avenue, Ames, USA, 361-366.
5. Perianu T., (2012) – *Tratat de boli infecțioase ale animalelor, Viroze și boli prionice, vol. II*, Ed. Universitas, XXI, Iași.
6. Schat K.A., Nair V., (2013) – Marek's disease, În *Disease of poultry,* 13th Edition, Editor in Chief: David E.Swaine, J. R. Glisson, L. R. McDougald, L. K.Nolan ,D. L. Suarez, V.L. Nair, Ed: Wiley Blackwell, State Avenue, Ames, Iowa, USA, 515-551.

THE GREY PARTRIDGE *(Perdrix perdrix)* – AN INDICATOR OF THE ROMANIAN AGRO-SYLVO-PASTORAL ECOSYSTEMS

MATIUȚI M.,[1] LERA C.,[2] HUȚU I.[1]
[1] Banat University of Agricultural Science and Veterinary Medicine *"King Michael I of Romania"* from Timisoara, Faculty of Veterinary Medicine Timisoara, C. Aradului 119
[2] County Association of Hunters and Fishermen,Timis
Correspondent author: matiutimarcel@yahoo.com

Abstract
The grey partridge (Perdrix perdrix), a bird which is included in the cynegetic biodiversity of Romania, is a sedentary species, who may vary in number according to the quantity and the quality of the food. This species can be seen as an biobarometric indicator which offers information about the level of pollution in the geographic areas. This study provides a retrospective analysis on the evolution of the number of partridges in Romania between 1937-2016. The average number of partridges in that period is 110,309,36±22.549,13 and their distribution suggests that there were three peaks: in 1937 (273.000 specimens), in 1971 (300.000 specimens) and in 1998 (132.000 specimens). In the West Plain the number of specimens decreased starting with the year 1971 but then in 2003 there was another increase which had its peak in 2011 when the large monoculture areas were replaced with smaller ones with variety flora. This study highlights the factors which lead to the small numbers of partridges and some measures which can be taken in order to protect this species.
Key words: *grey partridge, biobarometer, cynegetic biodiversity, ethnozootechny*

Introduction

The Grey partridge (*Perdrix perdrix*) is a sedentary bird that prefers vegetable crops and which is specific to Romania's wildlife biodiversity. They are useful to agriculture as they consume weed seeds and harmful insects, being attached to the place of residence and "*highly dependent on the quantity and quality of food*" (Kiss, 2003). *"The seeds of weeds passed through the partridge's digestive tube lose their germinal capacity in a rate of 99.75 %"* (Cristescu, 2007). The ethnozootechnycal studies demonstrate the existence of a large number of partridges in the early 20th century, when "in the Transylvanian and Banat plains there were between 300,000 and 500,000 specimens" (Alaci, 2009). By the mid-20th century the partridge occupied the same areas as the great bustard (*Otis tarda*) (photo 1).

Photo 1. Great bustard from Beba Veche 2015 (orig. Lera C).

Today the great bustard disappeared from Romania (incidentally there are some specimens reported in the border region of western Romania) while partridges, even if there are still declining numerically, are still seen in lowland areas, especially in the areas that monocultures have been replaced with multicultures. In Banat this species is not regarded as a

hunting trophy, the pheasant being preferred to the partridge. The number of partridges is declining not only in Romania but also and across Europe (DST, 2016).

Materials and methods

This study was conducted on the hunting funds in Timis county, and the data came from the County Association of Hunters and Fishermen (CAHF) Timis, the Timis Forestry Department (DST) and the Transilvanian Rare Breeds Association (TRB). The evaluation of the flocks was done on sample areas, which were randomly chosen, representing a percentage of the size of the hunting funds area, during the spring breeding when there were counted how many pairs there were, and in winter guiding after the snow tracks. Due to the difficulties related to the impossibility of evaluating all existing specimens, with all the imperfect appreciation there have been observed only part of individuals present on the spot, for example in Giarmata in 2016 when wheat crops have been reported cca.12-15 cm high, a flock of approx. 40 specimens has been observed; in the same period near Fibiș there were 11 specimens and these are the only data that can be analysed (TRB, 2016). An inventory of populations on a large scale involves specific difficulties related to limited human and financial resources.

Results and discussions

In fig. 1 there is the evolution of the number of partridges in Romania during 1937-2016.

Fig. 1. The evolution of the number of partridges in Romania during 1937-2016.

For that period one can calculate an average of 110,309.36 ± 22549.13 birds; their distribution suggests the existence of three peaks: in 1937 (273 000 units), 1971 (300,000 units) and 1998 (132,000 units).

This graph demonstrates the connection between the numerical evolution of partridges and the changes in agriculture in the mid of 20th century. Until the mid-20th century there was a practice of an agriculture "based on the use of manure for fertilization, using animal traction" (Alaci, 2009) and the existence of a number of areas with protective curtains favoured in general a large number of birds.

In the period between 1950-1968 we passed to intensive farming based on large areas of monoculture, the use of noxious chemicals (ex. Dichloro-diphenyl-trichloroethane abbreviated DDT), land systems, destruction of protective curtains, the introduction of agricultural machinery with increasing working speeds (the noise disorients the birds), poaching, stray dogs and cats led to a significant drop in the number of partridges; in 1961 there were approx. 8,000 specimens in Romania. The same factors influenced the decline of partridges in the Timis county (figure 2).

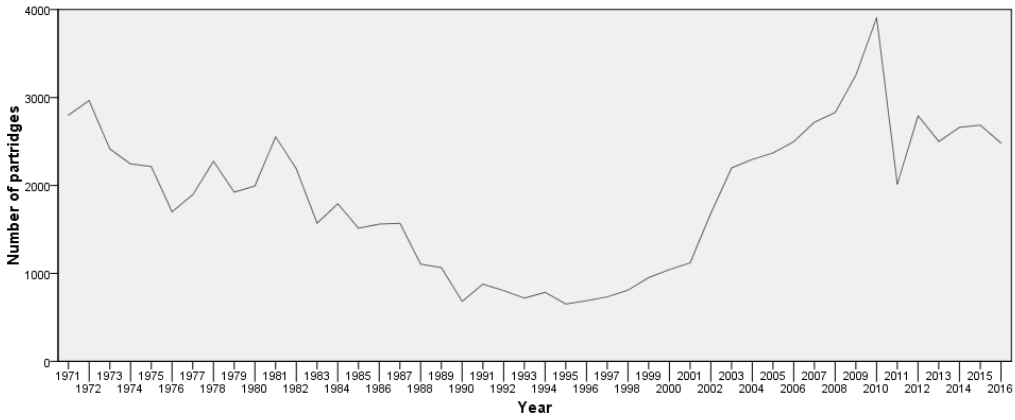


Fig. 2. The evolution of the number of partridges in the Timis county between 1971-2015

The descriptive statistics is in table 1.

Table 1.

Descriptive Statistics of partridges during 1971-2016 period

	N	Minimum	Maximum	Mean	Std. Deviation
Number of partridges	46	652,00	3908,00	1872,0870	817,42900
Valid N (listwise)	46				

In photos 2 and 3 is images from Hunting Complex Pischia.

Photo 2. The breeding of grey partridges in Pischia. Orig. Matiuti M. 2016

The chemical treatment of the seeds has led to the sharp decline of the entomofauna which forms the basis of the offspring in the first 10 days of life, "because at this time they do not have the necessary enzymes to digest cellulose" (Polverejan, 2008).

From the observations that have been made, the authors of this study believe that another important cause that has decreased the number of partridges in the western area of Romania is related to the introduction of the pheasant (*Phasianus cholchicus*). We will list some important data related to the introduction of the pheasant in western Romania: after O. Witting (1960), the first data about pheasants appear in 1504 with the introduction of a law on hunting in Transylvania, then in 1718 in a letter in which Karoly family speaks of the existence of some pheasant farms in Transylvania (Nedici, 1940). The data published by G. Olteanu in 1934 prove that in 1884 in Arad, Timis and Bihor counties a number of 1775 pheasants was harvested. In 1918 two pheasant farms appear: one in Timişoara at the Green Forest and the other at Somos (within the Chisineu Cris Forest District).

It is the beginning of a numerical increase of the pheasant and the start of the decline for the partridge. In Timis county in 1918 pheasant farms were located in Banloc, Pischia, Green Forest, Bistra, Macedonia and Chevereș. The biological peculiarities of the pheasant makes it a serious competitor for partridges: they use a wider range of habitat, their nesting starts 2-3 weeks before the partridge's, "the number of partridge eggs is higher by 6 pieces, meaning that it needs 6 extra days to complete the nest" (Castiov, 2014).

Photo 3. The pheasant from Pischia. Orig. Matiuti M. (2016)

"The pheasant is more resistant to cold and lack of food" (Lera and Bura, 2015). *"The winters of 1939-1940 and 1953-1954 with cold rains and flooding caused a disaster among partridges"* (Ivănescu *et al*., 1987).

In the past 10 years there has been a relative comeback of the number of partridges and this can be linked to a better control of the chemical substances used, combating poaching, reducing large monoculture of fields. There are good conditions for the existence of partridges in the uncultivated hills of Banat due to the higher slopes. The Pischia pheasant farm where there are approx. 3000 specimens annually for populating the hunting partridge funds do not solve the problem of their number, as the specimens bred here lose their instinct to hatch when they are released at the rate of 62-71%.

Conclusions

In order to protect the partridges, the following should be done:

- In situ conservation through the creation of a protective curtain along the perimeter of the agricultural area of farms (at least 7 m). Moreover, "the building of small draws of Spartium scoparium (*Sarotamus vulgaris*) on surfaces of a few tens of square meters is an excellent shelter for partridge " (Castiov, 2014);
- Rational use and control of chemicals;
- making agriculture ecological;
- Encouraging animal breeding in a pastoral system. Pastoral practices take into account entomofauna as an indicator for grazing;
- using alternative veterinary prophylactic measures which are efficient in maintaining the health status of animals for entomofauna and grazed ecosystems;
- Combating poaching and stray dogs and cats;
- Encouraging scientific studies on this species and introducing modern methods of numerical evaluation.

References

1. Alaci A., 2009 Potarnichile, Rev. Diana no. 2, printed in SC Nicola Mazzolin SRL, Timisoara, p. 10-11
2. Castiov Fr., 2014 Despre potarniche, Rev. Diana no. 3, printed in SC Nicola Mazzolin SRL, Timisoara, p. 30-31
3. Castiov Fr., 2014 Chestiunea potarnichilor, Rev. Diana no. 3, printed in SC Nicola Mazzolin SRL, Timisoara, p. 6-7
4. Cristescu P., 2007 Potarnichea. Crestere si valorificare, Ed. Regina din Arcadia, Bucuresti
5. Kiss A., 2003 Homo Kinegeticos si trofeele de vanatoare, Ed. Artaress, Timisoara
6. Ivanescu St., Olteanu M., Barbulescu St., 1987 Pagini despre vanatul romanesc, Ed. Sport-turism, Bucuresti
7. Lera C., Bura M., 2015 Fazanul – metode de crestere si apreciere a insusirilor pentru productia de carne, Ed. Eurostampa, Timisoara
8. Nedici, Gh., 1940 Istoria vânătoarei şi a dreptului de vânătoare, Tipogr. „Universul", Bucureşti.
9. Olteanu, G.,1934 Vânatul în Ardeal, înainte cu 50 de ani, Rev. Carpaţii, no. 10, Cluj.
10. Polverejan St., 2008 Ecologia in folosul vanatului mic, Rev. Diana no. 3, printed in SC Nicola Mazzolin SRL,Timisoara, p. 12
11. Witting O., 1960 Economia vanatului, Ed. Agro-Silvica de Stat, Bucuresti
12. *** Data base Association Transilvanian Rare Breeds (TRB) Timisoara, 2016
13. *** Data base County Association of Hunters and Fishermen (CAHF) Timis, 2016
14. *** Data base Timis Forestry Department (DST), 2016

BIOECONOMIC SOLUTIONS FOR PRESERVING THE LOCAL BREEDS IN THE BANAT EUROREGION

MATIUȚI M.,[1] HUȚU I.[1], MATIUTI C.[2], MORARIU F.[1]
[1] Banat University of Agricultural Science and Veterinary Medicine *"King Michael I of Romania"* from Timisoara, Faculty of Veterinary Medicine Timisoara, C. Aradului 119
[2] Technical College Azur Timisoara, C. Martirilor nr. 64
Correspondent author: matiutimarcel@yahoo.com

Abstract
This paper is based on previous studies which are dedicated to the inovativ concept of bio-economic solutions derived from ethnozootechny and associated to climate change which have an impact upon the domestic animals' resistance and production. The descriptive study presents the reasons and the challenges for which the local breeds must be preserved; for the first time, rusticity is approached differently, being no wone of the desirable features which has never been approached in animal selection in Romania. The arguments of the paper plead for maintaining the local breeds – this proves the cultural intelligence of the communities that created them. The products obtained from these animals are the base of the ethnogastronomy of various ethnies in Transylvania and Banat and can contribute to a raise in economic competitiveness, becoming an added value in this niche market and having immediate socio-bio-economic effects: a high level of occupancy in the labour force, diminishing the rural poverty and a certain bioeconomic independency in certain conditions. This study claims that in the strategy for bio-economic development of Banat and Transylvania in 2016-2030 there should be taken into consideration the results of the ethno – animal husbandry research as a part of the territorial capital.
Key words: *local breeds, biodiversity, ethnozootechny*

Introduction

"Genetic diversity is a critical basis for food security and rural development. It enables stock to be selected in response to changing market conditions or societal needs, new knowledge of human nutritional requirements, changing environmental conditions and new or resurgent disease threats" (Hoffman, 2013).

"Animal genetics industry represents one of the ways in which food can be ensured in the future. The local animal breeds have a key role in this industry and they are the main advantage in the competition with the transnational corporations in this domain. ... It is important that the local animal breeds be preserved since they are part of Romania's Zoogenetic Patrimony" (Matiuti *et al*., 2013).

Materials and methods

This scientific paper represents a continuation of other zootechnical studies, which promotes the sustainable bioeconimic development of the Banat euroregion (Matiuti *et al*., 2009, 2013, 2015; Matiuti and Matiuti, 2012; Matiuti, 2012).

The aim is that of preserving the old local breeds and of promoting economic competitiveness with the goal of maintaining the development of some rural areas in Banat. This region must have areas with wonderful landscapes in alignment with the general aim promoted by the EU.

Specific obiectives of this research is the development of advanced tools for mapping, protecting and managing local breeds in Banat Euroregion. „It is important to preserve the biodiversity and genetic resources through landscape management in this Danube region, because biodiversity increases pilot investments"(grain.org, 2012).

By family farm in Europe one understands a small dimensions farm, capable of being run by the members of a family, sometimes with 1-2 seasonal employees.

„Small farms represent approx. 17,7% out of Europe's farmland" (grain.org, 2014).

„Family farming model in Europe and thus of great importance in the EU" (EU web). "EU policies promote agriculture in the family farms, the ambition of keeping amazing landscapes in Europe, which have a good quality of the soil, air and water" (OECD, 2006). These policies represent a counterattack on the multinational corporations' domination, which many a times have environmental problems" (grain.org, 2014).

Results and discussions

In Romania "family farms are 99% of all farms and have 53% of the land with an average of 1.95 hectar/farm. They keep: 99% sheep, 99% goats, 99% of bees, 90% of cattle, 70% of pigs, 61% of poultry" (National Institute of Statistics, 2012). The data of FAO 2013 in Romania "small farms as 74-83% of all farms, 78% of agricultural land in the hands of small farmers 30.9%".

In Romania the local breeds are property of the farmers, the majority of whom have insufficient financial power (Matiuti, 2013).

Here is the SWOT analysis regarding the keeping, using, making efficient and exploiting the local breeds (table 1) – the strengths, the opportunities, the weaknesses and the risks or problems that might appear in the raising of local breeds.

It is possible that some yet unknown genes or combination of genes in the local breeds genome to be of interest in the near future and to contribute to the research programme.

It is pleasant to see various local breeds as you pass from one region to another. But it is a pitty that in order to see authentic breeds in small numbers one has to identify the area in which they live.

The area must be under observation, to see if the landscape has remained authentic for encouraging the local breeds development.

From the economic point of view, one has to be careful when speaking of concepts such as intensive-extensive, because they need to be redefined.

For instance, "extensive grazing in the mountainous areas where no tractors or agricultural machines are used can be financially rewarding in what the fodder resources are concerned" (Leger, 2013). One has to know which is the necessary amount of fodder for the animals, the quantity and the quality of the available fodder.

Independence and food sovereignty is achieved also through a circular economy of the food bioresources and necessary to combat and eradicate poverty and hunger (Matiuti, 2015).

"Breeds of animals that can be easily grown on pastures must have an important feature: rusticity" (Guintard and Denis, 2013). Specialists from the Ethnozootechny Society of Toul (France) recommend rusticity "as a criterion to improve animals and to maintain multipurpose breeds." In zone of Banat in animal selection rusticity was not included in the methodology.

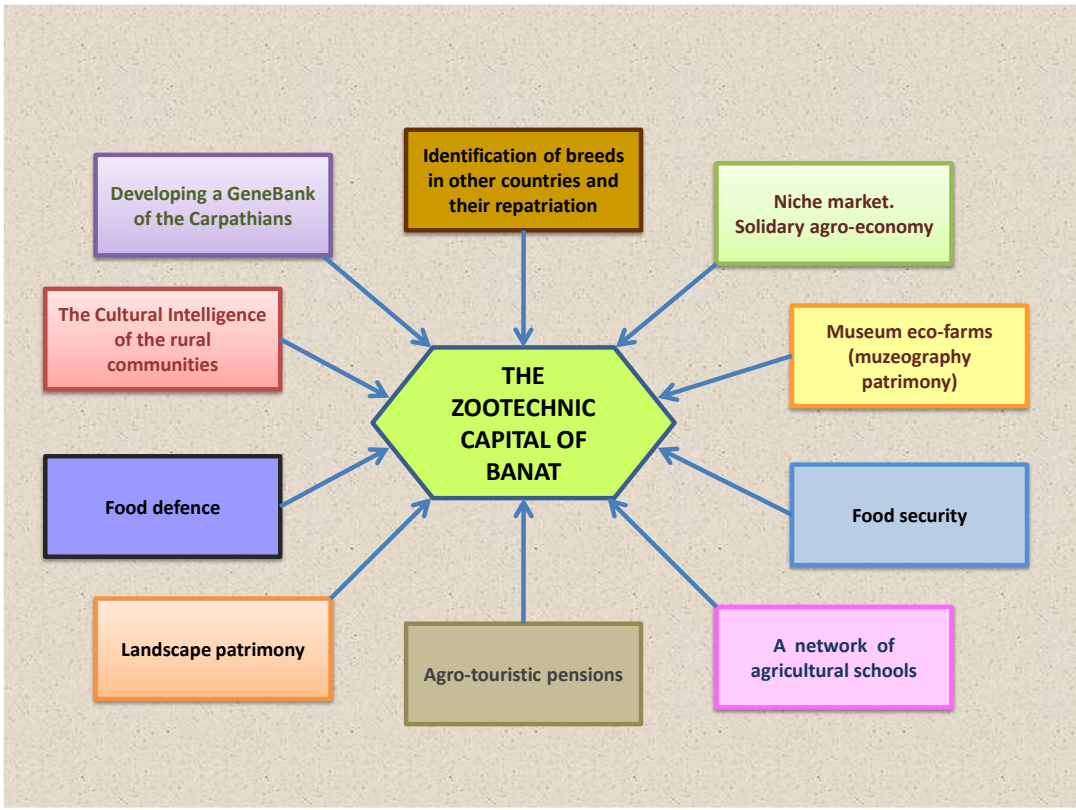
Along with the concepts "creative economy" and "creative industry" we can introduce the concept of "creative zootechny" in implementing high-impact nature friendly techniques and technologies in Banat's zootechny. (Matiuti *et al.*, 2014)

Table 1

SWOT analysis of local breeds

STRENGTHS	**WEAKNESSES**
- they react very well to the interaction between nourishment, reproduction and genotype; - geografic location; - tradition in breeding of local breeds; - the possibility for them to be raised by people with low financial power;	- the lack of certain census data that could reflect the number, the breeds and the areas in which they are raised, the lack of the Area Map of animal breeding; - low quality of management;

- they may be very well raised in the farms of specialised schools; - they adapt well to environmental-friendly technologies; - rusticity; - sturdiness; - flexibility; - plasticity; - resilience (a new criterion of quality); - they can be raised by pastoral practices which take into account the entomofauna as an indicator for the quality of the pastures; - alternative and efficient veterinarian methods can be applied to these animals for their health and respect for the floral mosaic from the regional landscape; - access to nroad network and knowledge through the Data Basis of Transilvanian Rare Breeds Association; - their existence contributes to their communitie's prestige, being part of their cultural intelligence; - productive longevity.	- the continuous decrease of number of animals; - lack of genetic animal industry; - the youth is not interested in local animal breeding; - insufficient of non-existent data regarding the productions from the past years.
OPPORTUNITIES	**THREATS**
- adaptability to the climate change; - excellent adaptability to the low quality pastures, for example in the mountainous regions; - the identification of a market niche; - the existence of an ethnogastronomy which is based on recipes that make use of the products obtained from the local breed animals; - the possibility to process in different ways the animal products from local breeds; - glocalization by the import from other areas; - the genes from the local breeds can be used to create other breeds; - in certain conditions they can offer a somewhat economical independence; - research in biotechnologies can lead to unexpected discoveries; - fertilization transfer; - collaboration with other related European networks.	- national interests often prevail; - climate change; - erosion of the domestic biodiversity; - lake of a Datebasefor zootechny; - lake of rural websitework; - lack of a GeneBank, with the exception of the one created for old chicken breeds with the help of the Transilvanian Rare Breeds Association; - lack of an agrifood industry in villages; - lack in genetic biodiversity, as part of the education.



