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CURPINS

DETERMINATION OF SOME ANTIMICROBIAL RESIDUES IN CHICKEN MEAT AND GIBLETS

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SUMMARY
Forty five samples of muscle, gizzard and liver (15 of each) were collected from poultry shops in Kalyobia governorate and examined for the presence of tetracycline and sulflaquinoxaline using four plate test (FPT) and high performance liquid chromatography (HPLC) . Also, the effect of boiling as a method of cooking on these residues was studied.
The results showed that 13.33%, 13.33% and 26.67% of muscle, gizzard and liver samples were positive for the presence of tetracycline residues by FPT method, respectively, however, 13.33% , 20% and 40% were positive for such residues by HPLC method, respectively. Moreover, non of the examined muscle samples were exceeded the maximum permissible limit of tetracycline while, 6.67% and 20% of the examined gizzard and liver samples were exceeded, the maximum permissible limit, respectively. Concerning, sulfaquinoxaline residue, it was found that 20% , 26.67% and 33.33 % of the muscle, gizzard and liver samples were positive by FPT method, while 26.67%, 33.33% and 46.67% by HPLC, respectively. Accurately , 33.33% , 20% and 40% of the examined muscle, gizzard and liver samples were exceeded the maximum permissible limit of sulfaquinoxaline, respectively.
On boiling , the levels of tetracycline residues were reduced by 100% for muscle samples while this percentage reached to 82.35% and 79.67% reduction for the examined gizzard and liver samples, respectively. Also, the highest reduction% for Sulfaquinoxaline residues was 100 for muscle sample after boiling, while 78.18% and 73.04% reduction were shown in gizzard and liver samples, respectively. The public health significance of such antimicrobial agents and some recommendations to control their presence in chicken meat and giblets were discussed.

Introduction

Chicken meat, derived mainly from broiler is considered as the favorite food for Egyptians due to its relatively low price n relation to the red meat. A large number of drug used to control or prevent infections or to promote growth are essential in modern poultry
production system. Antibiotics and sulfonamides are the two of the oldest groups used in veterinary medicine. ***(National Registration Authority for Agricultural and veterinary chemicals, 2000).***

Antibiotics are widely used in poultry farms as a dietary supplements. Tetracycline is one of these antibiotics which has a broad spectrum action on a variety of infection. ***(Schenck and Callery, 1998).***

About 60% of an ingested dose of tetracycline was absorbed from the gastrointestinal tract of human beings and then widely distributed in the body, particularly liver, kidney, bones and teeth. However, the use of this compound may result in residues in poultry meat especially if proper withdrawal times have not been used ***(Czeizel et al., 1998).***

The second commonly used drug in chicken broilers is sulfaquinoxaline which used for the treatment of coccidiosis and a wide range of bacterial disease. Producers may be unwilling to withhold the drug for a sufficient long period to ensure that all residues have been eliminated before slaughter , so the residue of sulfaquinoxaline may be retained in the edible tissues of marketed chicken broiler (***Kozarova et al., 2001a and kozarova et al., 2001b) and (Sutiak et al., 2000).***

Sulfaquinoxaline is also administered to poultry at a rate in excess of that recommended or at more frequent interval than specified. Therefore, other negative effects of sulfonamide on human body

have been reported after long period of consumption of animal products containing trace amounts and resulted in a build up of resistance and the development of hypersensitivity to sulfonamide ***(A garwal, 1992 and Spoo and Riviere, 1995)***. The maximum residual level of sulfonamide all compound of sulfonamide group was 0.1 mg / kg in food of animal origin, therefore in order to overcome the problem of drug residues, the dugs must be administered only in recommended concentrations and their withdrawal times must be observed together with the original 7 days withdrawal period must be in creased to 15 days ***(Augsburg, 1988).***

There fore, the in of the current study is to determine the levels of both tetracycline and sulfaquinoxaline residues in muscle, liver and gizzard of chicken broilers that were freshly dressed and purchased randomly in poultry shops at Kalyobia governorate by using four plate test (FPT) and high performance liquid chromatography (HPLC) .Also, study the effect of boiling as a method of cooking on these residues.

MATERIAL AND METHODS

A total of forty five random samples of muscle, liver and gizzard collected from 15 chicken broiler carcasses after being dressed and purchased at different poultry shops in Kalyobia governorate. Each sample was individually wrapped in a polyethylene bag and analyzed for detection of antibiotic (tetracycline) and salfonamide (sulfaquinoxaline) residues either by four plate test or HPLC technique.

1. Four plate test " FPT" (Microbiological menthod):.

The method recommended by ***Bogaerts and wolf (1980)*** was carried out. Each sample was divided and applied to four plates of antibiotic agar medium, three of which were inoculated with Bacillus subtilis spores at PH 6,7.2 and 8. Moreover, trimethoprim was incorporated into the medium at PH 7.2 to enhance the test for sulfonamide residue. Diffusion of the the active tetracycline and sulfaquinoaline were detected by the formation of inhibition zones on one or more plates after incubation at 37c^{o} overnight.

The sensitivity of the test was monitored by applying 6mm – diamegter discs containing standard quantities of known antimicrobial agents in each run.

2. Application of HPLC technique:.

Quantitative analysis of antimicrobial agent in the examined samples of chicken muscle, liver and gizzard was done accordling ***to Pieckova and Van Peteghem (2001) and Oka et al., (2003).***

Accurately, 5 gm of each sample and 10 gm of alhydrous sodium sulfate were blended with 20 ml of ethyl acetate and then centrifuged. The supernatent was evaporated and dryed under reduced pressure at 40 c^{o} .

The residue was dissolved in 5 ml of ethyl acetate – n- hexane and the solution was applied to a bond Elute previous washed by 5 ml n – hexane, The cartridge was washed with 3ml n – hexane and air – dried by aspiration.