Scheme 1. A solution for preserving the local breeds in the Banat euroregion according to the Bioeconomic development strategy for 2016-2030 (orig. authors)

Scheme 1 shows the proposed strategy for maintaining zoogenetic diversity in the Euroregion Banat for the period 2016-2030. This strategy is in line with the Bioeconomy development strategy for the period 2014-2030 developed by the European Union. The scheme is also based on the work of two researchers from France (Denis and Eglin, 2013).

"Conservation in vitro, in situ and on farm are ways of preserving the genetic heritage; finding economic value in the context of ecological growth, of solidary agriculture or of niche markets can be solutions" (Huţu, 2015). For example, the gene bank is one way of keeping local breeds verified; such a bank is the one for the oldest breeds of chickens in Banat, created with the help of the Transylvanian Rare Breeds Association. These breeds are found in Romania only in this farm and they possess genes which offer them rusticity, disease resistance and quality of the meat which is recommended for traditional dishes. TRB Association has a very large number of documents, photos, etc that will help in the near future to create ecological farms, local breeds museum, traditional processing of animal products rich and emphasizing the ethnogastronomy of the Banat Euroregion.

"Marketing products from local breeds has two advantages: traditional technical processing - production or manufacturing clothing with a characteristic design" (Mathias, 2010).

"Obviously the conditions for the persistence of a production must satisfy three criteria (Hutu and Onan, 2008): to be economically viable, to be sustainable in the environment and to be socially acceptable": would local breeds be able to satisfy these conditions successively? It's a question answered desirable but it requires study, internal analysis (virtuous circle of quality, the proposed SWOT analysis may be the first steps in making the transition from theory to practice) external analysis dependent on factors legislation and policies (laws and subsidies)

economic factors, social and cultural factors (ethnozootechnie) and technological factors (alternative technologies, cheap, suitable for the animals and the living environment).

Conclusions

- Preserving zoogenetic biodiversity is difficult, but that is an assurance for the food security of the population in a certain area;
- The contribution of local breeds to food security and food defense is major, for example through the contribution of genes that may confer special properties, resistance to disease, climate change, etc.;
- Zootechny development and hence rural areas development can be achieved through cross-border clustering for a combination between farmers - tourism (including rural tourism) - agrifood local industry - manufacturing industry;
- To maintain these local breeds is important to create a National Foundation for Research in Zootechny in order to facilitate the necessary fundraising for research. This research will enable the development of animal genetic industries in Romania;
- The management of peri-urban areas where there is a population with limited material possibilities who does not afford animals breed performance. Populations of breeds resistant to maintenance, poor quality feed assortment, including some waste from households; generally resistant to disease;
- Glocally rethinting.

References

1. Denis B. and Eglin J. E., 2013 Les races locales d'animaux domestiques: un element peu connu du patrimoine, Rev. de la Societe d'Ethnozootechnie Toul (France), Etnozootechnie no. 94, p. 13-18.
2. Digard J. P., 2013 La biodiversite domestique, une composante meconnue et negligee de la biodiversite animale, Rev. de la Societe d'Ethnozootechnie Toul (France), Etnozootechnie no. 94, p. 5-12.
3. Guintard C., Denis B., 2013 Notion de la rusticite chez les bovines; cas particulier de l'Aurochs – Reconstitue, Rev. de la Societe d'Ethnozootechnie Toul (France), Etnozootechnie no. 94, p. 19-29.
4. Hoffman I., 2013 Climate change in context: Implications for Livestock Production and Diversity, FAO, Rome, p. 33-34
5. Huțu I., 2015 Farm Animal Productions, Course for veterinarians in Animal Production and Husbandry, Ed. TVT Publishing - River Falls, Wi, SUA, Mirton - Timişoara, RO
6. Huțu I., Onan G. W., 2008 Alternative Swine Management Systems – Hoop Structures for Swine, Ed. TVT Publishing - River Falls, WI, Mirton - Timişoara, RO
7. Leger F., 2013 Redéfinir les concepts d'intensif et d'extensif, Rev. de la Societe d'Ethnozootechnie Toul (France), Etnozootechnie no 95, p. 11-18
8. Mathias E. coordonator, 2010 Adding value to livestock diversity. Marketing to promote local breeds and improve livelihoods, FAO Rome
9. Matiuți M., Bogdan A. T. and Crainiceanu E., 2009 *A case study of Transylvanian Pinzgau in Banat areal*, Lucrari stiintiifice Zootehnie si Biotehnologie, Timisoara, vol. 42 (1), p. 169-174.
10. Matiuți M., Matiuți C. L., 2012 Resurse genetice animale din spațiul carpato-dunărean. Etnozootehnie, Ed. Tempus, Timișoara.
11. Matiuți M., 2012 Methodology for the Elaboration of Sectorial Studies for Territorial Capital by Synergy of the sequential components based on Eco-BioeEconomy with a view to form the rural Web Network of zoogenetic Biodiversity in the Banat region, Danubius Journal of Regional Cooperation Belgrad, bilingv, sept internet
12. Matiuți M., Bogdan A.T., Matiuți C. L., 2013 A strategy for development in the danubian area from Bazias to the Delta, based on a new bio-ecoeconomic approach in order to integratively value the authentic danubian territorial capital between 2014-2020, Lucrări științiifice Medicină Veterinară Timișoara, vol. LXVI, p. 100-113.

13. Matiuți, M., Bogdan A.T., Matiuți C. L.,2013 The new concept of eco-bioeconomy in the elaboration of strategies for safety food and defense food, Danubius Journal of Regional Cooperation Belgrad, bilingv, ian internet.
14. Matiuți M., Bogdan A.T., D. Diaconescu D., Matiuți C. L., 2013 The scientific characteristics and the practical signification of the possible ecological biohazardous form in Euroregion Banat, Buletin AGIR nr. 3/2013, supliment 1, Ed. AGIR, Bucuresti, p. 115-122
15. Matiuți M., Bogdan A.T., Matiuți C. L., 2013 Valorization of the zootechnical potential within the territorial capital of Babat, through eco-bio-geo-economic strategy, The 12th International Symposium Prospects for the 3rd Millenium Agriculture Cluj Napoca, vol. 70(1), p. 65-78.
16. Matiuți M., Bogdan A.T., Gaspardy A., Diaconescu D., 2015 An innovative application of the Bio-economic strategies and actions plans elabored by the European Union for 2030 at a crossborder level in the Danubian Euroregions, 1st International Medical Conference „Environment and public health" MED ENV Constanta 2014, published in Journal of Environmental Protection and Ecology 16, no.1, SciBulCom Ltd, p. 280-291
17. Matiuți M., Bogdan A. T., Matiuți C., 2014 Ethnozootechny a Component of Geoeconomy, Bulletin UASMV-CN Animal Science and Biotechnologies, vol. 71(2), Ed. AcademicPres Cluj, p. 179-191
18. Organization for economic co-operation and development (OECD), 2006 The new rural paradigm: policies and governance, OECD Publishing Paris.
19. FAO, 2013 Developing the Institutional Framework for the Management of Animal Genetic resources, Rome.
20. *** Data Base Association Transilvanian Rare Breeds.
21. .*** National Institute of Statistics, 2012 press release no.149 of July 2012 "General agricultural census 2010"
22. web paghttpllec.europ.eu/agriculturefamilly-forming/index.eu.htm)
23. www.grain.org 2012
24. www.grain.org 2014

STUDIES CONCERNING THE DEVELOPMENT OF THE MYXOMATOSIS MONITORING AND SURVEILLANCE PROGRAM FOR *ORYCTOLAGUS CUNICULUS*

Marius DAN, Stelian BĂRĂITĂREANU*, Daniela NIȚĂ, Doina DANEȘ*
Institute for Control of Biological Products and Veterinary Medicines, 37 Dudului, 060603, Bucharest, Romania
e-mail: marius.dan@icbmv.ro
*Faculty of Veterinary Medicine of Bucharest, 105 Splaiul Independenței, 050097, Bucharest, Romania

Abstract

Myxomatosis is one of the most important infectious diseases of rabbits. Large economic losses caused by myxomatosis require the development of effective surveillance and monitoring programs. These programs must have an active and passive surveillance based on the best mix of diagnostic methods. In this paper we propose a combination of passive surveillance of myxomatosis (based on "the definition of the case of myxomatosis") with active sero-surveillance of myxomatosis (based on ELISA). A count of 1463 rabbits from 120 farms was epidemiologically and clinically evaluated. Out of these, 233 rabbits (15.93%), housed in 22 farms (18.33% of the households under monitoring), with signs of myxomatosis have been recorded. During 2010-2014, competitive ELISA or indirect ELISA assessment of blood serum samples taken from rabbits within the species Oryctolagus cuniculus from the exploitation units in which the passive surveillance was used, generated the following results: positive – 26.99% (105/389), negative – 70.44% (274/389) and inconclusive – 2.57% (10/389). The serum samples taken from rabbits previously vaccinated and tested using ELISA came back with 93.55% (58/62) positive results, 6.45% (4/62) negative ones, and none considered inconclusive. The serum samples taken from unvaccinated rabbits and tested using ELISA came back with 16.48% (45/273) positive results, 80.22% (219/273) negative results and 3.30% (9/273) inconclusive ones. Finally, the serum samples taken from rabbits with unknown history of vaccination and tested using ELISA came back with: 5.56% (3/54) positive results, 92.60% (50/54) negative results, and 1.84% (1/54) inconclusive ones. The passive surveillance of several rabbit breeding units allowed us to highlight the endemic character of myxomatosis in the area, along with an oscillatory character in as much as annual prevalence is concerned, and to pinpoint the value of "the definition of the case of myxomatosis". Also, the serological surveillance through ELISA techniques (competitive ELISA and indirect ELISA) can constitute an efficient control strategy for myxomatosis, and hence a successful alternative to the classical passive surveillance method, which becomes less efficient in endemic areas.

Keywords: *rabbits, epidemiological surveillance, diagnosis, competitive ELISA, indirect ELISA*

Myxomatosis is a viral disease of the European rabbit (*Oryctolagus cuniculus*), characterized by extensive internal and external injuries, immune dysfunctions and bacterial complications (9).

Myxomatosis is produced by the myxoma virus (*MYXV*), a pox virus that produces a benign cutaneous fibroma in rabbits of the genus *Sylvilagus*, considered the host of the *MYXV* (5, 7).

Along with the rabbit haemorrhagic disease, myxomatosis is one of the two diseases of lagomorpha included in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animal* (8). *MYXV* was initially used as a biological control agent for the population of wild rabbits in Australia (released into the environment between 1950 and 1951, and became endemic) and in France at Mallebois (released in 1952, it spread to the entire population of rabbits and become endemic throughout the European countries) (10). Progressively, the European rabbit population developed resistance to *MYXV*, along with the emergence and evolution of attenuated virus strains (5). The diversity of *MYXV* pathotypes, the variation of rabbit sensitivity and utilization of *MYXV*-vaccines in endemic areas led to the apparition of different groups of susceptible/resistant animals, such as: rabbits highly susceptible to *MYXV*, rabbits

with natural resistance to *MYXV*, rabbits immunized by vaccination, immunologically naive rabbits, and rabbits with undetermined sensitivity.

The epidemiological and pathological pattern of myxomatosis is the base of diagnosis in clinical practice (1), but sero-surveillance is the key approach of control strategies in endemic areas with several naturally immunized rabbits (2, 3). Also, the sero-conversion of exposed rabbits is used in the evaluation of myxomatosis prevalence in the rabbit population (2).

In this paper we evaluate the value of the association of passive surveillance of myxomatosis (based on "the definition of the case of myxomatosis") with active sero-surveillance of myxomatosis (based on ELISA), as part of the myxomatosis monitoring and surveillance program for *Oryctolagus cuniculus*.

Materials and methods

All rabbits included in this research were located in Romanian counties with history of myxomatosis (endemic area). One hundred and twenty family farms (table 1) of *Oryctolagus cuniculus* were epidemiologically and clinically investigated and 22 households (table 2) with history of myxomatosis were investigated by means of serological exams.

Table 1.

Distribution of *Oryctolagus cuniculus* rabbits and rabbit farms by year of study

Year	No. of rabbits	No. of farms
2010	360	36
2011	336	28
2012	392	28
2013	180	15
2014	195	13
Total	**1463**	**120**

Table 2.

Distribution of *Oryctolagus cuniculus* rabbits with Myxomatosis by year of study and number of farms

Year	No. of rabbits		No. of farms
	With specific signs	Without specific signs	
2010	108	42	9
2011	0	0	0
2012	0	0	0
2013	30	16	4
2014	95	15	9
Total	**233**	**73**	**22**

Samples used in active surveillance of myxomatosis by competitive ELISA

One hundred fifty-eight serum samples were collected. A count of 40 samples was from previously vaccinated rabbits, 79 samples were collected from unvaccinated rabbits and 39 samples from rabbits with unknown immunization history against myxomatosis.

Samples used in active surveillance of myxomatosis by indirect ELISA

Two hundred thirty samples were collected. A count of 22 samples was from previously vaccinated rabbits, 193 samples were collected from unvaccinated rabbits and 15 samples from rabbits with unknown immunization history against myxomatosis.

Table 3.

Monoclonal antibody and conjugate used in diagnostic of myxomatosis

Code	Utilization	Virus detected
1E5	Antibody 1	MYXV
1E5 HRP	Antibody conjugated with peroxidase	

Passive Surveillance of Myxomatosis

At the forefront of this research was the suspicion of myxomatosis conducted on the basis of the case definition set forth at the beginning of the studyies, and based on state of the art knowledge concerning the epidemiology and the signs/lesion patterns of myxomatosis in rabbits (*Oryctolagus cuniculus*).

Case definition: Any domestic or wild rabbit of the species *Oryctolagus cuniculus* which manifests fibrotic nodules with fleshy consistency on the nose, ears, and forefeet, adherents at cutis, originating in places with a history of myxomatosis, vaccinated or unvaccinated against this disease can be considered a clinical case of myxomatosis and laboratory investigations will be carried out to confirm the suspicion.

Active Surveillance of Myxomatosis

Serologic testing was conducted by two enzyme-linked immunosorbent assays: competitive ELISA and indirect ELISA.

Competitive ELISA is an in-house assay developed by Antonio Lavazza and Lorenzo Capucci at IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Umbertini"), Brescia (Italy). The method is based on the competition between antibodies from the serum sample (to be analyzed) and monoclonal antibodies 1E5 adsorbed to the wells of microplate to bind the antigen in the liquid phase (m71L membrane protein).

Table 4.

MYXV antibodies titres in correlation with different possibilities of induction
(IZSLER Brescia standard)

Antibody origin	Antibody titres
Post-infectious	1/2560 - 1/10240*
After vaccination (one vaccine)	1/40 - 1/320*
After hyper-immunization (several vaccine inoculation)	1/320 - 1/1280*

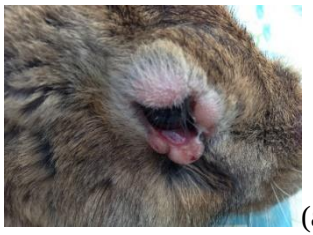
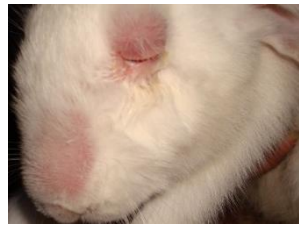
* Titres in accord with maximum period of Ab production: 5-30 days p.i. and 15-45 days post vaccination

Indirect ELISA is a commercial ELISA kit (INgezim MIXOMATOSIS R.17.MIX.K1, Inmunologia y Genetica Aplicada, S.A., Madrid, Spania, www.ingenasa.es). The method was performed in accordance with the manufacturer's recommendations.

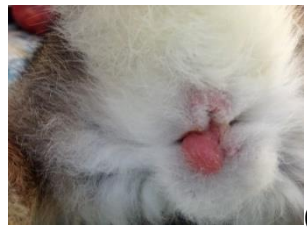
Both ELISAs were designed to detect *MYXV*-antibodies in rabbit blood serum, and they enable us to group samples into positive, negative and inconclusive ones.

Results and discussion

Passive surveillance of myxomatosis. The clinic and lesion pattern of the myxoma virus infections recorded in 22 carrier farms are described in fig. 1-3 and tables 5 and 6. The images (a) through (f) in fig. 1 present the main lesions developed by rabbits in the familial farms with outbreaks of myxomatosis.


(a)

(b)

(c)

(d)

(e)

(f)

Fig 1. Myxomatosis in European rabbits; (a, b, c): acute form of myxomatosis with head and eyelids tumefaction, mucous and purulent conjunctivitis and rhinitis; (d): first stage of multiple nodular lesions on pinna - ears become edematous; (e): nodular lesions on genito-urinary area, (f): last stage of multiple nodular lesions on pinna.

The distribution of rabbits with and without myxomatosis, after the clinical examination of 1463 animals, between 2010 and 2014, is presented in table 5 and fig. 2.

Table 5.

Distribution of *Oryctolagus cuniculus* rabbits with myxomatosis

Year	**Total**	**With myxomatosis**		**Without myxomatosis**	
		Nr.	%	Nr.	%
2010	360	108	30.00	252	70.00
2011	336	0	0.00	336	100.00
2012	392	0	0.00	392	100.00
2013	180	30	16.67	150	83.33
2014	195	95	48.72	100	51.28
Total	**1463**	**233**	**15.93**	**1230**	**84.07**

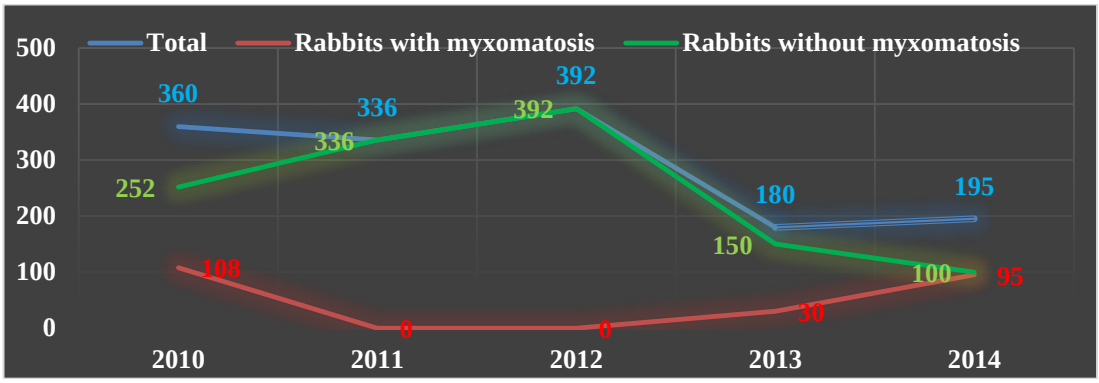


Fig 2. Annual myxomatosis events during 2010 – 2014

Similarly, the distribution of farms with and without myxomatosis, after the clinical examination of the animals in 120 farms, for the five-year span under analysis, is presented in table 6 and fig. 3.

Table 6.

Distribution of rabbit farms with myxomatosis by year of study

Year	Total	With myxomatosis		Without myxomatosis	
		Nr.	%	Nr.	%
2010	36	9	25.00	27	75.00
2011	28	0	0.00	28	100.00
2012	28	0	0.00	28	100.00
2013	15	4	26.67	11	73.33
2014	13	9	69.23	4	30.77
Total	**120**	**22**	**18.33**	**98**	**81.67**

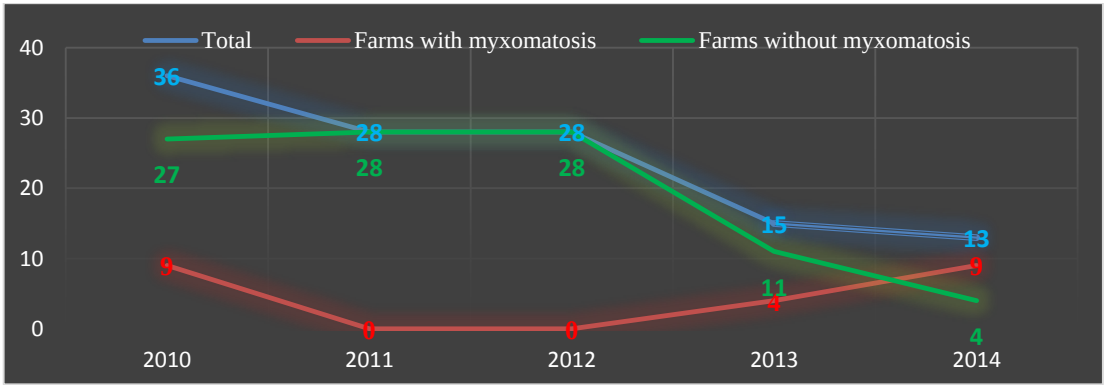


Fig 3. Annual myxomatosis outbreaks during 2010 – 2014

The annual myxomatosis events decreased from 30% in 2010 to 0% in 2011 and 2012, followed by a gradual recrudescence in the coming years, i.e. 16.67% in 2013 and 48.72% in 2014.

A similar developmental track can be observed in the case of annual myxomatosis outbreaks, which shrank from 25% in 2010 to 0% in 2011 and 2012, only to gradually increase thereafter in quite steep increments, amounting to 26.67% in 2013 and 69.23% in 2014.

During 2010-2014, myxomatosis was clinically diagnosed on 15.93 % (233/1463) of the rabbits included in the study, respectively in 18.33 % (22/120) from the rabbits breeding units included in the research plan; the case annual dynamics was oscillatory, with high values in 2010 (30.00 %; 108/360), moderately so in 2013 (16.67%, 30/180) and even higher in 2014 (48.72%, 95/195), and without any pathological events associated to this disease in 2011 and 2012.

These observations were consistent with other epidemiological studies, describing the evolution in waves of outbreaks of myxomatosis within the wild rabbit's population of *Oryctolagus cuniculus*, displaying decline periods of 2-3 years, followed by the re-emergence of the disease. This case scenario can be accounted for in terms of the variation of the rabbits' resistant and genetically susceptible populations, a phenomenon first described in the wild rabbit population in Australia (6, 11).

Active sero-surveillance of myxomatosis (based on ELISA).

The results of the serological surveillance by competitive ELISA are provided in fig. 4.

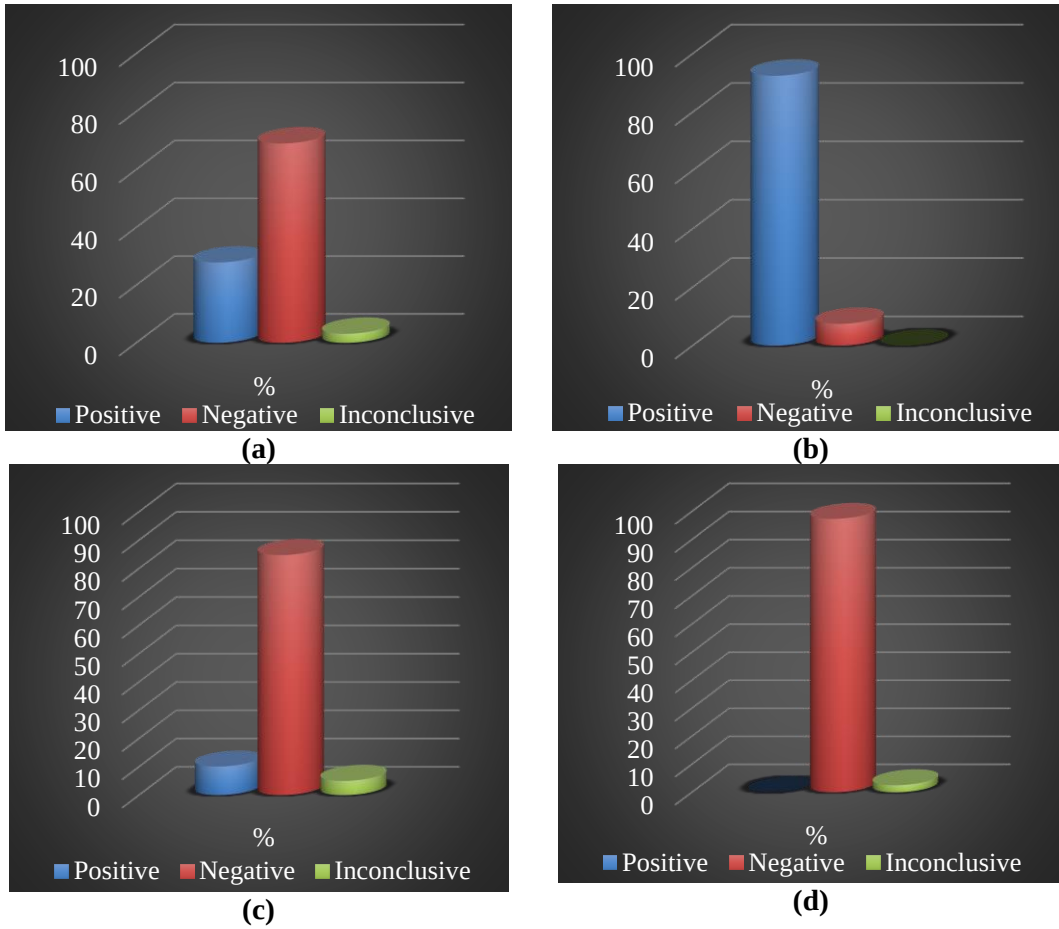


Fig 4. Myxomatosis active sero-surveillance by cELISA, in areas with history of disease: (a) in all serum samples; (b) in rabbits previously vaccinated for myxomatosis; (c) in unvaccinated rabbits; (d) in rabbits with unknown history of vaccination.

Competitive ELISA revealed 27.85% (44/158) positive, 68.99% (109/158) negative and 3.16% (5/158) inconclusive results (recommendation for retesting). Serum samples of the vaccinated rabbits gave 92.50% (37/40) positive and 7.50% (3/40) negative results. Serum samples of the unvaccinated rabbits revealed 10.13% (8/79) positive, 84.81% (67/79) negative results and 5.06% (4/79) inconclusive results. Finally, serum samples of the rabbits with unknown immunological status displayed 0% (0/39) positive, 97.44% (38/39) negative results and 2.56% (1/39) inconclusive results.

It is worth mentioning that inconclusive results might be due to the long period of time which allowed antibodies to develop (the highest titre was recorded at 20-60 days post infection) or to assay sampling after explosion of rabbits at low pathogenic virus strains (8). The results of serological surveillance by indirect ELISA are offered in fig. 5.

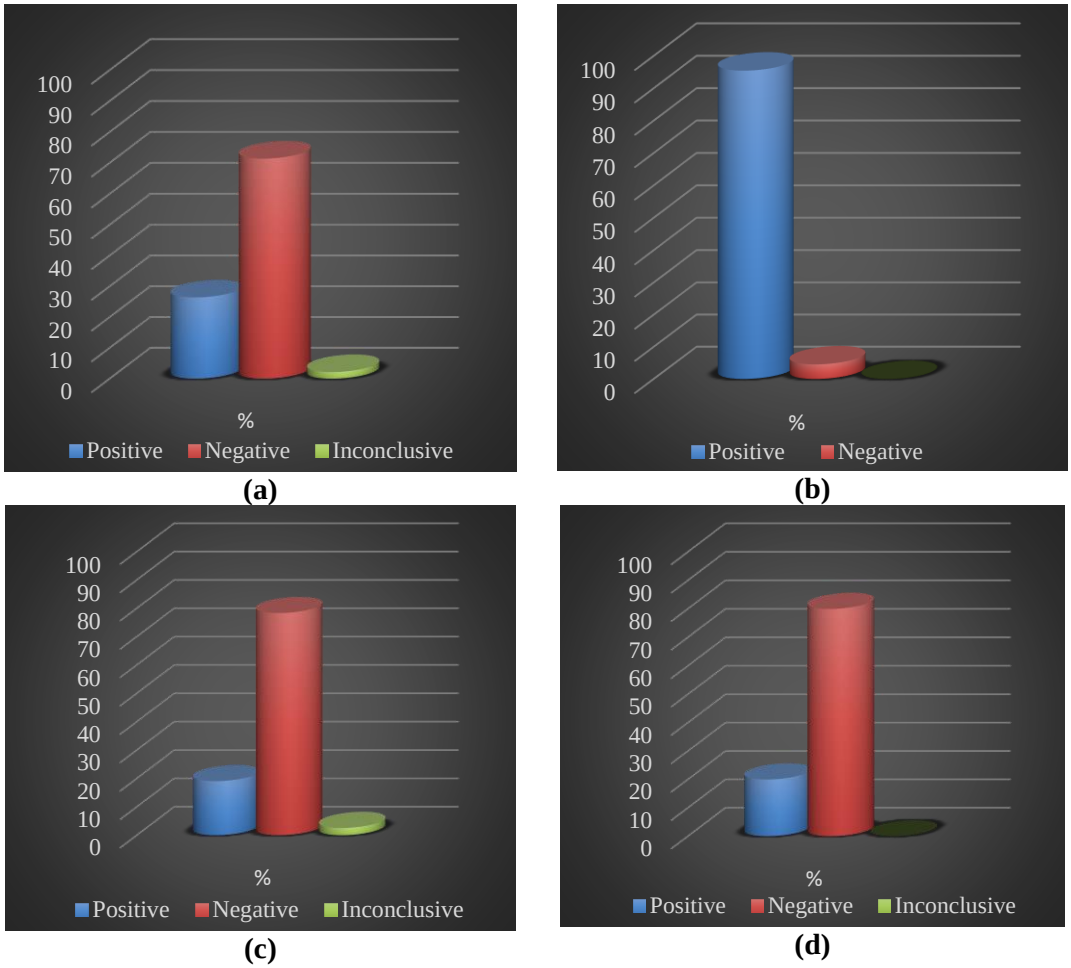


Fig. 5. Myxomatosis active sero-surveillance by iELISA, in areas with history of disease: (a) in all serum samples; (b) in rabbits previously vaccinated for myxomatosis; (c) in unvaccinated rabbits; (d) in rabbits with unknown history of vaccination.

Indirect ELISA revealed 26.41% (61/231) positive, 71.43% (165/231) negative and 2.16% (5/231) inconclusive results (recommendation for retesting). Serum samples of the vaccinated rabbits showed 95.45% (21/22) positive and 4.55% (1/22) negative results.

Serum samples of the unvaccinated rabbits revealed 19.07% (37/194) positive, 78.35% (152/194) negative and 2.58 (5/194) inconclusive results. Serum samples of the rabbits with unknown immunological status revealed 20.00% (3/15) positive and 80.00% (12/15) negative results.

As argued above in the case of competitive ELISA testing, one can tentatively ascribe inconclusive results to the long period of time of antibodies development or treat it as a consequence of the explosion of rabbits at low pathogenic virus strains. A similar situation can be observed when ELISA negative results are recorded in farms with myxomatosis history, since clinically recovered rabbits will provide negative ELISA results after 6-8 months (8).

During 2010-2014, Competitive ELISA assessment or Indirect ELISA assessment of blood serum samples taken from *Oryctolagus cuniculus*, from the exploitation units in which the passive surveillance was used, generated the following results: positive – 26.99% (105/389); negative – 70.44% (274/389), and inconclusive – 2.57% (10/389).

The serum samples taken from rabbits previously vaccinated and tested using ELISA came back with 93.55% (58/62) positive results, 6.45% (4/62) negative ones, and none considered inconclusive. The serum samples taken from unvaccinated rabbits and tested using ELISA came back with 16.48% (45/273) positive results, 80.22% (219/273) negative results, and 3.30% (9/273) inconclusive ones. The serum samples taken from rabbits with an unknown medical history considering vaccination and tested using ELISA came back with: 5.56% (3/54) positive results, 92.60% (50/54) negative results, and 1.84% (1/54) inconclusive ones.

Studies concerning serological surveillance of myxomatosis by means of ELISA testing have been carried out in Australia between 1980 and 1990. The studies were aimed at finding an alternative to the AGID method of diagnosis and at developing the serological surveillance of wild rabbits in Australia (4, 12). In addition, Kerr (1997) proved the efficiency of ELISA in the surveillance of myxomatosis in wild rabbits, and the sensitivity of the assay in the detection of *MYXV*-antibodies (4, 12).

Our data support the previous studies regarding the value of ELISA in serological surveillance of myxomatosis, mainly for *Oryctolagus cuniculus*, and recommend the utilization of this assay, along with passive surveillance, as part of the myxomatosis monitoring and surveillance program, mainly in endemic myxomatosis areas.

Conclusions

The passive surveillance of *Oryctolagus cuniculus* breeding units during 2010-2014 pinpointed the endemic trait of myxomatosis in this region; the oscillatory character of the annual prevalence, considering the number of affected animals as well as the number of outbreak points, with minimal values in 2011 and 2012, and its spectacular comeback in 2013 and 2013, highlights, once again, the risk of myxomatosis becoming a disease with an unxpected dynamics, given the epidemic evolution in the territories in which the number of receptive animals is high.

The positive results obtained from the unvaccinated rabbits suggests the circulation of a *MYXV* strain in the investigated areas, and, if this is the case, the introduction of an active surveillance plan for myxomatosis on the Romanian territory should be taken into consideration. The negative results obtained for 92.60% of the rabbits with an unknown medical history regarding vaccination suggest that these have not yet been exposed to the wild strain of the virus or introduced in an immunization program. The negative results obtained in the group of vaccinated rabbits could have different causes – varying from the maneuver to individual variability – and a new vaccination session is considered mandatory for these groups.

The serological surveillance of the *Oryctolagus cuniculus* population through ELISA techniques is considered an efficient control strategy for myxomatosis, an objective alternative to the classical method, (i.e.using passive surveillance), which proved inefficient for signalling the virus' circulation in endemic areas.

Acknowledgments

Part of the research was carried out by Marius DAN at Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Umbertini" (Italy). Special thanks to: Dr. Antonio Lavazza, Dr. Lorenzo Capucci, Dr. Patrizia Cavadini, Daniella Lavazza, Giuliana Botti, Cristina Palotta, Giovanni Bozzoni, Giuseppe Bertocchi, and Gabriela Brozbă.

References

1. Dan, M., Bărăităreanu, S., Daneş, D. 2013a. Mixomatoza. Etiologie, epidemiologie şi caracteristici clinico-lezionale. *Revista Română de Medicină Veterinară* 23 (3):11-26.
2. Dan, M., Bărăităreanu, S., Daneş, D. 2013b. Mixomatoza: diagnostic, profilaxie şi combatere. *Revista Română de Medicină Veterinară* 23 (4): 11-22.
3. Dan, M., Bărăităreanu, S., Daneş, D. 2014. Serosurveillance of Myxomatosis by Competitive ELISA. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine* 71 (1): 266-267. Available at: <http://journals.usamvcluj.ro/index.php/veterinary/article/view/10147>. [Accessed: July 2016].
4. Kerr, P. J., 1997 An ELISA for epidemiological studies of myxomatosis: persistence of antibodies to myxoma virus in European rabbits (*Oryctolagus cuniculus*). *Wildlife Research* 24 (1): 53-65.
5. Kerr, P. J., Best, S., M., 1998 Myxoma virus in rabbits. *Scientific and Technical Review OIE* 17 (1): 256-268.
6. Kerr, P. J., Merchant, J. C., Silvers, L., Hood, G. M., Robinson, A. J., 2003 Monitoring the spread of myxoma virus in rabbit Oryctolagus cuniculus populations on the southern tablelands of New South Wales, Australia. II. Selection of a strain of virus for release. *Epidemiology and Infection* 130 (1): 123-133.
7. McClure D., 2011 Viral Diseases of Rabbits. In: *Merck Veterinary Manual*. Merck Sharp & Dohme Corp., Whitehouse Station, N.J., U.S.A., http://www. merckmanuals.com/vet/exotic_and_laboratory_animals /rabbits/viral_diseases_of_rabbits.html?qt=myxomatosis&alt=sh, [Accessed: June 2016].
8. *OIE, 2008 Manual of Diagnostic Tests and Vaccines for Terrestrial Animal*. http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/ [Accessed: June 2016]
9. OIE, 2012a Myxomatosis. General Disease Information Sheets. http://www.oie.int/ fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/MYXO-EN.pdf [Accessed: June 2016].
10. OIE, 2012b Myxomatosis. General Disease Information Sheets. http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/MYXO-EN.pdf [Accessed: June 2016].
11. Saint, K. M., French, N., Kerr P., 2001 Genetic variation in Australian isolates of myxoma virus: an evolutionary and epidemiological study. *Archives of Virology* 146 (6): 1105-1123.
12. Wetherall, J. D., Clay, D. E., King, D. R., 1983 Humoral immunity to Myxoma virus in wild rabbits. *Australian Wildlife Research* 10 (2): 277-285.

THE ROLE OF NUTRIGENOMICS IN PROMOTING HEALTH BEHAVIOR

Silvia SOESCU, Violeta-Elena SIMION , Adriana AMFIM, Monica PARVU, Cristina ANDRONIE
Spiru Haret University,
Faculty of Veterinary Medicine,
256 Basarabia Ave., 30352 Bucharest, Romania,
e-mail: simion.violeta.elena@gmail.com

Abstract
It is well known as the diet regulates gene function and metabolism. Genetic differences play an important role in animal obesity (as the man otherwise) but the environment including diet play a major role in causing this disorder. The main objective of this review is investigating specialized literature to highlight how nutrigenomics influence pet obesity. In parallel with such studies, were also investigated several cases in cats with a tendency to obesity and some biochemical parameters were monitored. By dietary intervention was aimed to promote BW loss and formulated diets that can help to support the health of pets.
Keywords: *nutrigenomics, pets obesity, control food intake*

Introduction

The nutrigenomic approach in the control of some pathological affections through customized nutrition cannot be ignored in this day and age, whether we refer to human alimentation or that of pets and domestic animals. Nutrigenomics, a recent field of human medicine and a pioneering one in veterinary medicine, studies how food components affect gene expression and gene regulation; nutritional genetics is considered as the combination of nutrigenomics and nutrigenetics (German JB, 2005; Miggiano GA, 2006; De Busk et al., 2005; Hawkinson AK, 2007; Afman and Muller, 2006). It is not only the human genome that has been identified, genome which contains 20 000 – 35 000 genes, each with an average of around 3 proteins. The boxer Tasha was the first dog whose genome was sequenced and it should be highlighted that approx. 21 000 genes appear in the human genome as well, thus the two being similar in proportion of 90%. The genomic evolution at dogs and humans was parallel, especially the one for the genes associated with digestion and metabolism, neurologic processes and diseases such as cancer (Wang et al, 2013). Apparently without any connection, an entire series of diseases such as obesity, gastrointestinal affections, autoimmune diseases, heart affections, dermatitis, cancer, behavioral issues have something in common – at cellular level they result in inflammation, as a defense mechanism of the body in response to stress factors, including food.