The surveyed antimicrobial residues were eluted from the cartridge with 5ml acetonitrile (20%) and 0.05 M ammonium format. The preparation was injected into HPLC system (model LC – 10A series equipped with constant flow pump and variable wave length U/V detection***, Kyoto, Japan).***

Accordingly , tetracycline and sulfaquinoxaline residues were estimated by using their standard solutions specific for each of them. Operating conditions for analysis of tetracycline were eluant at 35c^{o} , flow rate 1 ml , injection volume, 10 ul; detection wave length 216 nm. While , the operating conditions for analysis of sulfaqunoxalilne were; eluant at 30 c^{o} ; flow rate, 1 ml / min.; injection volume, 20 ul and detection wave length 272 nm.

Statistical analysis of the obtained results was done by application of analysis of variance " ANOVA" according to ***Rosner (2002).***

RESULTS

Table (1) Incidence and levels of tetracycline residues (ug / kg) in the examined samples of broiler muscle and giblets (n = 15).

Method of detection / Examined samples	Positive Samples				Min	Max	Mean	S.E
	FPT *		HPLC **					
	No	%	No	%				
Muscle	2	13.33	2	13.33	20	85	52.50	9.42 ++
Gizzard	2	13.33	3	20.00	35	340	168.23	26.81
Liver	4	26.67	6	40.00	65	910	316.67	35.09

* = Four Plate Test.

** = High Performance Liquid Chromatography.

++ = High significant differences ($P < 0.01$).

Table (2) Acceptability of the examined samples of broiler muscle and giblets based on their levels of tetracycline residues.

Examined samples	No. of examined samples	Maximum permissible limit (ug/kg) *	Unaccepted samples	
			No	%
Muscle	15	100	-	-
Gizzard	15	200	1	6.67
liver	15	600	3	20.00

* FAO/WHO (1999)

Table (3) Incidence and levels (mg / kg) of sulfaquinoxaline residues in the examined samples of broiler muscle and giblets (n = 15).

Method of detection / Examined samples	Positive Samples				Min	Max	Mean	S.E
	FPT *		HPLC **					
	No	%	No	%				
Muscle	3	20.00	4	26.67	0.05	1.10	0.43	0.06+
Gizzard	4	26.67	5	33.33	0.08	2.25	1.02	0.19
Liver	5	33.33	7	46.67	0.10	3.45	0.81	0.11

* = Four Plate Test.

** = High Performance Liquid Chromatography.

+ = Significant differences ($P < 0.05$).

Table (4) Acceptability of the examined samples of broiler muscle and giblets based on their levels of sulfaquinoxaline residues.

Examined samples	No.of examined samples	Maximum permissible limit (mg/kg) *	Unaccepted samples	
			No	%
Muscle	15	0.1	2	13.33
Gizzard	15	0.1	3	20.00
liver	15	0.1	6	40.00

* Council Regulation (1990)

Table (5) Effect of boiling on the antimicrobial residues in the examined samples of broiler muscle and giblets.

	Tetracycline (ug/kg)			Sulfaquinoxaline (mg/kg)		
	Before boiling	After boiling	Reduction %	Before boiling	After boiling	Reduction %
Muscle	85	ND	100	0.46	ND	100
Gizzard	340	60	82.35	1.10	0.24	78.18
Liver	91 0	185	79.67	3.45	0.93	73.04

ND = Not Detected

DISCUSSION

Residues of veterinary drugs in food of animal origin represent a risks to human health and they have negative impact on the technological processes in the food industry. Accordingly, the improper use of veterinary drugs may result in drug residue in the poultry tissues causing allergic reactions in sensitive individuals consuming such food stuff (Kozarova et al., 2001a,b).

Results achieved in table (1) declared that 13.33% , 13.33% and 26.67% of the examined samples of broiler muscle, gizzard and liver were positive for the presence of tetracycline residue by application of FPT trial, respectively, however, such percentages were 13.33%, 20% and 40% by using HPLC, respectively. Thus, there was awide variation between the two methods of tetracycline determination Generally, the level of tetracycline residue were ranged from 20 to 85 with an average of 52.50 9.42 ug /kg for broiler muscle, 35 to 340 with an average of 168.23 26.81 ug/kg for gizzard and 65 to 910 with an average of 316.67 ug/kg for liver. The differences between the examined samples of broiler muscle and giblets were highly significant ($P < 0.01$) as a results of their levels of tetracycline residues.

Actually, **FAO/WHO (1999**) established the maximum permissible limits of tetracycline residues to be 100,200 and 600 ug/kg for broiler muscle, gizzard and liver, respectively. Therefore, 6.67% and 20% of the examined samples of gizzard and livers exceeded such permissible limits and unfit for human consumption. In contrast, all examined samples of broiler muscle were accepted (table, 2).

The obtained results come in accordance with previous studies, in Egypt, indicating the detection of antibiotic residues in chicken meat and giblets above the permissible limits (**Ahmed and Ahmed,1989 and Salem , 1998).**

Liver is the main organ for accumulation of antibiotic residues because liver is responsible for metabolism and detoxication of the drug by its microsomal enzymes, also the failure to observe the withdrawal period for antibiotics in poultry before they were slaughtered for human consumption may also responsible for the residue accumulation in its tissue such as muscle and liver.