Obesity is, at present, considered as one of the main threats for the health of pets, specifically cats and dogs. Unfortunately, its incidence is on the rise, with more than 50% of the cats and dogs being affected, as reported by the Association for Pet Obesity Prevention (APOP). Obesity is the most common nutrition-related disorder and is the core element of a group of metabolic syndromes (Ukkola and Bouchard, 2001). The causes are diverse and are related to several factors – age, sex, physiologic state, genetic predisposition, hormonal affections, lesions of the hypothalamus and external factors: level of activity, alimentation, behavior. In a previous study on both cats and dogs, a correlation was highlighted between the corporal status and the quantity of plasmatic leptine. It demonstrated the existence of a positive relationship between the quantity of fat tissue and the concentration of plasmatic leptine. Other previous studies have highlighted as well that an increase in the concentration of circulating leptine is correlated with the quantity of fat tissue, as well as an increase of the corporal score (Body-Mass Index?) at both cats and dogs (Appleton et al., 2000; Backus et al., 2000; Hoening

and Ferguson, 2002; Ishioka et al., 2002; Jeusette et al, 2005; Martin et al, 2006); by contrast, the reduction in fat tissue is strongly correlated with the reduction of the concentration of leptine at both species (Jeusette et al, 2005; Hoenig et al., 2007). At both species, the concentration of leptine increased considerably after the ingestion of rations with high lipidic or energetic value. Subsequently, we extended these results by further determining the level of apolipoproteins, in particular ApoB, which is important for maintaining the structural integrity and solvency of lipoproteins and with an important role in recognizing the lipoproteic receptors and regulating certain enzymes from the lipoproteic metabolism. At present we know six major classes of apolipoproteins and several sub-classes. The synthesis of apolipoproteins in the intestines is regulated, primarily, by the diet's fats content. Apolipoprotein B (ApoB) is the primary apopoliprotein from chilomicrons and LDL (low-density lipoprotein) and increased levels of this marker appear in disorders of the lipidic metabolism which entail excessive deposits of lipids (obesity, atherosclerosis, heart affections etc.). In the laboratory, ApoB concentration provides a good indicator of the number of particles in plasma of VLDL, LDL or IDL (intermediate-density lipoprotein). Moreover, the ratio of ApoB/ApoA1 is considered a good marker in estimating imbalances in the lipidic metabolism, indicating the evolution for aggravation of amelioration as a result of measures taken (therapeutic, dietetic) and the need for nutritive intervention for maintaining the animal's health state.

Material and method

The biochemical analyses were done on Analyser of dry biochemistry ARKRAY Spotchem EZ-SP-4430. The biochemical analysis of urine was done on Analyser with strips from Vielab U-11V West Medica. Apolipoprotein B was determined through the measurement technique using imunoturbidimetric kits (334nm, 340nm, 365nm).

Results and discussion

Clinical case No.1 was a European feline, female, 8 years old, sterilized at 1.5 years old, with 5.5 kg weight and after the first heat cycle. When presented, she had a very good general state. The cat does not have access outside (lives in an apartment), but occasionally enters in contact with other animals (dogs). It was chaotically fed throughout the first 3 years and was subjected to moderate stress factors (isolation, neglect), after which it was subjected to a strict diet for castrated cats. Without significant pathology up to now, but with occasional behavioral disorders (agressivity, sometimes isolation – hiding). Apolipoprotein B (principal component of LDL) was determined in 2016 and registered a value of 1.15, within the reference limit of 0.60-1.17 (Figure 1).

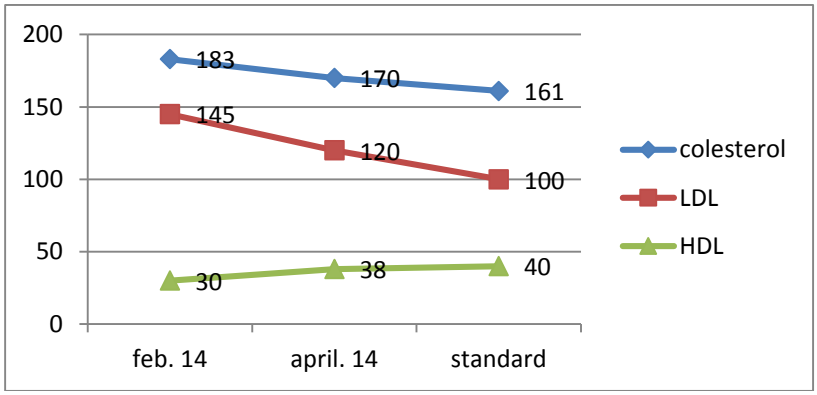


Figure 1. Comparative values Cholesterol, LDL, HDL; clinical case no.1

Other biochemical analyses done revealed as well a significant decrease of the values of the analyzed parameters, for the reference interval 2014-2016: thus, AST (UI/L) from 30 to 19 (reference limits 9.2-39.5); ALT (UI/L) from 60 to 38 (reference limits 8.3 to 52.5); triglycerides (mmol/dL) from 1.3 to 1.25 (reference limits 0.6 to 1.2). Alongside cholesterol and lipoproteins, triglycerides evaluate the risk of cardiovascular disease and have high values in hyperlipoproteinemy. A slight increase within normal limits of the values was registered for albumin (g/dL), from 2.9 to 3.1 (reference limits 2.4-3.7).

Recommendations involved the continuation of the diet for sterilized cats, taking into account that there are stress factors that can affect the endocrine metabolism and can subsequently determine disorders of the lipidic metabolism.

Clinical case No.2 was a European feline, female, 6 years old, sterilized at 3 years old, with 6.5 kg weight, with very good general health state, with unknown medical history up to the age of 2, as it was found and adopted at the age of 2.5 years old. The cat does not have access outside (lives in an apartment with two other cats), but occasionally has contact with other animals (cats and dogs). Since it was adopted and sterilized, it was fed only with a diet for sterilized cats and occasionally fish. Without medical history up to now. Apolipoprotein B (principal component of LDL) was determined in 2016 and resgistered a value of 1.25, which is above the reference limits of 0.60-1.17 (Figure 2).

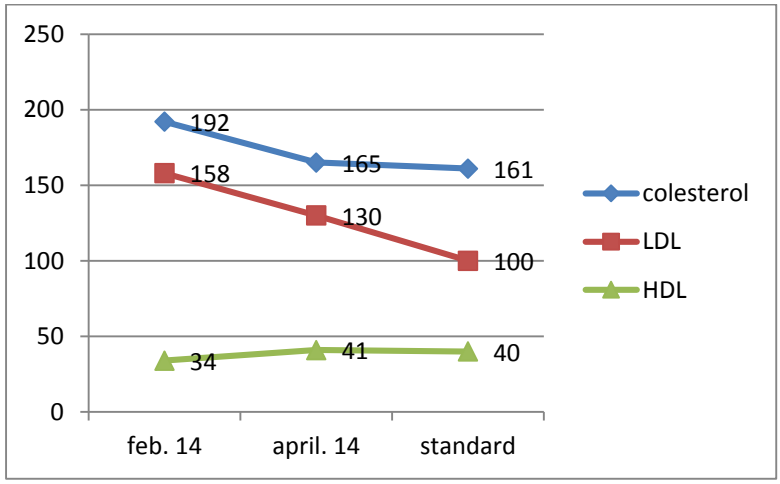


Figure 2. Comparative values Cholesterol, LDL, HDL; clinical case no.2

As in the previous case, the results obtained from other complimentary biochemical analyses revealed a significant decrease of the values of the analyzed parameters for the reference interval of 2014-2016. Thus, AST (UI/L) from 18 to 12 (reference limits of 9.2-39.5); ALT (UI/L) from 65 to 50 (reference limits 8.3-52.5); triglycerides (mmol/dL) from 1.5 to 1.2 (reference limits 0.6-1.2). A slight increase within normal limits of the values was registered for albumin (g/dL), from 3.2 to 3.4 (reference limits 2.4-3.7).

The moderately high value of ApoB indicates the risk for disorders of the lipid metabolism, conclusion supported as well by the increased value of LDL, cholesterol and ALT, as well as the low value of HDL. Because the value of ApoB in the blood is correlated with the presence of genetic modifications, it is possible that the genetic markers for ApoB (ApoB gene) are also modified. The high quality alimentation undoubtedly helped the evident amelioration

of the researched parameters, but the risk of obesity is still present (continuing the diet specific for castrated cats is obligatory).

Clinical Case No.3 is also a European feline, female, 9 years old, sterilized at 5 years old, having 4.5 kg in weight. It does not have access outside, but lives together with 2 cats and a small size dog since 2 years. Since the dog was adopted health problems started to appear (generalized loss of hair, diarrhea, obesity). Before castration the cat was fed with industrial dry and humid food from different brands, while after castration it didn't accept dry food and the owner offered her humid and cooked food at discretion without resulting in any drastic changes in weight. Two years back (August 2014) the dog was adopted and the metabolic changes started to appear: 3.5 kg before castration, with a doubling in weight afterwards reaching a maximum of 9 kg.; the diet was changed as a result by dropping the humid industrial food – resulting in weight loss over the next 6 months and thus reaching 4.5 kg, weight level that remained constant since.

Apolipoprotein B (principal component of LDL) was determined in 2016 and had a value of 1.24, over the reference limit (Figure 2).

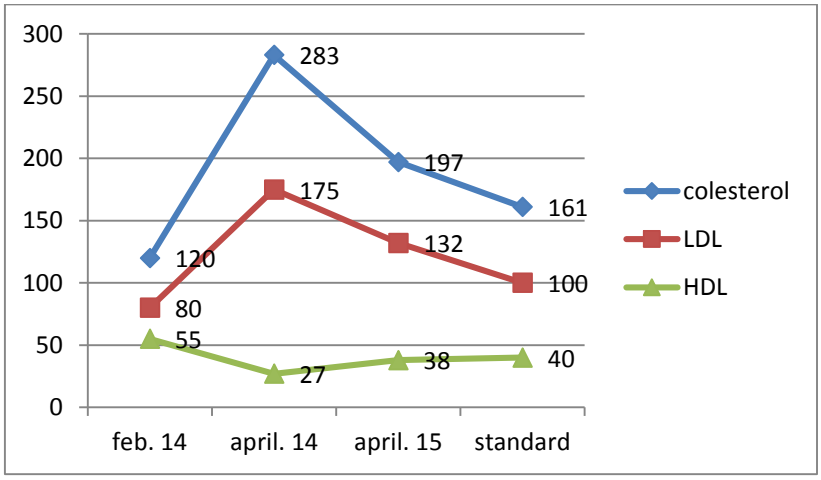


Figure 3. Comparative values Cholesterol, LDL, HDL; clinical case no.3

In this case the results obtained from other complimentary biochemical analysis have been monitored annually, throughout the interval 2014-2016, and showed: AST (UI/L) reached 50.5 in 2015 from 28 and then dropped to 31 in 2016 (reference limits 9.2-39.5); ALT (UI/L) from 29 in 2014 reached 71 in 2015 and then dropped to 49 in 2016 (reference limits 8.3-52.5); triglycerides (mmol/dL) from 0.8 in 2014 reached 1.7 in 2015 and then decreased to 1.1 in 2016 (reference limits 0.6-1.2). A slight increase within normal limits of the values was registered for albumin (g/dL), from 2.8 to 3.1 in 2016 (reference limits 2.4-3.7).

It can be observed that there were significant modifications of the biochemical parameters in the context of acute stress suffered by the animal: increase in weight, depression, other metabolic disorders. The ApoB value is not markedly high, although there is a moderate risk of obesity. The existence of stress and a non-corresponding diet made that this risk became reality. The metabolic diet allowed for the reduction towards normal physiologic values of the important parameters monitored, especially AST, ALT, total Cholesterol, LDL and HDL. The choice of a corresponding diet had an evident influence in this case, because of the additional existence of a predisposition towards obesity (possibly the positive ApoB gene), as well as the

apparition of stress factors that started evident metabolic disorders. Furthermore, there was also an extremely favorable response (clinical and biochemical) after the changing of the diet and the adoption of a metabolic diet.

Conclusions

Genetic, alimentary and behavioral factors surely contribute to the appearance and maintenance of obesity. Nutrigenetics and customized nutrition can offer to every particular individual advice/information regarding the diet, corresponding with thegenetic profile and behavioral factors that influence its activity.

The analysis of apolipoproteins can constitute a set of investigations necessary for the prevention of certain afflictions (obesity, atherosclerosis, diabetes etc.), for individuals with genetic predisposition for these pathologies.

Biliography

1. Afman L., Muller M. Nutrigenimics: from molecular nutrition to prevention of disease. *J Am Diet Assoc* 2006; 106(4):569-76.
2. Appleton DJ, Rand JS, Sunvold GD. Plasma leptin concentration in cats: Reference range, effect of weight gain and relantionship with adiposity as measured by dual energy x-ray absorptiometry. *J Feline Med Surg* 2000; 2:191-9.
3. Backus RC, Havel PJ, Gingerich RL, et al. Relantionship between serum leptin immunoreactivity and body fat mass as estimated by use of a novel gas-phase Fourier transform infrared spectroscopy deuterium dilution method in cats. *Am J Vet Res* 2000; 61:796-801
4. German JB. Genetic dietetic: nutrigenomics and the future of dietetic practice. *J. Am Diet Assoc* 2005:530-1.
5. DeBusk RM, Fogarty CP, Ordovas JM, Kornman KS. Nutritional genomics in practice: where do we begin? *J Am Diet Assoc* 2005; 105:589-98.
6. Hawkinson AK. Nutrigenomics and nutrigenetics in whole food nutritional medicine. Townsend letters for doctors and patients, Feb-March, 2007.
7. Hoenig M., Caffail Z, Ferguson DC. Triiodothyronine differentially regulates key metabolic factors in lean and obese cats. *Domest Anim Endocrinol* 2007; 34:229-37.
8. Hoenig M, Ferguson DC. Effects of neutering on hormonal concentrations and energy requirements in male and female cats. *Am J Vet Res* 2002; 63:634-9.
9. Ishioka K, Soliman MM, Sagawa M et al. Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 2002a; 26:1407-33.
10. Jeusette IC, Lhoest ET, Istasse LP et al. Influenceof obesity on plasma lipid and lipoprotein concentrations in dogs. *Am J Vet Res* 2005; 66:81-6.
11. Martin L, Siliart B, Dumon H , et al. Leptin, body fat content and energy expenditure in intact and gonadectomized adult cats: a preliminary study. *J Anim Physiol Anim Nutr* 2001; 85:195-9.
12. Miggiano GA, De Sanctis. Nutritional genomics: toward a personalized diet US National Library of Medicine, National Institute of Health. *Clin Ter* 2006, 157(4):355-61.
13. Ukkola O., Bouchard C. Clustering of metabolic abnormalities in obese individuals: the role of genetic factors. *Ann.Med* 2001; 33:79-90.
14. Wang G., Zhai W., Yang H., Fan R., Cao X., Zhong I., Wang L., et al (2013). The genomics of selection in dogs and the parallel evolution between dogs and humans. *Nature Communications*. Doi:10.1038/ncomms2814.

ARSUTRAT-PLAGOTRAT, NATURAL VETERINARY PRODUCT FOR CARE OF WOUNDS AND BURNS OF DIFFERENT CAUSES

Stefan MANEA[1], Viorica TAMAŞ[1], Emilia BALINT[2], Viorica CARABELA[1], Andreea DIMA[1]
e-mail: cercetare@hofigal.eu

Abstract

*The paper presents the research and the experimental results for a new phytotherapeutic product,**Arsutrat-Plagotrat** gel for veterinary use, designed for the care of burns and wounds of different causes, and also of some forms of dermatitis. As composition, the product is a balanced combination of natural compounds from indigenous medicinal and aromatic plants, namely sea buckthorn and lavender oils, concentrated extracts of Marigold and St. John's wort, and collagen hydrolysate; the product provides direct biocompatible necessary nutrients that increase local immunity, reconstruction and regeneration capacities of affected tissue. This paper focuses on two directions. Firstly, the entire used raw material was investigated for the main active compounds; the used vegetal materials are of organic provenience, form the cultures of HOFIGAL Company and processing is done in GMP conditions. On the other hand, 'Arsutrat-Plagotrat gel' was investigated for its action and proved encouraging experimental results when emplyed in the cases of 20 subjects (10 dogs and 10 cats) suffering of postoperative wounds, wounds with a high portion of dermal tissue missing, due to bites or entrapment or chemical burns, pyodermatitis or systemic lupus erythematous. The clinical trial and the most representative cases are described in this paper.*

***Key words**: burns, medicinal plants, phytotherapy, wounds*

Introduction

At the moment, not many veterinary products have been developed for the treatment of skin affections by natural, phythotherapic remedies and little information is available on the subject.

This paper presents the studies and the obtained results for a new phytotherapic product with topical use, Arsutrat-Plagotrat gel, designed for the treatment of burns and wounds of various causes (thermal, chemical, solar burns), cuts, bruises and also in the treatment of certain forms of dermatitis.

The study presented in this paper was conducted in two dirrections. Firstly, a detailed characterisation of the raw materials needed for the formulation is presented. Secondly, the product was submitted to a clinical trial involving feline and canine subjects, the procedure and the obtained results being disscussed.

Materials and methods

All used raw materials involved in the pharmacological effects of the Arsutrat-Plagotrat product were individually analyzed in terms of the major phytochemical components. Their chemical composition was largely discussed in literature, as well as their beneffits on damaged skin, but the actual association was never done for the stated purpose (Giurgiu E., Giurgiu O.C., 2012).

The vegetal materials come from plants cultivated without chemical treatments and their processing to finished product was made under GMP conditions (Muntean L.S., 1996). This include sea buckthorn, from which oil was extracted (obtained by cold pressing of sea buckthorn fruits), St. John's Wort and Marigold, from which extracts were obtained by maceration and concentration and lavender from which only the essential oil was used after hydrodistillation (European Pharmacopaea, 2005). Hydrolyzed collagen was purchased from Provital, Spain.

The gel was obtained by the combination of mentioned natural ingredients and collagen with specific formulation agents and the product is further reffered as **Arsutrat-Plagotrat gel.**

Destined for external administration for small animal companions (cats and dogs), clinical tests were driven in order to prove its efficiency. Those were conducted within the Faculty of Veterinary Medicine's clinic in Bucharest, under the guidance of physicians from different specialty clinics (Surgery Clinic, Medical Clinic, Obstetrics and Gynecology Clinic).

For each studied animal, a file has been created, which contains:

- The owner's accord to administer the gel product to their animal, following the attending physician's indications, for the entire treatment period.

- The animal's observation paper, accompanied by photographs of lesions from the beginning of the treatment, based on diagnosis, and from the end of the treatment.

- There were also noted the: administered doses, time of administration, eventual adverse reactions and other observations made by owners during the treatment. (Pop P., Cristina R.T., 1995).

The Arsutrat-Plagotrat gel has been used in surgical, accidental, decubital lesions, lesions auto-induced by grating, licking, puncturing or biting, over-infected lesions, dermatitis of different etiologies (for example, the eosinophilic plaque) , burns produced by fire, water and hot vapors, or by the action of caustic chemical substances.

In what the method of administration is concerned, the affected area has been sanitized by trimming the fur and washing the area with ordinary anti-septic substances. The gel was applied by light massage until it entered the skin, after which the area was protected by applying a sterile bandage (depending on case). It has been applied 2-3 times a day, for 5-7 days, or until the healing process was complete.

Results and discussion

Concerning the composition, the product is a well balanced combination between of plant extracts, with known favorable properties in dermatological practice and hydrolized collagen, also recognised for its broad therapeutic properties.

Sea buckthorn (*Hippophae rhamnoides)* contains: flavonoides (Quercitin, Isorhamnetin-3-Beta-D-glucosides); triterpens; sterols (sitosterols); vitamin C; carotenoids (α-βcarotene, criptoxantina, licopina, zeaxantina); vitamins: B1, B2, D, E, PP, P; proantocianides; lipids (glicerides of palmitic, linoleic and linolenic acids, minerales: Fe, Se, Zn, K, Na, Ca, Mg, Cu, Mn; etc. It has antioxidant; regenerating; cicatrizing antiinflamatory; tonifying and vitaminizing properties

Sea buckthorn oil has in its composition, aside from free and esterified essential fatty acids, complex structures like lipo- and glyco-proteins, lecithin, enzymes, carotenoids, phytosterols, vitamins and microelements (Brad I., 2002).

St. John's Wort extract *(Hypericum perforatum)* ***c***ontains, besides hyperin, hypericin and isomers (with regeneration properties), an important phytochemical complex (carotenes, flavones, vitamins - C, B, PP, saponins, sesquiterpenes, minerals, etc.) that improves local blood circulation by nourishing and relaxing the smooth muscle in blood vessel walls and helps the transport of essential nutrients that restore and release toxic metabolites of degradation.

Marigold extract (*Calendula officinalis*) has cortisone-like anti-inflammatory properties, given by the triterpene saponosides (ursolic acid, oleanolic acid etc.) and soothing, healing and antibacterial properties given by the terpenoids from essential oils. The whole complex of molecular species are working together to stimulate the restoration of normal dermal tissue (Pârvu C., 2000).

Lavender essential oil (*Lavandula angustifolia) contains* linalil acetate, geraniol, borneol, terpineol, izogeraniol, amilic alcohol, izoamilic alcohol, etc. and it is used for its calming, antimicrobial and healing properties. (Bojor O., 2003).

Hydrolysed collagen accelerates the regeneration of tissues enabling a wide variety of amino acids and short peptides to damaged skin and encreasing its elasticity.

The rich composition in natural components and the synergic activity of chemical constituents stipulate decongestive, anti-inflammatory, scarring, analgesic and regenerative properties useful for the affected cutaneous tissue.

Concerning the clinical trial, the lot of subjects rised to 33 animal patients of which 18 canine species and 15 belonging to feline species, which presented various types of skin ailments and possibly of tissue underlying the areas of interest.

Some of the cases are presented, with indications upon the most conludent differentiation criterias (breed, sex, age, diagnostic, treatment information, healing time) and the picure of the wound.

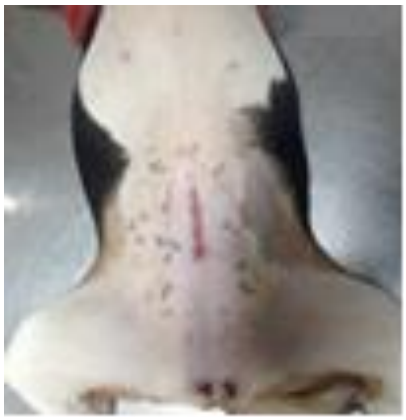


Fig.1 Canine, Beagle, female, 1 year; post-surgery neuter wound;
local treatment with Arsutrat-Plagotrat gel, 2 times/day after asepsis of the area;
healing after 2 weeks

Fig. 2 Feline European, male, 8 years; skin lesions on dorsal region;
local treatment with Arsutrat-Plagotrat gel, 2 times/day after asepsis of the area;
healing after 2 weeks

Fig. 3 Canine, Metis, male, 1 year 6 months; post-surgery wound after front-left limb amputation; local treatment with Arsutrat-Plagotrat gel, 2 times/day asepsis of the area, healing after 2 weeks

Fig. 4 Canine, Caucasian shepherd, male, 10 years; posterior limbs over-infected moist dermatitis; local treatment with Arsutrat-Plagotrat gel, 2 times/day asepsis of the area; healing after 2 months

Fig. 5 Canine, York Shire, female, 1 year 6 months; vasculitis; local treatment with Arsutrat-Plagotrat gel, 2 times/day asepsis of the area; healing after 1 month

Fig. 6 Canine, Metis, male, 11 years; chronic over-infected proliferative dermatitis; local treatment with Arsutrat-Plagotrat gel, 2 times/day asepsis of the area; healing after 1 month

During the treatment with Arsutrat-Plagotrat-gel product, in or without association with other products/solutions that favorise the asepsis of the area, it was proved that, because of the rich composition in natural components with synergic activity, it offered decongestive, anti-inflammatory, scarring, analgesic and regenerative properties to the affected cutaneous tissue.

The product, in the pharmaceutical form of a gel, it is easy to apply locally by spreading it with a sterile spatula after cleaning and disinfecting the wound.

In contact with the tegument, the product quickly enters the skin, acting upon the peripheral sanguine torrent, enhancing active principles by substance exchange on the tissue's level, contributing to the regeneration of the affected cutaneous tissue. The hydro- and lipo-soluble composition and by the small volume molecules (from essential oil, fatty oils, plant extracts, etc.) allow the crossing through cell membranes and the interaction with cellular receptors.

The pH of the product ranges between 5,5 and 6,5, similar to the normal pH of skin.

All patients received complete healing with good and very good results. Although most injuries were due to surgical incisions that were sutured, in which case, the Arsutrat-Plagotrat gel acted beneficial in terms of healing speed and final appearance of the scar (fine, discrete, inhibiting the reactions of a foreign body to the wire suture), the use of Arsutrat-Plagotrat gel has proved to be extremely beneficial to allergic or autoimmune dermatological diseases (side healings using Arsutrat-Plagotrat gel being superior in terms of the final appearance of the wound) or even oncologic, where besides cutaneous wound healing, delimitation of tumor formation increased, which is extremely useful for subsequent oncological surgery.

Conclusions

1. A new phytotherapic product with topical use, Arsutrat-Plagotrat gel, was developed.
2. The vegetal material's chemical composition used in the formulation of the product (sea buckthorn oil- obtained by cold pressing; concentrated extracts of St. John's Wort and Marigold; lavender essential oil) indicates a large number of structures with healing, anti-inflammatory and soothing properties; the hydrolyzed collagen contributes to the regeneration of affected tissue.
3. The product, in the pharmaceutical form of a gel, is easy to apply locally and it shows very good skin absorption;

4. Destined for external administration for small animal companions (cats and dogs),a clinical trial was conducted on a lot of 33 animals, for each, an observation file being created. The most suggestive cases are presented in the paper.
5. Driven clinical tests proved that the product has a very high efficiency; moreover, healing speed is enhanced and final appearance of the wounds is fne and discrete.

Bibliography

1. Bojor O., 2003 - , Ghidul plantelor medicinale și aromatice de la A la Z, Editura Fiat Lux, București.
2. Brad I., 2002 - , Cătina albă : O farmacie într-o plantă, Editura Tehnică, București.
3. European Pharmacopaea, 2005 - European Pharmacopaea 5th Ed., Strasbourg 2136-2137 Conncil of Europa
4. Giurgiu E., Giurgiu O.C., 2012 - Plantele medicinale importante in tratamentele naturiste, Ediția a II-a, ilustrată și adăugită – p. 187, 324, 1215, Bucuresti
5. Muntean L.S., 1996 - Cultura plantelor medicinale și aromatice, Editura Dacia, Cluj-Napoca.
6. Pârvu C., 2000 - Universul plantelor – Mică enciclopedie, ediția III, Editura Enciclopedică, București, p. 251,109, 328, 643.
7. Pop P., Cristina R.T., 1995 – Dermatologie medicala veterinară, Ed. Mirton, Timisoara.

THE USE OF PCR FOR THE DETECTION OF PRRS VIRUS VARIANTS

Larion FLUERAȘU[1], Virgilia POPA[2], Nicolae CATANA[1]
1- Faculty of Veterinary Medicine Timişoara, Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania" from Timișoara, Timisoara, Romania
fluerasu_larion@yahoo.com
2- SN Institutul Pasteur SA București

Abstract
In Romania the disease was first diagnosed in 1998 and nowadays the disease is spread in many swine farms (4). The etiologic agent is an ribovirus, initially called Lelystadt virus. Subsequent research has shown that this virus has two genotypes respectively, Type 1 (European) and type 2 (American) with an considerable variability of gene sequences, with an similarity of only 50-60%. For detection of this virus have been taken lymph nodes with pathological lesions, macroscopic characteristic forPRRS syndrome, from cadavers of youth porcine, from farms where this disease evolves. Finally were intended to RT-PCR technique, an number of 12 lymph nodes. By Real Time RT-PCR version, PRRS virus was detected in only one sample (0,08%) of the 12 samples examined. By the techniques recommended by GILBERT the virus was detected in all samples (100%) and by Nested RT-PCR techniques the virus was detected in five samples (41,66%). All positive samples provided by these techniques have demonstrated that the strains belong to type 1 (European).By using the technique recommended by TRUYEN, for the detection of type 2 (US) were not detected strains belonging to this type, all samples being negative
Key words: PRRS, lymphnode, RT-PCR, virus type 1

In 1987 in the USA and Canada, it has been reported a disease at pigs with viral etiology, which has expanded rapidly, starting at the end of 1990, in Germany and Netherlands, and later in other European countries. OIE recommended in 1992, that the name of the disease is "Porcine reproductive and respiratory syndromes - PRRS" (4, 8) .

In Romania the disease was first diagnosed in 1998 and nowadays the disease is spread in many swine farms (4).

The etiologic agent is an ribovirus, initially called Lelystadt virus. Subsequent research has shown that this virus has two genotypes respectively, Type 1 (European) and type 2 (American) with an considerable variability of gene sequences, with an similarity of only 50-60% (8).

Considering the fact that both types have a global distribution in populations of pigs reared in intensive system, research followed the detection of the type of virus responsible for outbreaks of disease development in young swine fattening farms.

Materials and methods

PRRS virus can be detected in pathological samples with the aid Polymerase Chain Reaction with Reverse Transcriptase (RT-PCR), in different variants, using two separate kits for each type of virus.

For detection of this virus have been taken lymph nodes with pathological lesions, macroscopic characteristic forPRRS syndrome, from cadavers of youth porcine, from farms where this disease evolves. Finally were intended to RT-PCR technique, an number of 12 lymph nodes (Table 1).

The viral genome was detected by Standard Operational Procedure of RT-PCR, Real Time version used in molecular biology laboratory of SN Institute Pasteur SA, Bucharest. For this purpose, were used four extraction kits (Qiagen and Roche, Germany) and 2 primers specific to ORF 7 area:

- Primer PRRS -2 ORF 7: 5' – GCG AAT CAG GCGCAC WGT ATG - 3' ;
- Primer PRRS-4 ORF 7: 5' – AGA AAA GTA CAG CTC CGA TGG - 3';

After completion of electrophoresis the gel was analyzed by the video documentation system, Gel Doc. Specific bands obtained by amplifying the viral genome have the size of 400 bp.

For the classification of the strains in one of two virus types were used different technique of RT –PCR as following:

- rRT-PCR PRRS ORF7 MARDASSI (1step) (3)
- rRT-PCR PRRS ORF7-(EU) TRUYEN (1step) (5)
- rRT-PCR PRRS ORF7-(NA) TRUYEN (1step)(5)
- RT + rPCR PRRS ORF1B-(EU) GILBERT (2steps)(2)
- RT + rPCR PRRS ORF1B-comun GILBERT (2steps)(2)
- Nested PCR: RT-PCR PRRS ORF7 MARDASSI + rPCR PRRS ORF7-(EU) TRUYEN (3, 5).

Results and discussion

RT-PCR technique was used for the detection of PRRS virus in pathological materials and in cell culture. Because this virus has two types (European and American), this technique has been used to characterize and differentiate the isolated strains in several variants.

The obtained results after conducting my own research concerning the detection of PRRS virus in lymph nodes, are shown in Table 1.

Table 1

Results provided by RT-PCR

Crt. No	Sample type	Sampling date	Age (Weeks)	Result
1	Lymph node	15.11.2013	6	-
2	Lymph node	15.11.2013	6	-
3	Lymph node	15.11.2013	6	-
4	Lymph node	22.11.2013	6	-
5	Lymph node	22.11.2013	6	-
6	Lymph node	12.12.2013	6	-
7	Lymph node	24.01.2014	6	-
8	Lymph node	24.01.2014	6	-
9	Lymph node	24.01.2014	8	+
10	Lymph node	13.03.2014	8	-
11	Lymph node	13.03.2014	8	-
12	Lymph node	21.01.2015	8	-

By Real Time RT-PCR version, PRRS virus was detected in only one sample (0,08%) of the 12 samples examined.

Given that the virus strains isolated from outbreaks can belongs either to type 1 or type 2, most authors recommend two or three versions of RT PCR technique (1, 3, 5).

To complete the obtained results, the lymph nodes samples were examined through other 5 different variants of work mentioned above, recommended by GILBERT, MARDASSI and TRUYEN (2,3,5).

The results from these variants are shown in Table 2.

Analysis of these results, demonstrate that there are differences between differents work variants, this appearance being confirmed by the literature. By the techniques recommended by GILBERT the virus was detected in all samples (100%) and by Nested RT-PCR techniques the virus was detected in five samples (41,66%). All positive samples provided by these techniques have demonstrated that the strains belong to type 1 (European).

By using the technique recommended by TRUYEN, for the detection of type 2 (US) were not detected strains belonging to this type, all samples being negative. These results confirm that the lymph samples, taken from the corpses of youth swine from several farms, were present only one type of strains, belonging to type 1 (European).

The obtained results are similar with the existing data from the literature in our country or foreign countries, concerning the phylogenetic origin of strains of PRRS virus.

In Romania, ZAULEȚ MIHAELA et al., conducted a lot of studies and by similar variants of RT PCR technique, studied strains isolated from different outbreaks of PRRS. The authors also identified an attenuated vaccine strain, and a virulent strain was similar with the EU071264 (6, 7).

Table 2

Results provided by different variants of RT-PCR

rRT-PCR PRRS orf7-EU Truyen (1step)			rRT-PCR PRRS orf7-NA Truyen (1step)			RT + rPCR PRRS orf1b-EU Gilbert (2steps)			RT + rPCR PRRS orf1b-comun Gilbert (2steps)			nested PCR: RT-PCR PRRS orf7 Mardassi + rPCR PRRS orf7-EU Truyen		
Ct	Tm	240bp	Ct	Tm	337bp	Ct	Tm	186bp	Ct	Tm	255bp	Ct	Tm	240bp
/	/	/	/	/	/	21,88	83,35	+	22,17	80,10	+	NoCt	85,50	d
/	/	/	/	/	/	21,94	83,35	+	22,44	83,33	+	NoCt	83,85	d
/	/	/	/	/	/	20,70	83,35	+	21,13	83,33	+	NoCt	85,50	-
/	/	/	/	/	/	20,50	83,35	+	20,53	83,33	+	NoCt	84,40	-
/	/	/	/	/	/	21,54	83,35	+	21,43	83,33	+	NoCt	84,95	-
/	/	/	/	/	/	23,21	83,35	+	22,83	80,10	+	12,90	85,50	+
/	/	/	/	/	/	21,99	83,35	+	21,74	83,33	+	13,98	85,50	+
/	/	/	/	/	/	21,51	83,35	+	21,03	83,33	+	14,36	85,50	+
25,37	83,35	+	26,69	81,30	N	21,35	83,35	+	21,56	83,33	+	8,20	84,90	+
/	/	/	/	/	/	22,19	83,35	+	22,40	80,10	+	NoCt	83,33	-
/	/	/	/	/	/	22,95	83,35	+	22,88	83,33	+	NoCt	83,90	-
/	/	/	/	/	/	23,26	83,35	+	22,97	83,30	+	20,51	84,95	+
22,65	82,85	+	27,28	81,30	N	26,20	83,35	+	24,74	83,33	+	11,74	85,05	+
NoCt	72,25	-	NoCt	72,25	-	29,43	72,25	-	35,49	74,80	-	NoCt	90,85	-

Conclusions

By the Real Time RT-PCR technique PRRS virus was detected only in one sample.

By Nested PCR technique PRRS virus was detected in 5 samples.

The strains detected were classified only in Type 1 (European).

The samples intended for examination by these techniques have been negative for the type 2 (US).

Bibliography

1. Dee, S. A., Philips R. E. (1999) - *Use of polymease chain reaction to detect vertical transmission of PRRS virus in piglets from gilt litters,* Swine Healt Prod, 7, 237-239.
2. Gilbert,S., A., Larochelle, R., Magar, R., Cho, H., J., Deregt D. (1997) - Typing of Porcine Reproductive and Respiratory Syndrome Viruses by a Multiplex PCR Assay, JOURNAL OF CLINICAL MICROBIOLOGY, p. 264–267
3. Mardassi, H., S. Mounir, S. Dea, (1994): Identification of major differences in the nucleocapsid genes of a Quebec strain and European strains of porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 75, 681–685.
4. Stănuică, D., (2005) - *Sindromul de Reproducţie şi Respirator Porcin*, Lucrare realizată în cadrul Proiectului „Sprijinirea Serviciilor din Agricultură".
5. Truyen, U., Wilhelm S., Genzow, M., Schagemann G., (2006) - Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): A Ring Test Performed in Germany to Assess RT-PCR Detection Methods, J. Vet. Med. B 53,p. 68–74
6. Ză uleț Mihaela, Iuliana Nicolae, Toana Andreea, Baraitareanu S., Otelea Maria Rodica, Stadejek T., (2011) - *Genetic characterization of Romanian Porcine Reproductive and Respiratory Syndrome Viruses based on ORF7 nucleoprotein gene Sequences*, Romanian Biotechnological Letters Vol. 16, No. 6, 2011.
7. Ză uleț Mihaela, Gurau Maria Rodica, Petrovan V., Buburuzan Laura (2012) - *Genetic Diversity Characterization of Porcine Reproductive and Respiratory Syndrome Virus Isolates in Romania, Based on Phylogenetic Analysis, Int. J. Mol. Sci.*, *13*, 12046-12061; doi:10.3390/ijms130912046.
8. Zimmerman, J. J., Benfield, D. A., Scott, A. D., Murtaugh, M. P. Stadejek, T., Stevenson, W. G., Torremorell M. (2012) – *Porcine reproductive and respiratory syndrome virus (Porcine arterivirus) in Disease of swine* edited by Zimmerman J.J. 10 th edition, Wiley-Blackwell.

CORRELATIONS BETWEEN SOME PHYSICOCHEMICAL PARAMETERS, ELECTRICAL CONDUCTIVITY AND SOMATIC CELLS COUNT IN SUBCLINICAL MASTITIS IN GOAT

Ştefan CIORNEI, Dan DRUGOCIU, Petru ROŞCA, Florin NECHIFOR, Carmen CREȚU, Liliana (CIORNEI) GHINEȚ, Gabriel AGAPE*

University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Reproduction, Aleea Sadoveanu 8, 700489-Iași, Romania

* Suinprod Roman SA - Războieni

stefan_ciornei@uaiasi.ro

Summary

Correlation between some biochemical parameters, electrical conductivity and somatic cells in goat subclinical mastitis. Within a goat farm, subclinical mastitis represents one of the main causes that leads to economical loss. The evolution of subclinical mastitis occurrence leads to changes of milk quality parameters (acidity, NTS, NTG, salts). The milk becomes out of line and inappropriate for commercialization. If the mammary gland disease is not discovered in time and the milk is collected in the storage tank, the whole quantity of milk could be compromised. Our study was made during the summer season (july -august) within a goat farm with 40 goats (80 quarters) for milk. The problem incriminated by the farmer was the milk acidity over the maximum limit. This was the reference point of our study. To identify the causes, we kept track of the following parameters: milking quality, milk transportation and preservation and identifying the goats that suffer from subclinical mammary gland diseases. Out of all this parameters, the problem that came into proeminence was the mammary gland level of health. Mammary gland level of health was clinically evaluated through quality determination of milk secretion. On the collected milk we highlighted the next results: acidity 19 degrees Turner, chlorides >2g‰, CMT+ (medium positive), electrical conductivity 280 units. The determination made on every animal, individually, proved that 82,5% of the examined goats were registered with positive and high positive out of line NCS values (CMT).

Key words*: goat, mastitis, CMT*

The quality of goat milk is already regulated by Law, which takes into consideration its physical-chemical and microbiological composition parameters. Several factors contribute for the alteration of the physical-chemical and microbiological parameters of caprine milk. These include breed, age, lactation phase and diet, among others. The health status of animals may interfere in the quality of the milk produced, both regarding food safety and milk processing and, for that reason, the identification of mastitis-causing agents is important, so that prevention and control measures can be implemented (Carina Morais Correa 2010).

Mastitis is a general term which refers to inflammation of the mammary gland, regardless of cause. It is characterized by physical, chemical, and usually bacteriological changes in the milk and by pathological changes in the udder. Early recognition and prompt treatment are important for limiting tissue damage and production losses. However, since treatment is often unrewarding, emphasis should be on mastitis control and prevention (Shearer J.K 1992).