Withdrawal times of antibiotics is considered as a safe way for avoiding its residues in human food of animal and poultry origin. (**Arichimbault et al., 1978, Anadan et al., 1990 and Mignot et al., 1993).**

Improper dosage of tetracycline may lead to presence of its residue in broiler tissues and have side effects to consumers in the from of toxic, allergic and teratogenic reactions as well as emergence of resistant bacteria to this antibiotic (**Oka et al., 2003).**

In man, approximately 60% of the ingested dose of tetracycline and its derivatives as oxytetracycline and chlortetracycline were absorbed from the gastrointestinal tract and distributed in the body, particularly liver, kidneys, bones and teeth (**Czeizel et al., 1998).**

Table (3) revealed that the mean values of sulfaqinoxaline residues in the examined samples of broiler muscle, gizzard and liver were 0.43 0.06, 1.02 0.19 and 0.81 0.11 mg/kg, respectively. More over, there was variation in accuray of FPT and HPLC for detection of sulfaquinoxaline in broiler muscle (20% and 26.67%) , gizzard (26.67% and % 33.33%) and liver (33.33% and 46.67%) . Significant differences ($P < 0.05$) were appeared between the concentrations of sulfaquinoxaline in the examined muscle, gizzard and liver samples.

Acceptability of the examined samples of broiler muscle and giblets based on their levels of sulfaquinoxaline was shown in table (4) . Thus, 13.33%, 20% and 40% of broiler muscle, gizzard and liver were unfit and exceeded the maximum permissible limit (0.1 mg/kg) stipulated by **Council Regulation (1990).**

Nearly similar were recorded by **Davitiyanada et al. (1996) and ya-Min et al. (2001)** who estimate this residue in broiler muscle and liver by levels of 0.58 and 1.23 mg/kg, respectively. While, higher levels of this residue in broiler muscle and liver (1.98 and 3.80 mg/kg) were reported by **El-Gazzar and El-Lawendy (2005**). On the other hand, **Salem (1998)** could not detect sulfaquinoxaline in surveyed chicken muscle and liver samples.

There is concern that sulfaquinoxaline may be retained in the edible tissues of broilers and the clearance rate may be varied
according to age of bird, route of administration, preslaughter with-holding time and husbandry practices which can result in violative tissue residues at slaughter even when the drug is used within the recgonized dose **(Sutiak et al., 2000).**

Direct toxic or allergic reactions after consumption of chicken tissues containing sulfonamide residues have been reported (**Spoo and Rivieri,1995)**. The accumulation of these residues inside the human body may cause disruption of normal flora in the intestinal tract **(Doyle,2006).** Also, sulfonamides have negative effects on the thyroid gland in relation to the development of the thyroid gland tumors (**Davitiyananda et al.,1996).**

Effect of boiling on the tetracycline and sulfaquinoxaline residues in the examined samples of broiler muscle and giblets was indicated in table (5). Concerning tetracycline residues, the reduction percentages were 100%, 82.35% and 79.67% for the muscle, gizzard and liver, respectively. On the other hand, the reduction percentages in sulfaquinoxaline residues in the examined samples of muscle, gizzard and liver were 100%, 78.18% and 73.04%, respectively. Furthermore, all examined broiler muscle samples became free from tetracycline and sulfaquinoxaline residues by boiling due to their lowest levels of such residues as compared with gizzard and liver samples.

The present study allow to confirm the bad hazard using of antimicrobial drugs inside the poultry farms and the failure in monitoring the withdrawal periods of such drugs. Accordingly, the concerned authorities must take extra efforts for corrective use of veterinary drug for solving the problem of such residues in broiler tissues.

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RESEARCHES REGARDING THE SEROPREVALENCE OF SWINE HEPATITIS E VIRUS INFECTION IN THE EAST OF ROMANIA

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Hepatitis E virus (HEV) is ubiquitous in european swine herds, but does not cause clinical illness in swine. Hepatitis E is a zoonotic disease risk and cross-species infection occurs, for which pigs play a role as a reservoir. In 2003 the first direct evidence of zoonotic transmission of HEV was documented in two outbreaks involving human consumption of raw wild boar liver and raw deer meat. HEV infection spreads by the fecal-oral route, usually through contaminated water.
The objective of this study was to investigate the prevalence of serum antibodies to hepatitis E virus in swine from household system in the east of Romania. A total of 170 serum samples collected from healthy pigs were examined by enzyme immunoassay for IgG anti-HEV. Because there is no vaccine for preventing hepatitis E virus in swine, the pigs found seropositive were the one naturally infected with the virus. IgG anti-HEV was detected in 29 serum tested, representing 17%.

Key words: *HEV, swine, enzyme immunoassay.*

Hepatitis E virus (HEV) is an enteric virus that is transmitted basically through contaminated water. Hepatitis E virus (HEV) is a small non-enveloped virus belonging to the Genus Hepevirus (Emerson et al., 2004), the Hepeviridae family. The virus presents a single-stranded positive sense RNA molecule of 7.2 Kb containing 3 open reading frames (ORFs). The ORF 2 encodes the capsid protein of the virus, that is the most immunogenic and conserved protein. Nowadays, available systems for HEV diagnostic are based on recombinant peptides of the ORF-2 and ORF-3 proteins of genotype 1 and 2 strains and are designed for use in humans. Hepatitis E is one of the main causes of acute hepatitis in tropical areas with inadequate sanitary conditions, where it causes large epidemics. In the last decade, there is an increase of reported autochthonous cases of hepatitis E in developing countries. HEV has been classified in 4 genotypes, all of which infected humans. In addition, genotype 3 and 4 have been isolated in pigs. (Emerson and Purcell, 2003; Clemente-Casares et al., 2003). Swine and human strains of a given geographic region are genetically more closely related to each other than strains of the same species from different geographic regions (Banks et al., 2004; Lu et al., 2006). Fifth type has been detected in birds and it is called Avian HEV. This type is distinct from the other four and does not seem to infect non-human primates. It is not known whether Avian HEV represents a fifth genotype of HEV or belongs to a separate genus (Huang et al., 2002). In addition to pigs and deers, so far HEV has been detected only in horses (Saad et al., 2007) and mongooses (Nakamura et al., 2006). Antibodies against HEV have been also demonstrated in species other than the abovementioned such as cows, sheeps, goats, dogs, cats and rodents (Arankalle et al., 2001; Zhang et al., 2008) but the epidemiological role of those domestic species is uncertain. Most of these serological studies in domestic animals other than pigs have been carried out in Asia and information about Europe is still lacking.