Material and methods

Our study was made during the summer season (july -august) within a goat farm with 40 goats (80 quarters) for milk. The problem incriminated by the farmer was the milk acidity over the maximum limit. This was the reference point of our study.

Determination of acidity of milk

Determining the acidity of the milk was carried out according to the method Thörner, STAS 6353/1985. Thörner method has as a basic principle neutralizing lactic acid derived from

bacterial fermentation of lactose with 0.1 N sodium hydroxide, in the presence of phenolphthalein as an indicator, forming sodi.um lactate and water.

Thorner acidity in degrees (°T) is within the range of 19-20 °Tfor goat milk.

Assessing freshness milk sample with alcohol 68°

The principle is based on the precipitation of casein in an acidic medium, alcohol precipitation by speeding up the removal of water from the casein. Of precipitation of casein is an indication that the acidity of the milk to be analyzed exceeds 19 °T.

California Mastitis Test (CMT)

California Mastitis Test (CMT) is a rapid, accurate, to determine the somatic cell count (SCC) of milk. The test was designed to determine the presence of sub-clinical mastitis. The test was designed to determine the presence of sub-clinical mastitis. Mastitis milk are identified by highlighting content in leukocytes.The test has the advantage that it can be applied both on individual milk, thus enabling discovery milk mixture mastitic normal milk.

As a result of negative and reactive mixture of milk and homogeneous assay remains positive and strongly positive aspect has a gel consistently.

Determination of milk chloride

In the case of mastitis in milk increases chlorides and lactose decreases. The chlorides of milk acidified with nitric acid combines with the silver nitrate by forming silver chloride.The excess silver nitrate is titrated with ammonium thiocyanate in the presence of ferric alum (indicator). End of the reaction is marked by a red brick appearance persistent than 2 minutes. In normal milk amount of chlorides expressed as sodium chloride varies between 0.7 - 2 g‰.

Determination of electrical conductibility (DRAMINSKI Mastitis Detector):

Readings below 250 units: This is a clear indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states or the high risk of passing to subclinical states of mastitis.

Readings above 300 units: The milk sample is of high quality and is healthy. Usually the readouts are placed in the range 330-360 units. The incidence of subclinical mastitis is very low. Among young milk goats (1-2 lactations) the most common readings approach the vicinity of 370- 400 units whereas the old goats will show readings at lower level of 300-320.

Readings between 300 and 250 units: A progressively increasing incidence of subclinical infection as readings decrease. Due to physiological differences it is extremely difficult to define border between healthy and sick quarter. The readings at the level of 250-300 units can be taken as normal readings and quarters as healthy, especially when results in a particular goat does not show higher values. However, if the sudden drop is noticed down to 250-300 units whereas all the previous

Result and discussion:

On the collected milk we highlighted the next results: acidity 19 degrees Turner, chlorides >2g‰ , CMT+ (medium positive), electrical conductivity 280 units. The determination made on every animal, individually, proved that 82,5% of the examined goats were registered with positive and high positive out of line NCS values (CMT).

Table 1

Interpretation of California Mastitis Test scores on goat milk

CMT score		Somatic Cell Range	Interpretation
Negative	-	0 -1 x 10^6	Healthy Quarter
Positive	+	1 – 1,5 x 10^6	Subclinical Mastitis
	++	1,5 - 2 x 10^6	Subclinical Mastitis
	+++	>2 x 10^6	Serious Mastitis Infection

Table 2

Relationship between CMT, electric conductibility acidity, clorides in goat milk

The mean values parameters of the milk		Quarters examined		Goats (no)	Goats with healthy milk	
		No	%		No	%
CMT Average Somatic Count (Cells per milliliter)	**-**	17	21,3	80	7 (40)	17,5
	+, ++, +++	63	78,7			
Electric conductibility (units)	**< /=250 UT**	15	18,7	80	3 (40)	7,5
	>/= 300 UT	8	10			
	250 - 300 UT	57	71,3			
Acidity	**<19°T**	9	11,2	80	4 (40)	10
	>19°T	71	88,8			
Chlorides (g Na Cl ‰)	**< 2 g ‰**	10	12,5	80	5 (40)	12,5
	> 2 g ‰	70	87,5			

Conclusions

- According to the CMT, in the case of goat's subclinical mastisis diagnosis, 78,7% of the breast quarters had mastisis, with a somatic cell count of over 1 million.
- Measuring electrical conductivity of the goat's milk yields no relevant results in the case of subclinical mastisis. Only 18,7% respectively 7,5% of the goat's appear healthy according to this approach.
- Physical and chemical determinations (acidity and chlorides), yield similar values (88.8% and 87,5%) in the case of diagnosing subclinical mastisis.

Biblography

1. Carina Morais Correa, Raquel Michaelsen, Maria Edi da Rocha Ribeiro, Andrea Troller Pinto, Maira Balbinotti Zanela, Verônica Schmidt. Milk Composition and Mastitis Diagnosis in Goats - Acta Scientiae Veterinariae. 38(3): 273-278, 2010.
2. Shearer J.K., Harris, B. Jr. Mastitis in Dairy Goats Florida Cooperative Extension Service DS 85 November, 1992
3. Suarez, V.H.; Martinez, G.M.; Gianre, V.; Calvinho, L.; Rachoski, A.; Chavez, M.; Salatin, A.; Orozco, S.; Sanchez, V.; Bertoni, E. Relations between somatic cell count, California mastitis test, electrical conductivity and the diagnosis of subclinical mastitis in dairy goats. RIA. Revista de Investigaciones Agropecuarias, vol. 40, no. 2, 2014, pp. 145-153

SEASONALITY OF CLINICAL ESTRUS IN BUFFALOS

Liliana GHINEȚ (CIORNEI), Dan DRUGOCIU, Petru ROŞCA, Florin NECHIFOR, Gabriel AGAPE* Ştefan CIORNEI
University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Reproduction, Aleea Sadoveanu 8, 700489-Iași, Romania
* Suinprod Roman SA - Războieni
l.ciornei@yahoo.com

Summary

Seasonality is a variation on changes that are recorded during the year, being modulated by microclimate factors and macroclimat. At buffaloes, seasonality is manifested during certain times of the year. The literature are described seasonal changes in the female genital tract, especially differences in the volume of ovaries, depending on the period of the sexual cycle. Regarding of occurrence estrous phase in the winter (November to March), anestrous installs in around 90% of cases. In this study have been monitored a total of 65 buffalo concerning: timing of estrus, estrous clinical signs and its duration. Estrus detection was performed by clinical methods while buffalos were stimulated by using male buffalo. Mode of occurrence of oestrus and its manifestations have been grouped into seasons. Results showed that at 9.23% (6 buffaloes) of the effective were not identified clinical signs of heat. As to the clinical manifestation of the estrous phase, her characterization is poorly expressed clinical, also vulvar edema is reduced, less abundant mucus and reduced volume. Behaviourally buffalo in estrus are characterized by reduced appetite, slightly agitated and curious. On the seasons, spontaneous estrus was diagnosed in 50.8% (30) in spring, 30.5% (18) summer, 13.6% (8) autumn and 5.1% (3) in winter. Seasonality manifestation estrus at buffalo, is concretized by the antagonistic results obtained in May and December. It was found that spontaneous estrus in May was diagnosed in 28.8% (17) of buffalo herd, and in December no female has been diagnosed in estrus. Low values were observed in January, November and August (1.7%).

***Key words**: buffalo, spontaneous estrus, seasonality*

The breeding season for buffalo in the major buffalo rearing countries appear to extend from September to March (Fahimuddin M.1986, Ingawale M.V. 2004). Compared to cattle, buffaloes exhibit delayed puberty in both males and females, poor estrus expression in a greater proportion of females, delayed postpartum estrus and prolonged inter-calving intervals. Female buffaloes inherently have lower population of primordial follicles in the ovaries and evidence two to three follicular growth waves during the estrous cycle. The estrus persists for 24-36 h and ovulation occurs 24-48 h after estrus onset (Ingawale MV 2004, Awasthi M.K. 2013, Runceanu L. et al 2016).

The normal anatomy of the female and male genital system is important to understand in view of the physiological clinical procedures that need to be undertaken and also to understand the various structures. The female reproductive tract comprises of the tubular genitalia and the generative organs (Hafez ESE 2004, Luktuke S.et al 1962) whereas the male reproductive tract consists of the testes and the tubular structures and the accessory sex glands. Cattle and buffalo appear to be similar in the anatomy of the female and male reproductive tracts yet some differences such as a narrower cervix, smaller ovarian and testicular dimensions and smaller male accessory sex glands in the buffalo render the species slightly different from cattle. (Tonizza de Carvalho N. A. et al. 2014).

The age at which estrus is first detected is referred to as puberty. Buffalo heifers attain puberty at about 24-30 months of age and at 225-275 kg body weight, i.e when animals attain 55–60% of their adult body weight, however, swamp buffaloes attain puberty at 21–24 months of age. Reaching puberty is more related to body weight than to age. However, individual's

genotype, nutrition, management, season of birth, climatic factors, occurrence of disease and the presence or absence of a mature male can influence the age at puberty (Awasthi M.K. 2013).

Tabel 1

Seasonality of clinical estrus in buffalos

Buffaloes		**Spontaneous estrus**					
		Spring					
No.	**%**	March		April		May	
65	**100**	**No**	**%**	**No**	**%**	**No**	**%**
59	90,7	3	5,0	10	16,9	17	28,
Total		**30**					
	%	**50,8**					

Buffaloes		**Spontaneous estrus**					
		Summer					
No.	%	**Jun**		**July**		**Ougust**	
65	100	**N**	**%**	**No**	**%**	**N**	**%**
59	90,7	1	18,6	6	10,1	1	1,69
Total		**18**					
	%	**30,5**					

Buffaloes		**Spontaneous estrus**					
		Autumn					
N	%	Septem.		Octom.		Novem.	
65	100	**No.**	**%**	**No**	**%**	**No.**	**%**
59	90,7	3	5,08	4	6,77	1	1,69
Total		**8**					
	%	**13,6**					

Buffaloes		**Spontaneous estrus**					
		Winter					
No.	%	December		January		February	
65	100	**N**	**%**	**No**	**%**	**No.**	**%**
59	90,7	0	0	1	1,6	2	3,3
Total		**2**					
	%	**5,1**					

Table 2.

Morphological characteristics of indigenous Romanian Buffalo

Other names	**Bivol românesc**	
Country of origin	**Romania**	
Type	**River**	
Use	**draught; dairy; meat**	
Traits		
Weight	**Male:**	**650-680 kg**
	Female:	**530-560 kg**
Height	**Male:**	**140-142 cm**
	Female:	**131-133 cm**
Coat	**black**	
Water buffalo *Bubalus bubalis*		

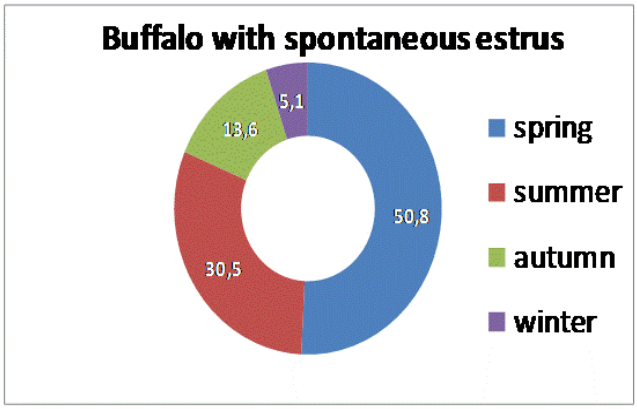


Fig. 1 Graphic presentation of estrus in buffalo cows

Material and methods

In this study have been monitored a total of 65 buffalo (***Terra de Bufalo*** farm) concerning: timing of estrus, estrous clinical signs and its duration. Estrus detection was performed by clinical methods while buffalos were stimulated by using male buffalo. Mode of occurrence of oestrus and its manifestations have been grouped into seasons.

Result

Regarding of occurrence estrous phase in the winter *(November to March), anestrous* installs in around 90% of cases. In this study have been monitored a total of 65 buffalo concerning: timing of estrus, estrous clinical signs and its duration. Estrus detection was performed by clinical methods while buffalos were stimulated by using male buffalo. Mode of occurrence of oestrus and its manifestations have been grouped into seasons. Results showed that at 9.23% (6 buffaloes) of the effective were not identified clinical signs of heat. As to the clinical manifestation of the estrous phase, her characterization is poorly expressed clinical, also vulvar edema is reduced, less abundant mucus and reduced volume. Behaviourally buffalo in estrus are characterized by reduced appetite, slightly agitated and curious. On the seasons, spontaneous estrus was diagnosed in 50.8% (30) in spring, 30.5% (18) summer, 13.6% (8) autumn and 5.1% (3) in winter. Seasonality manifestation estrus at buffalo, is concretized by the antagonistic results obtained in May and December. It was found that spontaneous estrus in May was diagnosed in 28.8% (17) of buffalo herd, and in December no female has been diagnosed in estrus. Low values were observed in January, November and August (1.7%).

Discution

The reproductive physiology of the buffalo is similar to cattle in many aspects yet there are subtle differences in other aspects. Buffaloes exhibit marked influence of season on the expression of estrus, conception and calving in females and depressed libido during hot summer months in males. It has been mentioned that the timing of reproduction in buffalo species is highly variable (Barile VL 2005, Marai FM 2010, Cockrill WR 1997).

The estrous cycle in buffaloes is regulated by the hypothalamic-pituitary-gonadal axis consisting of hypothalamus, pituitary, and the ovary. The hypothalamus produces GnRH in response to neuro-endocrine signals and circulating reproductive steroids. GnRH has a trophic action on the pituitary stimulating the production of gonadotropins; FSH and LH. These

hormones stimulate ovarian follicles to grow and ovulate. Furthermore, upon ovulation the follicle transforms to form the corpus luteum (CL) under LH influence. The CL is responsible for progesterone production in cyclic and pregnant animals. The primary hormones produced by the ovary are estrogen and progesterone, in addition to other local hormones.

Many authors (Taneja M. 1996, Baruselli PS, 1997, Manik RS, 1998 , Awasthi M.K. 2013) have shown that buffaloes typically had two and three follicular waves during an estrous cycle, with the first wave beginning around Day 0 (day of ovulation). In each wave of follicular growth, one dominant follicle develops and suppresses the other follicles. The dominant follicle grows and reaches maximum diameter in the middle of the estrous cycle.

The most clearly definable sign of this rhythmic pattern is estrus, a period of sexual receptivity, which recurs every 21 days (range 19 -23 days). Broadly, the estrous cycle can be divided into four phases: proestrus (3 days), estrus (24 h), metaestrus (3-4days) and diestrus (12-15 days). In river buffaloes estrus lasts an average of 24 h (10-48 h) in comparison to a shorter time period in swamp buffaloes (19.9 ± 4 h). The female accepts the male for mating during this period. During estrus, an ovum matures within the ovarian follicle under the influence of LH and ovulates approximately 11 h after disappearance of estrus signs in river buffaloes and 13.9 hrs in swamp buffaloes.

Signs of estrus in buffalo are less overt than in the cow (Adams GP 2000), homosexual behavior between females is rarely seen. The main behavioral signs are restlessness, bellowing, tail raising, vulvar swelling, decreased feed intake and frequent voiding of urine. The willingness of the female to stand for mating is regarded as a true sign of estrus.

During summer, estrus is exhibited only during the night or in the early morning hours. Silent heat is common during summer months. The estrus signs are accompanied by changes in external genitalia swelling of the vulva and reddening of the vestibular mucosa and changes in internal genitalia such as good uterine tone and coiling of the uterine horns. Due to vulval swelling, the horizontal wrinkles which are present on its external surface disappear in estrus animal. Secretion of mucus from the cervix during estrus is less copious than in cattle and does not usually hang as strands from the vulva, although a proportion of buffaloes may show mucus strands, but can be seen by trans-rectal back racking of genitalia or when the buffalo sits.

Acknowledgement

This study was done in ***Terra de Buffalo*** farm, particularly like to thank for the biological material.

Conclusions

At buffaloes, seasonality is manifested during certain times of the year. Results showed that at 9.23% (6 buffaloes) of the effective were not identified clinical signs of heat. As to the clinical manifestation of the estrous phase, her characterization is poorly expressed clinical, also vulvar edema is reduced, less abundant mucus and reduced volume. Behaviourally buffalo in estrus are characterized by reduced appetite, slightly agitated and curious. On the seasons, spontaneous estrus was diagnosed in 50.8% (30) in spring, 30.5% (18) summer, 13.6% (8) autumn and 5.1% (3) in winter.

References

1. Awasthi M.K. 2013 - Dynamics in Female Buffaloes In: Bubaline Theriogenology, Purohit G.N. (Ed.). International Veterinary Information Service, Ithaca NY; A5704.061
2. Barile VL. Review article: improving reproductive efficiency infemale buffaloes. Livest Prod Sci 2005; 92:183–194.
3. Baruselli PS, Mucciolo RG, Vistin JA, et al. Ovarian follicular dynamics during the estrous cycle in buffalo (*Bubalus bubalis*). Theriogenology 1997; 47:1531–1547.

4. Brar PS, Saigal, RP, Sharma RD, et al. Histology and histochemistry of broad ligaments in buffaloes. Indian J Anim Sci 2008; 78:464-467.
5. Chantaraprateep P, Prateep P, Lohachit C, et al. Pelvimetry in swamp buffalo and indigenous cattle cow. J Thai Vet Med Assoc 1984; 35:307-316.
6. Cockrill WR. The Husbandry and Health of the Domestic Buffalo. FAO, Rome, Italy 1977.
7. El-Sheikh AS. The reproductive performance of the buffalo in Egypt. Indian J Dairy Sci1987; 89-95.
8. Fahimuddin M. Domestic water buffalo. Oxford and IBM Publishing Company, New Delhi 1986.
9. Hadi MA, Sane CR. Critical observations on certain anatomical features encountered during study of female Murrah buffalo pelvis. Indian Vet J 1965; 42:327-330.
10. Hafez ESE, Hafez B. Reprodução animal. 7th ed. São Paulo: Manole, 2004; 513.
11. Ingawale MV, Dhoble RL. Buffalo reproduction in India – A review. Buffalo Bull 2004; 23:4-9.
12. Luktuke SN, Rao ASP. Studies on the biometry of the reproductive tract of the buffalo-cow. Indian J Vet Sci 1962; 32:106-111.
13. Manik RS, Singla SK, Palta P, et al. Ovarian follicular dynamics monitored by real-time ultrasonography during estrous cycle in buffaloes (*Bubalus bubalis*). Asian Aust J Anim Sci 1998; 11:480–485.
14. Marai FM, Habeeb AMM. Buffaloes reproductive and productive traits as affected by heat stress. Trop Sub Trop Agro Eco Systems 2010; 12:193-217.
15. Runceanu L., Groza I. Șt., Șonea Al., Zamfirescu Stela, Drugociu D., Roșca P., Ciornei Șt., Runceanu Margareta, Iuga P., Drugociu Dana, Nechifor F. 2016. Sezonalitatea reproducerii animalelor. Editura Ion Ionescu de la Brad Iași, ISBN: 978-973-147-226-3
16. Sharma R, Jerome A. Purohit G. 2014. Reproductive Physiology of the Male and Female Buffalo.
17. Singh J, et all. The reproductive pattern and efficiency of female buffaloes. Anim Reprod Sci 2000;
18. Taneja M, ei al. Ovarian follicular dynamics in water buffalo. Theriogenology 1996; 46:121–130.
19. Tonizza de Carvalho N. A.,. Soares J. G, Kahwage P. R., Garcia A. 2014 -Anatomy of the Reproductive Tract of the Female and Male Buffaloes. In: Bubaline Theriogenology, Purohit G.N. (Ed.). International Veterinary Information Service, Ithaca NY.