Swine HEV isolated in Europe are mainly of genotype 3. Due to the potential risk of zoonotic transmission, it is important to establish the seroprevalence of hepatitis E in the romanian swine from household system in the east region of the country.

MATERIALS AND METHODS

One hundred seventy pig blood samples collected from five counties in the east region of Romania were used in this study; all samples were from household system, pigs sampled appeared to be clinically healthy. The swine serums were tested for IgG antibodies against HEV (anti-HEV) by enzyme immunoassay. For the detection of antibodies anti-HEV was used the HEV ELISA kit produced by MP Biomedicals, according to the manufacturer's recommendations and modified using of anti-swine IgG horseradish peroxidase enzyme conjugate (P.A.R.I.S. anticorps). Blank solution, non-reactive and reactive controls were included for each plate.

RESULTS AND DISCUSSION

The swine blood samples were collected from five counties placed in the eastern region of the country. The number of the samples collected from each county is different, ranging from 20 to 59 blood samples, about 10 samples for each village.

Table .1

Results of the serologic exam for the detection of IgG anti-HEV on pig sera

County	No. of serum tested	No. of positive serums	No. of negative serums
Iași	38	5	33
Botoșani	59	10	49
Neamț	23	4	19
Tulcea	20	3	17
Brăila	30	11	19
Total	170	33	137

Thirty-three out of 170 pigs (19,41%) were IgG anti- HEV reactive by EIA (enzyme immunoassay). Anti-HEV seroprevalence ranged widely over the counties, from 13,15% to 36,66%. Fig. 1 shows the distribution of the seroreactivity to HEV according to the kit interpretation for the swine serum samples collected at different counties when tested by EIA. It could be observed that most of the serum samples from Iași and Tulcea counties were non-reactive to HEV.

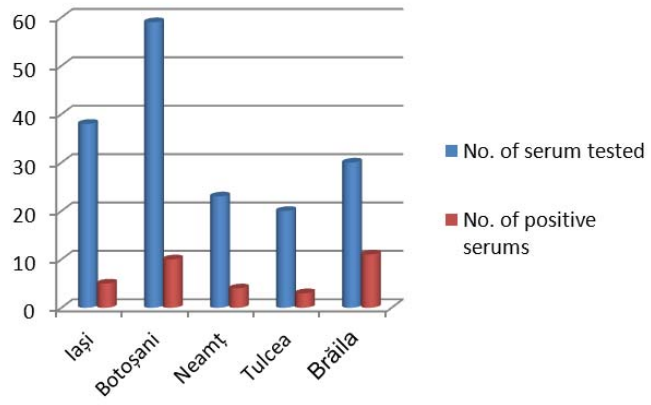

Fig.1. Distribution of the seroreactivity to HEV

Specific antibodies against HEV are only rise from natural infections because of the lack of existence of a vaccine against swine hepatitis E virus infection. Our serological data point out that circulation of HEV occurs in household system, demonstrating that the hepatitis E virus infection is spread over the region. The number of seropositive animals can be explained by the fact that the privet owners may have 3 to 5 pigs, and disease transmission (through oral-fecal) is hardly possible between households.

The importance of HEV in romanian pigs as a cause of the diseases is unknown, but the abundant presence of infected pigs raises the concern of potential zoonotic transmission of the virus infection. Raising the awareness of the importance of the adequate cooking of meet, the boiling of drinking water and washing of vegetables are likely to be effective public health intervention to prevent hepatitis E infection.

The research in this study was made by fund type project agreement CNCSIS type IDEI nr.1104/2009.

CONCLUSIONS

- The 33 positive serums, representing 19,41%, in domestic pigs from household system were detected using un human ELISA kit adapted for swine.
- Antibodies evidence show that hepatitis E virus is circulation among domestic swine in the five counties from eastern region of Romania.

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SEROEPIDEMIOLOGICAL STUDY REGARDING INFECTIOUS BOVINE RHINOTRACHEITIS IN COUNTIES FROM NORTH OF MOLDOVA REGION

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Infectious bovine rhinotracheitis (commonly called IBR or red nose) is an acute, contagious virus disease of cattle. This infection usually occurs in the air passages of the head and the wind pipe. However, in females this virus also causes inflammation of the vulva and vagina and abortion. Abortion occurs about 20 to 45 days after infection. Infectious bovine rhinotracheitis (IBR) is caused by bovine herpesvirus-1 (BHV-1) and is recognized as one of the major cattle diseases of economic importance. Wherever cattle are confined, or groups are permitted to commingle as in feedlots and collection points, the disease is rapidly spread to new arrivals from cattle already infected or those recovered carriers that serve as virus reservoirs and shedders of infection.

Our study was made during 6 month, from October 2009 until April 2010 in three counties from north of Moldova region (Botoșani, Suceava and Iași county). Blood samples were taken from cows, bulls and calves from small farms and household system. From 339 blood samples tested we found 131 positives representing 38,64% and 208 samples were found negatives (61,36%). The serologic exam was made using an enzyme linked immunosorbent assay test produced by Svanova Veterinary Diagnostics – Svanovir® IBR-Ab (confirmation format).

Key words: IBR, ELISA, antibody, cattle

Bovine Herpesvirus Type 1 (BHV1) is the aetiological agent of a number of diseases and not only of IBR, namely infectious pustular vulvovaginitis (IPV), infectious balanoposthitis (IBP), conjunctivitis, encephalomyelitis, mastitis, abortion, enteritis, and lesions in the interdigital space. (Straub O.C., 2001) The serological identical strains differ, however, in some aspects. Typical genital strains usually cause a mild illness, sometimes not even detected clinically, but serologically. Prevalences vary greatly depending on herd size and management. Because seronegative cattle play a role in international trade a number of European countries have eradicated BHV1, with very high costs involved. For the detection of humoral antibodies the ELISA is widely used.