THE SURGICAL APPROACH IN THE DIAPHRAGMATIC HERNIAS IN CATS

Eusebiu Viorel ȘINDILAR, Diana-Alexandra BUSUIOC
University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Reproduction, Aleea Sadoveanu 8, 700489-Iași, Romania
esindilar@yahoo.com

Abstract
A diaphragmatic hernia occurs when one or more organs of the abdominal cavity protrudes through a gap or opening into the thoracic cavity. The hernias can be congenital (usually before the kitten reaches the first year) or acquired, because of a trauma (occurs at any age). The standard treatment is the surgical procedure which includes the topographical arrangement of the organs in the abdominal cavity, the suture of the detached portion of the diaphragm and the establishment of the negative pressure in the thoracic cavity. Usually the first two organs to herniate are the liver and the stomach, but we had cases when all the organs of the digestive tract until the small intestine were herniated.
Keywords: *Diaphragmatic hernia, surgical procedure, trauma*

Introduction

The diaphragm is a musculo-membranous organ that separates the thoracic cavity from the abdominal cavity and functions like a barrier and helps the breath. A diaphragmatic hernia consists in the rupture or tearing of the diaphragm, allowing the organs to protrude into the thoracic cavity. Very frequent, the diaphragmatic hernias occur after a trauma, most often like a car accident. Thes animals can suffer several lesions that need medical examination and an increased atention from all the points of view.

There are two types of diaphragmatic henias that can be found in dogs and cats:

- Post-traumatic acute and chronic- caused by a traumatic event that provoked the rupture of the diaphragm
- Congenital- these animals are born with this defect (the most frequent type is the peritoneo-pericardial diaphragmatic hernia PPDH)

Materials and Methods

The casuistry taken into this study was formed by a number of 72 cats of different rases, sex and ages. The period taken into consideration was September 2000- September 2016. For those we used two surgical methods that aim to remedy the defects of integrity of the diaphragm and the topographical defects.

A diaphragmatic hernia can cause semnificative difficulties in breathing. The trauma that cause the hernia can also determine rib fractures, pulmonary lacerations and pulmonary bleeding. These lesions can take to pneumothorax or hemothorax. In the case that the abdominal organs protrude into the thoracic cavity, the capacity of the lungs to increase their volume during inspiration is reduced and the respiratory capacity is decreased.

The surgical procedure

Using the abdominal approach we performed an incision from the xifoid appendix to the navel. This incision can be easily expanded if necessary. Once the peritoneal cavity is open, the diaphragm is exposed and can be evaluated. Some of the hernias were not easy to visualise, especially those from the area of the dorsal pillars and of the aortic hiatus. These areas were carefully examinated even though other regions of the diaphragm were perforated. The

herniated organs were placed topographically and inspected for secondary lesions. Some of the complex lesions that were a great challenge for the surgical team were represented by the cases when we observed the torsion of one of the hepatic lobes, ruptured organs, intestinal invagination, intercostal hernias. In the cases when we found adhesions, these were undone using the dissection tehnique of dilaceration, in order to evoid a haemorrhage and the accidental deterioration of the vital tissues. Ocasionally, in the chronic cases, the hernial ring had to be excised because of the adhensions between it and the herniated organs. The segments of the diaphragmatic ring with hepatic adherences had to be separated from the diaphragm to evoid the bleeding, which is one of the most to fear enemy in this situations.

Using large pledgets and special instruments, the liver and the intestine were retracted one by one, laterally and caudally. From this moment, the diaphragmatic rupture was exposed so the examination of the thoracic cavity can be performed. All the thoracic fluids were aspirated. From this moment, the pulmons started slowly to recover their initial volume during inspiration.

The re-expansion of the pulmonary parenchyma produced very slow. Pulmonary edema can be a complication during re-expansion, especially for the cats with chronic diaphragmatic hernias. In the case that the hernia is older the 48 hours, the margins of the hernia had to be debridated. The rupture of the diaphragm was sutured using resorbable material, in one layer. The size of the resorbable wire varied from 4/0 to 2/0 with atraumatic needle. Also, for the reconstruction of the diaphragm architecture we used a suture in separate points. When the rupture is around the orifice of the caudal vena cava, large sutures have to be avoided to prevent the vascular constriction.

The same principle was applied for the aortic hiatus and esofagian hiatus. The chronic diaphragmatic hernia can require the utilisation of a muscular or fascial flap or of a flap from the epiploon to close the defect.

To evacuate the pleural space we used a tube that has to be placed before closing the diaphragm. After this moment, the closure of the defect can be finalised. Using a valve with three positions and a 50cc syringe the air from the thoracic cavity was evacuated until negative pressure was aquiered. The celiotomy was closed using the routine procedures. In the moment when the compete closure of the abdomene was realised the tube was again aspired. The pacients were consecutive placed in different positions while they were allowed to breath spontaneous. The pacients were monitored during the intervention and after using vital monitors and pulsoximeters.

The postoperative care included monitoring the breath of the pacient, it's temperature and the colours of the mucouses and the systemic administration of amoxicillin (10-15 mg/ kg body weight) for 5- 7 days. The small cats were held on a heating system for at least 24 hours. The use of a bandage around the thorax is not advised because it can prevent the animal from breathing well. As an analgesic we used meloxicam (0.1mg/ kg body weight) to calm the pain so the animals can breath more easy. The thoracostomy tube is checked every hour for the first 4 hours, then every 6-8 hours and is removed usually 24 hours after the surgery.

In most of the cases (78%) the cats started to fell well after the surgery and tend to be very active. It's important to encourage the repose and to avoid making effort in the postoperative period.

The prognostic for the animals that suffered a diaphragmatic henia varies according to the lesions. We estimated that approximate 15% of the animals that suffer a traumatic transdiaphragmatic hernia will die before being examinated by a doctor. A treatment against shock should be established before the surgical intervention to increase the survival rates.

Animals that had the intervention performed after more then an year from the trauma had a bad prognostic because of the adherences to the other tissues or organs.

The mortality rate of the cats with PPDH that underwent the surgery was low and the prognostic for a normal functionality was excellent, with the specification that the intervention is performed after 6 months of age.

Results

According to the size of the animals, the age and the acute or chronic nature of the hernia, we used the same surgical tehnique that we consider optimum.

The age of the animals was between 6 months and 12 years. Abdominal and thoracic radiography were taken for a radiographic diagnosis. At least two radiographs in different incidence were taken. The functional recovery of the cats was extremely fast.

In 36 of the cases we performed laparatomy on the medial line of the abdomen, and in 3 cases we had to perform a median sternotomy and laparatomy.

In 22 animals we had to perform the detachment of the adhesions between the pulmons and the diaphragm and the herniated organs to permit the reduction of the hernia and the topographical repositioning.

In the case of 14 cats we had to make the resection of segments of the pulmons, liver and intestine. All the hernias were sutured without using flaps, tissue graft or other implants. 21 of the cats developed transitory complications in the postoperative period.

The mortality rate was 14%. In 78% of cases none of the initial clinical signs were present during the postoperative controls.

In two cases of female cats we found the uterus with fetuses herniated threw the diafragm because of the pressure put on the diaphragm due to the advanced gestation. This kind of hernia is very rare in the specialized literature.

Discussion and conclusion

The diaphragmatic hernia due to trauma occurs mainly in the cats that live outdoor, or indoor and outdoor, indifferent of the age or breed. It is a certain predisposition in the overweighted animals and the entire males.

The first clinical sign is severe dyspnoea that can worsen taking the animal to exitus, before any surgical intervention. On the other side, a chronic hernia can occur spontaneaous without other known episodes. The acute traumatic cases are usually accompanied by other lesions like different wounds, fractures or nervous system lesions.

The medical intervention must be correlated with the size of the animal, the age, the presence or absence of other lesions and the pathophysiological complications of the cardiovascular system.

A precocious diagnostic can help by limiting the ischemic complications that occur to the topographically moved organs.

The result of a well-performed surgery can be compromised by the neglected surveillance of the owners or their carelessness in the convalescent period of the animal.

The postoperative evolution of the pacients was generally good in the first days, in this time the pacients have been monitorized by pulse oximetry.

References

1. Crowe DT. The acute and delayed diaphragmatic hernia, in Proceedings. 10th Int Vet Emerg Crit Care Symp 2004;795–799.

2. 2. Fossum TW. Surgery of the lower respiratory system: pleural cavity and diaphragm. In: Fossum TW, Hedlund TS, Hulse DA, et al, eds. Small animal surgery. 2nd ed. St Louis: CV Mosby Co, 2002;795
3. Hyun C. Radiographic diagnosis of diaphragmatic hernia: review of 60 cases in dogs and cats, J Vet Sci. 2004 Jun;5(2):157-62.
4. Minihan AC, Berg J, Evans KL. Chronic diaphragmatic hernia in 34 dogs and 16 cats, J Am Anim Hosp Assoc. 2004 Jan-Feb;40(1):51-63.
5. Omer BESALTI 1 , Zeynep PEKCAN 2 , Murat CALISKAN 1 , Z. Gamze AYKUT. A retrospective study on traumatic diaphragmatic hernias in cats, Ankara Üniv Vet Fak Derg, 58, 175-179, 2011.
6. Sullivan M, Reid J. Management of 60 cases of diaphragmatic rupture. J Small Anim Pract 1990;31:425–430.
7. Thomas W. G. Gibson, DVM ; Brigitte A. Brisson. Perioperative survival rates after surgery for diaphragmatic hernia in dogs and cats: 92 cases (1990–2002), JAVMA, Vol 227, No. 1, July 1, 2005, 105-109.

INTERNAL PARASITE COMMUNITY AND EPIDEMIOLOGY OF PARASITIC INFECTIONS IN WORKING HORSES, ROMANIA

Marius Cătălin BUZATU, Ioan Liviu MITREA, Mariana IONITA
University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Dep. of Parasitology and Parasitic Diseases
buzatumariuscatalin@yahoo.com

Abstract

Equine gastrointestinal parasites, and particular helminthes, are ubiquitous. Horses that graze contaminated pasture can burden large numbers of parasites which can lead to serious clinical disease. The aim of the present study was to describe the parasite community and epidemiology of intestinal parasitic infections in working horses in Romania. For this, a total number of 459 working horses originated from 38 villages belonging to 18 counties in Northeastern, Center, and Southeastern Romania were included in the study. Horses were allocated by age and gender category. Fresh fecal samples were collected from individual horses and analyzed qualitatively for the presence of intestinal parasites using sodium chloride flotation technique and quantitatively for fecal worm eggs counts, described as the number of eggs per gram (EPG) of feces, using a modified McMaster technique. 75.40% of working horses were positive for strongyle infection, followed by Parascaris equorum (7.40%), Eimeria leuckarti (7.20%), Strongyloides westeri (3.70%), and Anoplocephala spp. (2.61%). Young animals (of 1 to 5 years old) were the most infected category for all parasite species identified, except for Anoplocephala spp. for which animals over 10 years old were more frequent infected. Positive horses for strongyles were found in all villages and counties, respectively, where studies were performed. The positive horses for P. equorum, S. westeri, Anoplocephala spp., and E. leuckarti originated from 19 (50.00%), 13 (34.20%), 6 (15.80%), and 7 (18.40%) of villages, respectively, distributed in 11 (61.10%), 8 (44.40%), 3 (16.70%), and 5 (27.80%) counties, respectively. The results showed that strongyle infections are highly prevalent in Romanian working horses and provide further evidence that the parasite burden is influenced by age of horses and other factors, such as anthelmintic treatments of animals or degree of pasture hygiene. These findings represent a base for further studies to design sustainable control program of horse parasites.

Keywords: *internal parasites, epidemiology, working horses, Romania.*

Introduction

Equine gastrointestinal parasites are ubiquitous in grazing horses. The main internal parasites traditionally considered to be important in horses are ascarids, large and small strongyles, bots, and pinworms (Lyons et al., 1999; Mitrea, 2011). Other species, such as tapeworms, stomach worms, and intestinal threadworms also can be of clinical importance. Some of these parasites, usually depending on their abundance, are known to cause problems ranging from reduced performance and condition up to abdominal disease such as colic, severe diarrhoea or even death (Love et al., 1999).

Within the equine parasites, strongyles (Nematoda: Strongylidae) represent a complex group which is separated in two groups called large strongyles (Strongylinae) and small strongyles (Cyathostominae, cyathostomins), respectively; they have a huge impact on equine health. Studies in different horse establishments world-wide have shown a highly occurrence of these parasites under largely different geographic and climatic conditions (Lyons et al., 1999). Their clinical importance is well-documented, as infection can lead to weight-loss and unthriftiness, disease symptoms dominated by colic and diarrhea, and in severe cases, death (Drudge and Lyons, 1986).

In the last years, it is well accepted that the assessment of helminth distribution patterns in different equine populations will yield useful information for developing improved control methods that are less reliant on chemical compounds (Nielsen, 2012). Moreover, within the new strongyle control strategies, identification of high egg shedders within the herd is an

essential goal for developing control programmes based on targeted selective treatment (Nielsen, 2012; Relf et al., 2013).

In Romania, horses are raised for various purposes, such as for agriculture as working horses, for sport, recreational activities, or breeding. However, working horses represent a great majority (up to 98%) of the total horses in Romania. They are breed in small individual farms in rural areas. Usually, working horses are raised in households with 1 or 2 horses / yard, for working purposes. Nonetheless, limited data about the parasite infections in Romanian working horses are available. Therefore, the aim of the present study is to describe the parasite community and epidemiology of intestinal parasitic infections in working horses in Romania.

Materials and methods

A total number of 459 working horses originated from 38 villages belonging to 18 counties in Northeastern, Center, and Southeastern Romania were included in the study for copro-parasitological investigations (Figure 1).

Horses were twelve months to 26 years of age. All the horses had access to pasture grazing. The animals were assigned in age and gender groups (Table 1)

Fresh fecal samples were collected from individual horses and analyzed qualitatively for the presence of intestinal parasites using sodium chloride flotation technique and quantitatively for fecal worm eggs counts, described as the number of eggs per gram (EPG) of feces, using a modified McMaster egg counting technique with a sensitivity of 25 eggs per gram of faeces (EPG) (Ionita and Mitrea, 2013).

For analysis of age- and gender- related differences, the animals were assigned in age (1 - 5, 6 - 10, 11 - 15, 16 - 20, and >20 years-olds) and gender groups. Additionally, to analyze the proportion of the high parasite eggs shedders, horses were stratified by classes of intensity (0, 25 - 200, >200), based on fecal worm eggs counts (FWECs).

Results and discussion

Overall, of the 459 fecal samples analyzed, 351 (76.50%) were positive for at least one parasite species. Of these, the most prevalent infection was with strongyles (75.40%), followed by *Parascaris equorum* (7.4%), *Eimeria leuckarti (*7.20%), *Strongyloides westeri* (3.70 %), and tapeworms – *Anoplocephalidae* (2.61%).

A high intensity rate for strongyles was registered, with the EPG counts varying from 0 to 6725, while for *P. equorum* FECs ranged from 0 to 1475, respectively.

Details about the infection rate of each parasite species according to different parameters (i.e. age category, originating area) are presented in Table 1 and Table 2.

Young animals (of 1 to 5 years old) were the most infected category for all parasite species identified, except for *Anoplocephala* spp. for which animals over 10 years old were more frequent infected.

Positive horses for strongyles were found in all villages and counties, respectively, where studies were performed. The positive horses for *P. equorum, S. westeri, Anoplocephala* spp., and *E. leuckarti* originated from 19 (50.00%), 13 (34.20%), 6 (15.80%), and 7 (18.40%) of villages, respectively, distributed in 11 (61.10%), 8 (44.40%), 3 (16.70%), and 5 (27.80%) counties, respectively (Table 2).

Table 1.

Epidemiological data on parasite infection in working horses, according to the age category (helminth infections are expressed as eggs per gram (EPG) of feces; the mean EPG values within each age category are given)

	Age (years) category of horses					
	1 – 5	**6 – 10**	**11-15**	**16 – 20**	**>20**	All ages
Number of horses tested	127	**172**	**99**	**51**	**10**	**459**
Positive for:						
- *strongyles*						
number positive	108	130	**66**	35	7	**346**
mean prevalence (%)	85.00	75.60	66.70	68.60	70.00	***75. 40***
			[χ^2= 11.848; *P*-value = 0.019]			
- *Parascaris equorum*						
number positive	22	10	1	1	0	**34**
mean prevalence (%)	17.30	5.80	1.00	2.00	0	***7.40***
- *Strongyloides westeri*						
number positive	*7*	*3*	*5*	*2*	*0*	*17*
mean prevalence (%)	5.50	1.74	5.05	3.92	0	***3.70***
- *Anoplocephala* spp.						
number positive	*1*	*4*	*5*	*2*	*0*	*12*
mean prevalence (%)	0.90	2.40	5.20	4.10	0	***2.61***
- *Eimeria leuckarti*						
number positive	*15*	*16*	*2*	*0*	*0*	*33*
mean prevalence (%)	13.30	9.50	2.10	0	0	***7.20***

Table 2.

Epidemiological data on parasite infection in working horses, according to their originating location

	Geographical location							
	North-eastern		Center		South-eastern		**Total**	
	county	village	county	villag	county	village	**county**	**village**
Total	6	11	2	3	10	24	**18**	**38**
Number of originating location of positive horses for:								
- strongyles	6	11	2	3	10	24	**18**	**38**
- *Parascaris equorum*	4	6	2	2	5	11	**11**	**19**
- *Strongyloides westeri*	**3**	**5**	**0**	**0**	**4**	**7**	7	12
- *Anoplocephala* spp.	**0**	**0**	**1**	**1**	**2**	**5**	3	6
- *Eimeria leuckarti*	*0*	***0***	2	***3***	3	***4***	5	7

Table 3.
Distribution (number and percentage) of horses based on strongyle fecal eggs per gram (EPG) values, according to the age category

EPG	Age category											
	1 – 5 years		6 – 10 years		11-15 years		16 – 20 years		>20 years		All ages	
	No.	%	No.	%	No.	%	No.	%	No.	%	**No.**	***%***
0	19	*15.00*	42	*24.40*	33	*33.30*	16	*31.40*	3	*30.0*	**113**	***24.40***
25 - 200	31	*24.40*	46	*26.74*	27	*28.30*	16	*31.40*	2	*20.0*	**122**	***26.80***
>200	77	*60.60*	84	*48.83*	39	*39.40*	19	*37.20*	5	*50.0*	**224**	***48.80***
Total	*127*		*172*		*99*		*51*		*10*		***459***	

The proportion of strongyle positive horses, based on the EPG value, stratified by the age category is presented in Table 3. Overall, 48.8% of the working horses exceeded the cut-off value of 200 strongyle EPG. Analysis of the distribution of working horses with positive strongyle EPG counts by classes of intensity was undertaken to help identifying of high strongyle egg shedders. The proportion of horses with EPG >200 varied between the age category groups from 37.20% to 60.60%. The higher proportion of horses with the EPG values >200 was registered in the young horse category (1-5 years), of which about 60.60% of the individual animals exceeded the cut-off value. In contrast, the higher proportion of negative horses was in horses aging between 6 and 15 years, which represented up to 58.0 % of the total negative horses.

Discussion

In the last decades, due to the increasing prevalence of the anthelmintic resistance phenomenon, it is well recognized that there is a stringent need to develop strategies for parasite control that are less rely on anthelmintic medication and more reliant on management-based control (Nielsen et al., 2006; Becher et al., 2010). In this respect, a prerequisite for developing such programmes is a better and improved understanding of the distribution and prevalence of the most relevant parasite species in the field settings (Relf et al., 2013).

The present study adds new insights into the epidemiology of internal parasite infections in working horses. The study was based on extensive investigations covering areas in North-eastern, central and southeastern of the country. A large number of Romanian working horses were included and valuable data were obtained.

The results generated from this study inform on the prevalence of the targeted parasite species and their distribution in working horses of different age categories on small households/yards. The findings are consisting with previous studies in Romania reporting that strongyle infections are highly prevalent, with levels varying as 70.30%, 80.70%, 87.90% up to 100% (Cernea et al., 2003; Covasa and Miron, 2011; Ionita et al., 2013; Buzatu et al., 2013, 2014, 2016;). It is well known that strongyles are the most common and prevalent parasites in grazing horses. This fact is registered including in well managed farms, but the cyathostomins might be considered to be the most important parasite group, if their anthelmintic resistance was reported (Kaplan, 2002, 2004).