Efficient methods of diagnosis and prophylaxis of infectious bovine rhinotracheitis must consider the concept of latency of the etiological agent, infectious bovine rhinotracheitis virus (Bovine herpesvirus 1; BHV 1).

MATERIAL AND METHODS

The investigations were made during 6 month, from October 2009 until April 2010 in three counties from north of Moldova region (Botoșani, Suceava and Iași county). The bovine blood samples were collected from small farms and household system. For the preservation of samples prior to analysis, the blood serums was obtained after centrifugal separation and was stored at -20^{0}C.

The serologic exam was made using an enzyme linked immunosorbent assay test produced by Svanova Veterinary Diagnostics – Svanovir® IBR-Ab (confirmation format) according to the manufacturer's recommendations.

RESULTS AND DISCUSIONS

During this study were collected 339 blood samples from cows, bulls and calves from small farms and household system from tree counties: 102 samples from Iași county, 210 from Suceava county and 24 samples from Botoșani county.

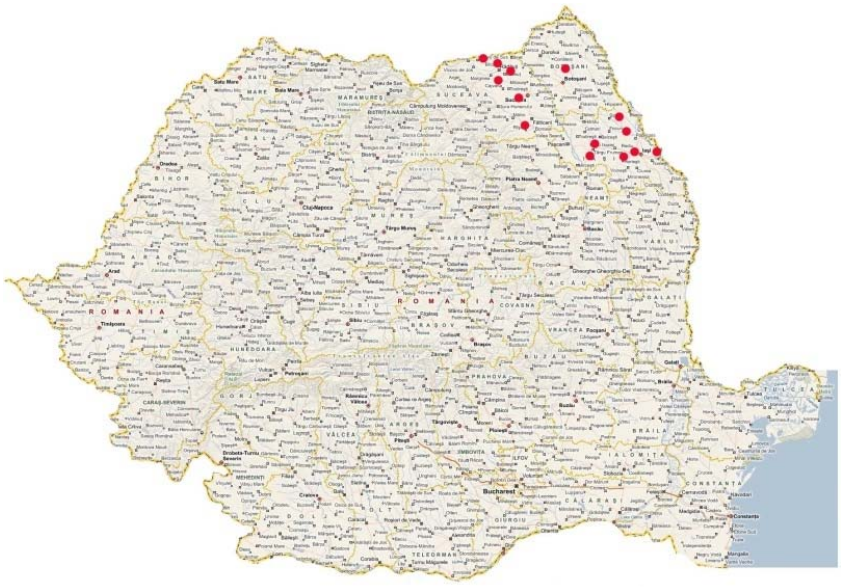

Fig. 1 Distribution of the localities from where were collected the serum samples

Table no. 1. Results of the serologic exam on bovine serum samples

Date of the examination	Location	Samples analyzed	Positive results	Negative results
13.11.2009	Movileni (IS)	12	**5**	7
	Bosia (IS)	13	**0**	13
	Strunga (IS)	19	**15**	4
20.11.2009	Strunga (IS)	3	**3**	0
	Bălțați (IS)	22	**0**	22
	Miroslava (IS)	23	**23**	23
12.02.2010	Milișăuți (SV)	30	**8**	22
	Bilca (SV)	12	**0**	12
17.02.2010	Bilca (SV)	40	**0**	40
03.03.2010	Moara (GP) (SV)	29	**22**	7
	Bilca (SV)	11	**0**	11
01.04.2010	Moara ferma (SV)	25	**0**	25
	Baia ferma	5	**0**	5

	(SV)			
	Moara ferma (tauri) (SV)	5	**5**	0
	Vlădeni (IS)	4	**4**	0
22.04.2010	Iacobești (SV)	36	**18**	18
	Dumești (IS)	6	**5**	1
26.04.2010	Baia fermă (SV)	4	**0**	4
	Milișăuți (SV)	4	**0**	4
	Iacobești	9	**9**	0
	Ungureni (BT)	24	**15**	9
Total		**339**	**131**	**208**