Age had a significant influence on the strongyle prevalence in working horses, with the highest value registered in 1 - 5 year olds (85%). Of the strongyle positive horses, 64.70% exceeded the EPG 200 value. Of them, 34.40 and 37.50% were 1-5 years and 6-10 years olds, respectively, while the remainders were 11-15 years old (17.4%) and >16 years (8.4 and 2.2% respectively). These findings indicate a heterogenous distribution of the horses in the three EPG categories, with almost half of the investigated horses in the category of high egg shedders. Similar situation was reported by Traversa et al. (2010), where the proportion of the positive horses with EPG values >150 varied from 42.2% (Italy), 32.2% (U.K.) up to 27.3% (Germany).

P. equorum eggs were predominantly observed in samples from the first age category (1-5 years) (17.30%), whilst only one sample for each category of the samples from horses >10 years old were negative (mean prevalence of 1-2%). Commonly, many studies have reported prevalence and egg-shedding levels of this parasite greater in young animals (Lyons et al., 2008); therefore, it is always suggested that effective control is essential in young horses against this potentially pathogenic parasite (Laugier et al., 2012).

The findings and prevalence rates of *S. westeri, Anoplocephala* spp. and *E. leuckarti* are in line with similar studies. Of these, tapeworm infections, which are found in all ages of horses, may have a clinical significance, particularly *A. perfoliata*, which is considered the most pathogenic species, probably related to its location, the ileocecal junction. The prevalence of tapeworm-positive working horses was established at 2.60% on horse level, very similar to other studies (Hinney et al., 2011). However, despite the low frequency of this parasite, its pathogenic potential should not be underestimated; a high burden of infection is correlated with a marked increase in clinical symptoms (e.g. severe colics) (Gasser et al., 2005).

Conclusion

The results generated from this study indicate that different factors, including host- and management practice- associated factors had a significant influence on the prevalence, intensity and distribution of parasite infections in the investigated horses. Data recorded on individual fecal worm egg counts, in order to identify the higher egg shedders will assist as basis for further development of sustainable control programmes.

Acknowledgements

The authors would like to thank the horse owners and veterinarians that participated in this study. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS - UEFISCDI, project number PN-II-RU-TE-2014-4-1432.

References

1. Becher AM, Mahling M, Nielsen MK, Pfister K., 2010. Selective anthelmintic therapy of horses in the Federal states of Bavaria (Germany) and Salzburg (Austria): an investigation into strongyle egg shedding consistency. Vet Parasitol. 2010 Jul 15;171(1-2):116-22.
2. Buzatu M.C., Ionita M., Mitrea I.L., 2013. Coprological prevalence of intestinal parasites and strongyle EPG profiles of working horses from North-Eastern and South-Eastern Romania. Scientific Works. Series C. Veterinary Medicine, Vol. LIX (3), 62-67.
3. Buzatu M.C., Mitrea I.L., Lyons E., Ionita M. 2016, Epidemiological study on parasite infections in horses from different types of equine establishments, romania. AgroLife Scientific Journal, 5(1): 31-35.
4. Buzatu M.C., Mitrea I.L., Miron L., Ioniță M., 2014. Coprological investigations on strongyle EPG profiles in working horses and horses residing in stud farms in Romania. Journal of Biotechnology, 185, S42.
5. Cernea M., Cozma V., Cernea C., Gall A., 2003. The strongilidosis in horses from Mures district: epidemiology and diagnosis. Bulletin USAMV Cluj Napoca, 60: 196-201.
6. Covașă C.T., Miron L.D., 2011. Prevalence study of digestive and the serous cavities endoparasitosis in horses from Iassy city area. Lucr. St. USAMV Iasi, Seria Medicina Veterinara, 54 (13), 302-306.

7. Drudge J.H., Lyons E.T., 1986. Large strongyles. Recent advances. Vet Clin North Am Equine Pract., 2(2): 263-280.
8. Gasser R.B., Williamson R.M.C., Beveridge I., 2005. *Anoplocephala perfoliata* of horses - significant scope for further research improved diagnosis and control. Parasitology, 131:1–13
9. Hinney B., Wirtherle N.C., Kyule M., Miethe N., Zessin K.H., Clausen P.H., 2011. Prevalence of helminths in horses in the state of Brandenburg, Germany. Parasitol Res. 108(5): p. 1083-1091.
10. Ionita M., Buzatu M. C., Enachescu V., Mitrea I.L., 2013. Coprological prevalence and intensity of gastrointestinal parasites in horses in some Romanian studs: preliminary data. Agrolife Scientific Journal, 2(1): 207-212.
11. Ionita M., Howe D.K., Lyons E.T., Tolliver S.C., Kaplan R.M., Mitrea I.L., Yeargan M., 2010. Use of a reverse line blot assay to survey small strongyle (Strongylida: Cyathostominae) popu-lations in horses before and after treatment with ivermectin. Vet Parasitol., 168(3-4): p. 332-337.
12. Ionita M., Mitrea I.L., 2013, Diagnosticul parazitolozelor la animale. Ghid de laborator, vol. I: Tehnici si metode de diagnostic parazitologic. Diagnosticul protozoozelor. Editura Ceres, Bucharest.
13. Kaplan R.M., 2002. Anthelmintic resistance in nematodes of horses. Vet. Res. 33, 491–507.
14. Kaplan R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481.
15. Laugier C., Sevin C., Ménard S., Maillard K., 2012. Prevalence of *Parascaris equorum* infection in foals on French stud farms and first report of ivermectin resistant *P. equorum* populations in France. Vet Parasitol., 188(1-2): p. 185-189.
16. Lyons E.T., Tolliver S.C., Drudge J.H., 1999. Historical perspective of cyathostomes: prevalence, treatment and control programs. Veterinary Parasitology, 85: 97-112.
17. Lyons E.T., Tolliver S.C., Ionita M., Collins S.S., 2008. Evaluation of parasiticidal activity of fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate in horse foals with emphasis on ascarids (Parascaris equorum) in field studies on five farms in Central Kentucky in 2007. Parasitol Res. 2008 Jul;103(2):287-91. doi: 10.1007/s00436-008-0966-8.
18. Mitrea I.L., 2011. Parazitologie si boli parazitare. Ed. Ceres, Bucuresti.
19. Nielsen M.K., 2012. Sustainable equine parasite control: Perspectives and research needs. Veterinary Parasitology, 185, 32-44.
20. Nielsen M.K., Haaning N., Olsen S.N., 2006. Strongyle egg shedding consistency in horses on farms using selective therapy in Denmark. Veterinary Parasitology, 135 (3-4): 333–335.
21. Relf V.E., Morgan E.R., Hodgkinson J.E., Matthews J.B., 2013. Helminth egg excretion with regard to age, gender and management practices on UK Thoroughbred studs. Parasitology. 2013 Apr;140(5):641-52.
22. Traversa D., Milillo P., Barnes H., von Samson-Himmelstjerna G., Schurmann S., Demeler J., Otranto D., Lia R.P., Amodie D., Rohn K., Cobb R., Boeckh A., 2010. Distribution and species-specific occurrence of cyathostomins (Nematoda, Strongylida) in naturally infected horses from Italy, United Kingdom and Germany. Vet Parasitol., 168(1-2): p. 84-92.

ACTION OF BIOMAS OF STREPTOMYCETES ON IMMUNIOLOGICAL AND BIOCHEMICAL INDICES OF CHICKENS

Nicolae STARCIUC, Alexandr MANCIU, Natalia OSADCI, Olga JUNCU, Aurel CIUCLEA
State Agrarian University of Moldova, Chisinau, str. Mircesti 44, MD-2049
n.starciuc@uasm.md; alexandr.manciu@gmail.com

Abstract

The investigations were based on determining influence of the variation of intestinal microflora to post vaccination antibody titers against Newcastle disease. Were formed 5 groups of one day old chickens which were vaccinated against Newcastle disease through different methods using vaccine strains La Sota and M5 clone30. Was collected the serum samples and samples of intestinal content for serological and microbiological tests to determine the titers level of antibodies and intestinal microflora composition and diversity. As a result it was determined that the variation of intestinal microflora is composed from cultures that are part of digestive tract in chickens (streptococci, bifid bacteria, Escherichia coli, and others) and post vaccine antibody titers obtained by experimental chickens groups was quite high in the groups which in ration was administered biomass of Streptomyces's (23,8%)and increase the number of lymphocytes with 4.16% compared with control group of chickens. In same time haven't been established a significant change in the composition of intestinal microflora of chickens.

Key words: *chickens, virus, antibody titer, vaccination, microflora.*

Introduction

Newcastle Disease is caused by a paramyxovirus type I (APMV-1), which belongs to the genus Avulavirus family Paramyxoviridae. Paramyxoviruses isolated from avian species have been classified by serological testing from 9 serotypes 1 APMV 9.Virusul APMV Newcastle disease is APMV-1.

The virus strains of Newcastle disease varies greatly in relation to the severity of the disease. Less pathogenic strains can cause severe illness or other microorganisms are present due to poor environmental conditions. From the first mention in 1926, Newcastle disease is considered endemic in many countries. Prophylactic vaccinations were practiced in all countries, particularly in the international trade practice.

Materials and methods

The research aimed to study the variation of immunological post vaccination indices against Newcastle diseases, depending on the composition of intestinal microflora of chickens.

The investigations antibody titters determined post vaccine against Newcastle and intestinal microflora diversity was performed at the Department of Epizootology of State Agrarian University of Moldova and the State Veterinary Laboratory in Iasi, Romania.

As a experimental material were used one day old chicken Rhode Island breed. Were studied following indices as:

- post vaccination titers of antibody against Newcastle disease;
- the composition of intestinal microflora;
- blood biochemical indices;

For this purpose were formed 5 groups of one day age chickens 20 in each group. The research scheme was as follows:

- I group of chickens - control;

-II group of chickens in food ration was administrated biomass of streptomycetes, 1g /kg of feed and was vaccinated with vaccine strains M5 Clone30,

-III group of chickens in food ration was administrated cultural liquid product of streptomycetes 1ml/1 l water and was vaccinated with vaccine strains La Sota;

- VI group of chickens, in water was administrated enrofloxacin 1ml/2 l water and was vaccinated with vaccine strains La Sota;

- V group of chickens was vaccinated only with vaccine strains La Sota;

The chickens were maintained in optimal physiological conditions in accordance with levels forecast for this category of poultry. The administration of vaccines and medicinal preparations was done manually feed in nourishing and constant water access.

Selected chickens were the same age, weight and body development, free from infectious and parasitic diseases. The chickens were subjected to vaccination at age one day and then at 20 and 60 days. Samples were taken to determine titers of antibodies, evaluating hematological and intestinal microflora at the age of one day, 15, 55 and 75 days.

Assessment of antibody titers was performed by hem agglutination inhibition reaction. The method is based on the property of Newcastle disease virus to this activity hem agglutinin against poultry red blood cells. Hem agglutination inhibition indicates the presence of specific antibodies for Newcastle disease.

To determine the intestinal microflora were sampled at intestinal tract mucosa and carried seeding areas, which then were made the insemination on special and differential nutrient media subjected to incubation at 37^0C for a period of 48 hours.

Results and discussion

A blood serum is considered positive if hem agglutination inhibition occurs a serum dilution of 1:16 at 4HAU. The results are shown in table 1 which states that the maternal antibody titers against Newcastle disease are able to protect chickens up to an age of 14-17 days and then is recommended the first vaccination. After 15 days of vaccination in blood serum of experimental groups of chickens had a substantial level of post vaccination antibody. The heist level of antibody titers was in the second group of chickens which ranged from 1:90,67 until 1:171,33. In the group three the level of antibody on 15 days after vaccination was 1:74,67 that has grown to 1:106,67 at 75 days of examination. The antibody titers were lower in the fourth group of chickens which contend after 15 days of vaccination 1: 64 that has grown to 1: 128 at 75 days after vaccination. At 55 days after vaccination there is an increase in the levels titers of antibodies but if compare between groups was obtained values 40% higher in the group II compared to group III respectively 16.67% in group IV and 100% with group V.

Table 1.

The level of antibody titers against Newcastle disease virus

Nr. group of chickens	First day after vaccination	15 days after vaccination	55 days after vaccination	75 days after vaccination
1	1:74,77 ± 0,37	1: 17,33 ± 0,08	1 : 9,33± o,o	1: 10,6± 0,02
2	-	1: 90,67 ± 0,32	1: 149,33± 0,49	1: 171,33 ± 0,37
3	-	1:74,67 ± 0,24	1 :106,67 ± 0,18	1 : 106, 67 ± 0,18
4	-	1: 64 ± 0,32	1 : 128 ± 0	1 : 128 ± 0,55
5	-	1: 74,67 ± 0,37	1 : 74,67 ± 0.24	1 : 106,67± 0,18

$^{*}<, ^{**}>$

From intestinal lavages which was collected at 1, 15, 55 and 75 days were carried seeding on solid media, which have grown bacterial cultures that are part of bacteriocinosis of digestive tract of chickens (streptococci, bifid bacteria, Escherichia coli, bacteroides and others). They did not record differences between experimental bacterial cultures.

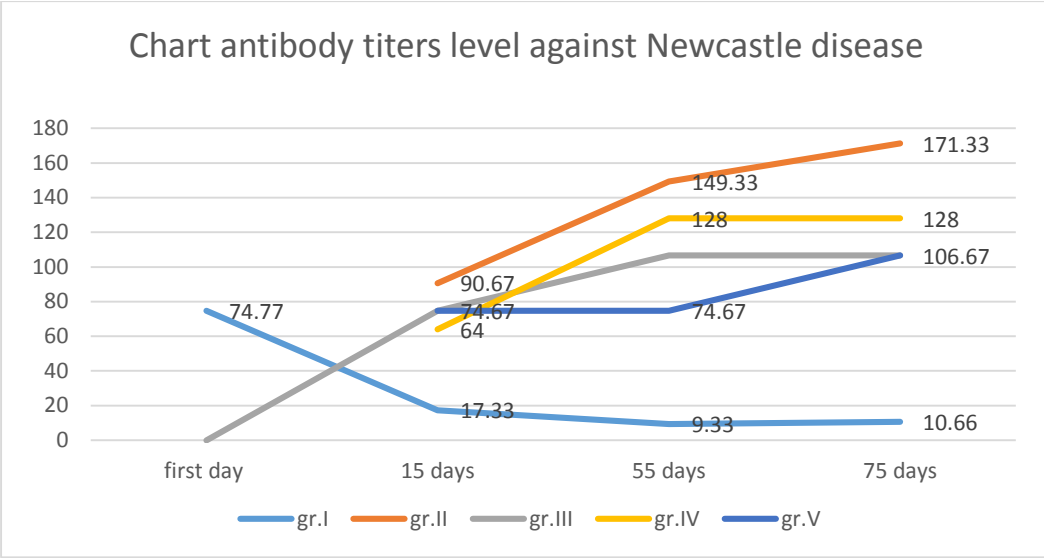


Figure 1. The graphics presentation of postvactination level of antibody titers

In this context for assessing variation in hematological consistent with post vaccine antibody titers values against Newcastle serological samples were taken which were subjected to laboratory investigations, hematological values in chickens are shown in tables 3-5.

Table 3.

Hematological values of chickens (one day old)

Indicators	Leukocytes $10^9/l$		neutrophil unsegmented %		neutrophils segmented %		eosinophils %		lymphocys %		monocytes %	
	- X	p	- X	p	- X	p	- X	p	- X	p	- X	p
Gr.I	63.53	0.04	1.33	0.07	11.33	0.57	1.53	0.07	80	4	4.33	0.22
Gr. II	67.46	0.04**	0.67	0.03	4.28	0.55	0.33	0.02	83.33	4.17	10.33	0.52
Gr.III	65.76	0.3	1	0.05	10.66	0.53	1.33	0.03	80.67	4.03	10	0.5

The investigation have shown that in the experimental chickens groups, the number of lymphocyte and monocyte had a significant upward trend similar to the average values of these parameters compared with control group. There were diametrically opposed downward changes highly significant average values of neutrophils and eosinophil compared with the control group.

The number of leukocytes of chickens from experimental groups showed a decrease of the level compared to the control group, but statistical difference between them is significant. From the data in tables may also shows that that the number of leukocytes in the blood of chickens age growths, which can be considered a physiological trend. If analyze leukocytes formula which results shown in the table 3 of all groups of chickens, it was found that lymphocytes from groups II-III have values higher than the reference group being 4.16% and 0.83%, 0.41 -1.25 % and according to table 5 - 2.48 to 0.42%.

Table 4.

Hematological indices of chickens (55-th day old)

Indicators	Leukocytes $10^9/l$		neutrophil unsegment ed %		neutrophils segmented %		eosinophils %		lymphocys %		monocytes %	
	- X	p	- X	p	- X	p	- X	p	- X	p	- X	p
Gr.I	771.13	0.36	3.67	0.02	12.33	0.06	4.33	0.02^{**}	79.67	0.39^{**}	3.33	0.01
Gr. II	83.77	0.42	3.67	0.01	9	0.04	4	0.02	80	0.4	8.67	0.04
Gr.III	73.1	0.37	4.33	0.02**	11.67	0.05^{**}	4	0.02*	80.67	0.4^{**}	4.67	0.02**

Distribution or reported unsegmented neutrophils with nucleus compared with control following values: table 3 from 80.23 to 40.11%; and in table 5 from 21.18 to 12.09%. Table 4 were poor results, record is identical between group I and II and higher values to 15.24% in group III. These values mention about of more favorable physiological condition on chickens of group II. The data presented in the tables showed the value of higher number of monocytes in the experimental groups being 51.89 ± 0.72% on average and 42.92 ± 1.85% compared with the chickens from the control group.

Table 5.

Hematological indices of chickens (70-th day old)

Indica-tors	Leukocytes 10^9/l		neutrophils unsegmented %		neutrophils segmented		eosinophils %		Lymphocys %		monocytes %	
	- x	p	- x	p	- X	p	- x	p	- X	p	- X	p
Gr.I	771.13	0.36	3.67	0.02	12.33	0.06	4.33	0.02**	79.67	0.39**	3.33	0.01
Gr. II	83.77	0.42	3.67	0.01	9	0.04	4	0.02	80	0.4	8.67	0.04
Gr.III	73.1	0.37	4.33	0.02**	11.67	0.05**	4	0.02*	80.67	0.4**	4.67	0.02**

Interpretation of these results relative to recent scientific data is therefore to the detriment of the product tested which states that the reduction of monocytes in peripheral blood may be a result of their migration into tissues, their transformation and maturation in macrophages [3].

Conclusions

Using liquid and cultural biomass of streptomycetes in combination with vaccines against Newcastle disease demonstrated a immunostimulatory action expressed by the increase of antibody titers with 23.8% and the number of lymphocytes with 4.16% compared with control group of chickens. At the same time, haven't been established a significant change in the composition of intestinal microflora of chickens.

Bibliography

1. Hailu M., Esayas G**.,** Evaluation of newcastle disease antibody level after different vaccination regimes in three districts of Amhara Region, Northwestern Ethiopia. 2010.
2. Patrizia Casagrande Proietti, Alessandro Dal Bosco, Friedrike Hilbert, et.all. Evaluation of intestinal bacterial flora of conventional and organic broilers using culture-based methods, Sci. 2010, p.51-62.
3. Карпуть И. М. и др. Микробные препараты в повышении резистентности и профилактике болезней молодняка. În: 35 ani de învăţământ superior med.vet. din RM: simpoz. şt. intern., Chişinău, 15-16 oct. 2009, pp. 115-119. ISBN 978-9975-4044-6-4.
4. Young M., Alders R., Grimes S., Controlling Newcastle disease in Village chickens, laboratory Manual. Australian Centre for International Agricultural Research (ACIAR).*2002,* Monograph. 87: 9-142.
5. Zelalem Gebretsadik A., Kidist T., Belachew B., Evaluation of the newcastle disease antibody level after vaccination regimes in chickens in Debrezeit Agricultural Research Center, Ethiopia. Debrezeit Agricultural Research Center, Ethiopia. 2013.
6. OIE. Diagnostic Techniques.In: Newcastle disease .Terresterial Manual, pp.557-86. 2009.
7. Falcă C., Mocofan E., Morar D. Influenţa unor surse şi doze de seleniu din hrana puilor de carne asupra parametrilor biochimici sanguini. În: 35 ani de învăţământ superior medical veterinar din Republica Moldova: simpoz. şt. intern., 15-16 oct. Chişinău. 2009, pp. 77-79. ISBN 978-9975-4044-6-4.