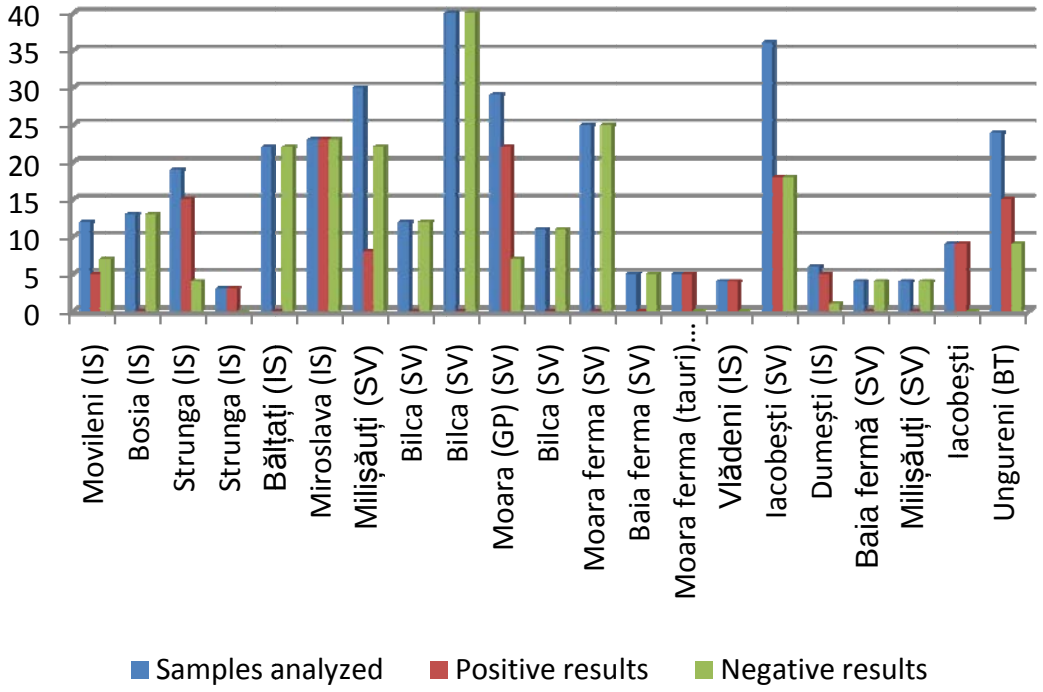

Fig. 2 Results of serologic test for each village and county

The serological exams using the ELISA to detect Infectious Bovine Rhinotracheitis specific antibodies (IgG_1) in bovine serum, showed a positivity of 38, 64% (131 samples) from 339 samples tested, and 208 samples were found negatives (61,36%).

CONCLUSIONS

1.The results of this study clearly established that IBR infection is prevalent in cattle. ELISA is required to solve this problem, which must be cheap, highly sensitive and could be used on large scale for screening and eradication programs.

2.The stock density and mixing of the animals in closed farms allow the virus to spread. In addition, due to the virus latency that is normal criteria of BHV-1, the identification of serologically positive apparently healthy animals, provide a useful indicator of infection status.

3.In this study may be explained the presence of high percentage of seropositive dams in endemically infected herds, despite some authors claimed that colostrum derived antibodies in young calves have protective effect.

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USING SOMATIC CELLS COUNT AND BACTERIAL COUNT TO EVALUATE MILK PRODUCTION IN ONE DAIRY FARM IN IASSY COUNTRY

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ABSTRACT
The most spread mammary gland diseases are mastitis, the inflammation of the mammary gland, often caused by bacterial infection (2). Most farmers associate mastitis with an inflamed quarter and with a change of milk appearance, but there are also subclinical mastitis, with no visible external changes, although infection is present in the mammary gland. In this case we can observe only a decreased in milk quantity, that varies with the severity and duration of the infection and the causative microorganisms. In general, the higher the SCC, the higher the milk loss (7). Analyzing the milk, we can determine compositional changes due to the increase in somatic cell following infection of the mammary gland and also the presence of bacteria. Somatic cell count is used as one of the most commonly indicator of mammary gland infection, as well as the total number of germs. Subclinical mastitis are desirable to be detected, so that milk should maintain its organoleptic properties (1,3).
The aim of this paper is to find the correlation between the number of somatic cells (NCS/ml), total number of germs (NTG/ml) and the milk quota.
Key words: milk production, mastitis, somatic cells, total number of germs

MATERIALS AND METHODS

To respect E.U. standards, quality milk must fulfill certain conditions, including the maximum total number of germs admitted under 100.000 germs/ml and and maximum somatic cells of 400.000/ml milk (Directive 92/46/EC, Decision 95/342/CEE). In our country through the farms self-control program imposed by ANSVSA, total number of germs (NTG / ml) and of the somatic cells (NCS / ml) in milk samples are determined in LSVSA.

The study was conducted in farm A, located in Iassy County, a herd of 290 cows in milking process, with high number of somatic cells from bulk tank milk and a high incidence of mastitis at different periods of lactation. Study material was represented by cows of breed Romanian Black Pond (NBR).

From January 2007 to November 2009 bulk tank milk samples were analyzed. Research was conducted on a lot of 75 bulk tank milk samples that have been collected each month, during these 3 years. Bulk tank milk samples were analyzed to determine milk quality, so the data including in the analysis represents BTSCC and NTG tests results and also the quota of milk corresponding to each month in this farm. Somatic cell count was performed using the "SomaScope MKII" (Delta), by the citometric method and fluorescence. To establish the total number of germs, plate count was performed using an automatic colony numerator "aCOLyte" after VR EN ISO 4833/2003.

RESULTS AND DISCUSSIONS

A total number of 77 composite milk samples were analyzed during the 3 years of study. There has been an increase in 2008 number of samples: 34 samples were analyzed, instead of 21 samples in 2007 and 22 samples in 2009. Each year, the milk quota increased, as shown in table 1. Research show high average milk production in 2008 (4122.4 l/day) and in 2009 (4422.4 l/day), comparing to the low average milk production in 2007 (only 1103.4 l/day).

Table 1. Average milk quota per month in Farm A,
from January 2007 to November 2009

Month	Average of milk quota/day		
	2007	**2008**	**2009**
1	1000	3825	4066
2	1000	3912	4350
3	1100	4350	4270
4	1100	4275	4283
5	1200	4300	4300
6	1400	4300	2850
7	1366	4225	4150
8	1100	4216	4750
9	1100	4200	4950
10	1000	3790	5500
11	950	4216	4850
12	925	4016	4750
Annual average	1103.4	4122.4	4422.4

In 2007, milk production was constant, with an average milking quota of 1100 liters/day. In 2008, the milk production fluctuated between the lowest value of 3790 liters/day, in October, and the maximum of 4350 liters/day, in March.

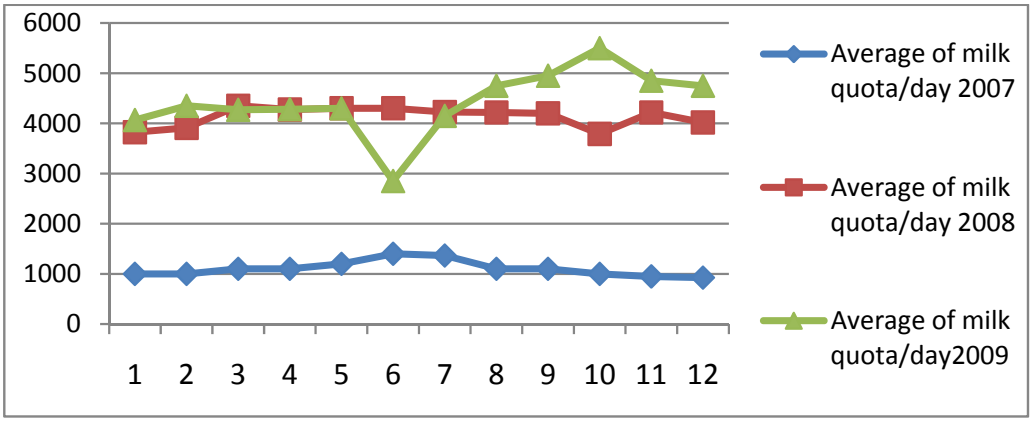

Fig. 1. Comparison between variation of milk quota average from 2007 to 2009 in Farm A

In 2009 were the most visible fluctuation in the milk quota, with an important decreased milk production in June, only 2850 liters/day. The highest milk production in farm A was observed also in 2009, in October, 5500 liters milk/day. In 2007 and 2008, an increased milk production was observed in the hot season, from May to June, but in 2009, the milk production was higher during winter, from October to December.

Table 2. Monthly average of SCC and NTG from January 2007 to December 2009 in Farm A

	2007		2008		2009	
Month	average SCC/ml	average NTG/ml	average SCC/ml	average NTG/ml	average SCC/ml	average NTG/ml
1	250000	150000	124037	73500	290869	284189
2	335757	117050	277611	57825	295900	54103
3	359000	123800	164215	66900	345900	59800
4	236767	74800	253080	69700	106833	49026
5	44950	12400	103830	41000	356700	182400
6	20000	47600	108830	57500	460850	183100
7	60386	523941	432560	67200	183445	68512
8	188870	80701	76053	110133	40000	87600
9	185000	82133	487453	529400	86570	45700
10	302381	52200	677454	890148	63793	59500
11	68165	65200	414100	92000	65700	56400
12	165476	77400	389719	243933	87600	87650
Annual average	184729.3	117268.8	292411.8	191603.3	198680	101498.3

Our study showed that SCC annual average in Farm A is within the limits imposed by the European Union, but the annual average of NTG in Farm A exceeds the maximum value admitted by the U.E. standards.

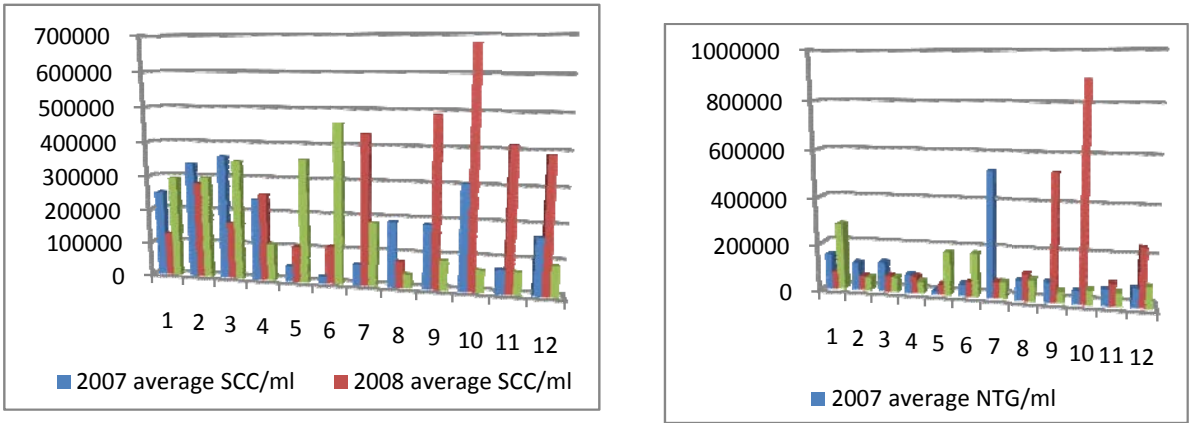

Fig. 2. Comparation between SCC average and bacteriological status in farm A, from January 2007 to December 2009

The statistics indicate that high NTG in Farm A was frequently observed during spring, autumn and winter in each of the 3 studied years, but an increased value of SCC was observed during all months, with high variation values, as shown in the Fig.2. High SCC values are associated with an increased NTG values, but there are months of winter and autumn where the NTG was high, but the SCC values were low. The highest values obtained for the average of SCC an NTG was in October and November, when both parameters showed a more pronounced increase.

Our data agrees with what others have demonstrated before (4,6,7), that milk production is affected by the number of somatic cells and by the number of germs. In November 2008 was recorded the lowest value of milk quota (3790 liters), 9% lower than the average of milk quota for that year (4122.4 liters). This decreased can be correlated with the high SCC and NTG. Also in June 2009, an increased number of germs and somatic cells affected the average milk production (only 2850 liters). Milk quota in June decreased by 36% compared to the average of annual milk production (4422.4 liters).

CONCLUSIONS

1. Each year, from 2007 to 2009, the milk quota in Farm A increased and a decreased annual average of SCC and NTG was observed.

2. Annual average of SCC in Farm A was within the limits imposed by the European Union, but the annual average of NTG in Farm A exceeded the maximum value admitted by the U.E. standards.

3. High NTG values in Farm A were frequently observed during cold, rainy seasons in each of the 3 studied years, but instead, an increased value of SCC was observed during all seasons, with high variation values.

4. Milk production was affected by the number of somatic cells and by the total number of germs. An increased lactational SCC and NTG showed a decreased milk quota.

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SELECTION RESPONSES OF ALABIO DUCK (*Anas platirinchos Borneo*) PRODUCTION IN INTENSIVE MAINTENANCE SYSTEM

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ABSTRACT

*This study aims to determine the Performances of **Alabio** duck (**Anas platirinchos Borneo**), which maintened in an intensif system and the annually selection responses. This study used 164 Alabio Duck aged 22 weeks, and the research conducted for 12 months in uniform environment. The parameters are number of egg production, egg weight and duck day production. Research method is Completely Randomized Design (CRD) and Harvey's Test Range Analysis. Results indicated that maximum egg production are 304 eggs, and minimum 75 eggs per duck per year. Maximum egg weight 73.4 grams and minimum 55.0 grams. Production intensity is 56.66 percent with a peak production of 84.33 percent. From the population, there are selection response of 30.11 eggs per year and annually 20.07 eggs.*

Keywords: Alabio duck (Anas platirinchos Borneo), Egg Weight, Duck day Production.

Duck is a potential poultry that has promising prospects for further development, because in addition to a productive poultry egg producers also have a diseases high durability enough.

Alabio duck is a type of high egg production, was originally from South Kalimantan (Alabio), with extensive maintenance system (traditionally), so the appearances is still very diverse. Still very high diversity, both phenotypic and genotypic, because it has not improved genetic quality. Increased productivity will occur when the above constraints have to be overcome, especially if maintenance pattern had change from extensive maintenance system into an intensive care system.

Selection is an effort to improve the genetic quality of birds while maintaining its purity. This program will be effective if it is already known parameters quantitative traits in an economic value. This parameter indicates the selection criteria that will be used to obtain duck with an adaptive genetic superiority with social conditions of local communities, thus providing many benefits for humans (Philipson and Rege, 2002).

Selection of mass or individual selection is the simplest selection of individuals selected on the basis of performance alone (Hardjosubroto, 1994). Performance is influenced by genetic and environmental factors; the general performance of bird is described through qualitative and quantitative traits. Quantitative trait is a trait controlled by many pairs of genes, environment and can be measured. This trait related to the ability of a bird including egg production, egg weight, body weight and so forth. While the qualitative nature of a trait controlled by only one or two pairs of genes, little or not influenced by environmental factors and can not be measured like feather patterns and colors shank (Warwick, et al, 1995).

Performance generated on animal that are kept in a uniform environment is the expression of genetic potential (Martojo, 1995).

With the knowledge of the Alabio production performance reared in an intensive care system as a selection criterion, then the prediction of genetic improvement of production traits can be calculated. The progress of a program selection in animal breeding can be known based on selection responses. Selection response is the average increase in phenotype from one generation to the next as a result of selection on the population. Selection responses is a selection of responses from one program to estimate the magnitude of genetic progress made in the next generation if the present generation of selection. By knowing the magnitude of selection response properties of Alabio production then we can determine the breeding policy to improve the productivity through genetic improvement.

MATERIAL AND METHOD

This research was conducted at the Animal Research Institute/Balai Penelitian Peternakan (Balitnak) Ciawi Bogor for 12 months. Declared free of disease Alabio, in individual cages which have been provided for one-year production. Feed and water were given ad-libitum, with intensive management system in an uniform environment maintenance.

Parameter and Statistical Analysis

The parameters that were measured include: Egg Production, Egg Weight and Day Duck Productions. Samples were taken randomly from its original place, while the analysis of variance was tested using Harvey's statistical model as follows:

$$Ykm = \mu + \alpha k + ekm$$

Where: Ykm an examination of the k individual, repeated to-m, m is General Average, α k: Influence of observations on individuals to - k , ekm: Error on the individual to - k, replicates to – m.

To calculate the response of selection used in selection intensity, because the selection of population has not been done. Total population is maintained from the overall population 20% or as many as 33 heads of 164 Alabio duck. The amount of the intensity of selection taken from Becker's research (1975) based on the total population is maintained. Heritability values of properties used as the selection criteria used heritability value obtained by Rahmat (1989).

The formula used:

$$R = h^2 \text{ x } I \text{ x } P\ \sigma$$

Where, R: Response Selection h^2 is heritability of the respective properties, I is intensity of selection, σ P is Standard deviation of the respective properties.

Selection response per year:

$$R / yr = (h^2 \text{ x } I \text{ x } \sigma P / L)$$

Where, R / yr response selection per year, L is Interval Generation.

RESULTS AND DISCUSSION

Number of Eggs

Egg production is the main parameter of the performance of laying birds and depicted in the number of eggs produced by individuals within a certain period of spawning period. Survey data by the Alabio number of eggs results of analysis of variance and ranking can be seen in Table 1.

Table 1. Total Egg Production Alabio per Year

Value	Number of Eggs
	... ... Grains ... ...
Average	214.72
Standard deviation	42.25
Variation Coefficient	19.68
Max	304
Min	75

Notes : Samples 164 duck

From the research results obtained by averaging the number of eggs per duck per one year of production as much as 214.72 ± 42.25 eggs, the result is smaller than the results of Gunawan (l987) who get an average number of Alabio eggs per duck per year 220 eggs. Similarly, when compared with the results of Rachmat (1989) who find the average number of Alabio eggs per duck per year were 227.92 ± 63.63 eggs, if we look at the overall data obtained Alabio population under study had a mean number of eggs a maximum of 304 grains per duck per year and a minimum of 75 grains per duck per year.

Egg weight

Survey data Alabio egg weight results from the analysis of variance and the rank can be seen in Table 2.

Table 2. Egg weight Alabio One Year of Production

Value	Egg Weight
	... ... grams ... ... …
Average	63.88
Standard deviation	3.30
Variation Coefficient	5.16
Max	73.4
Min	55.4

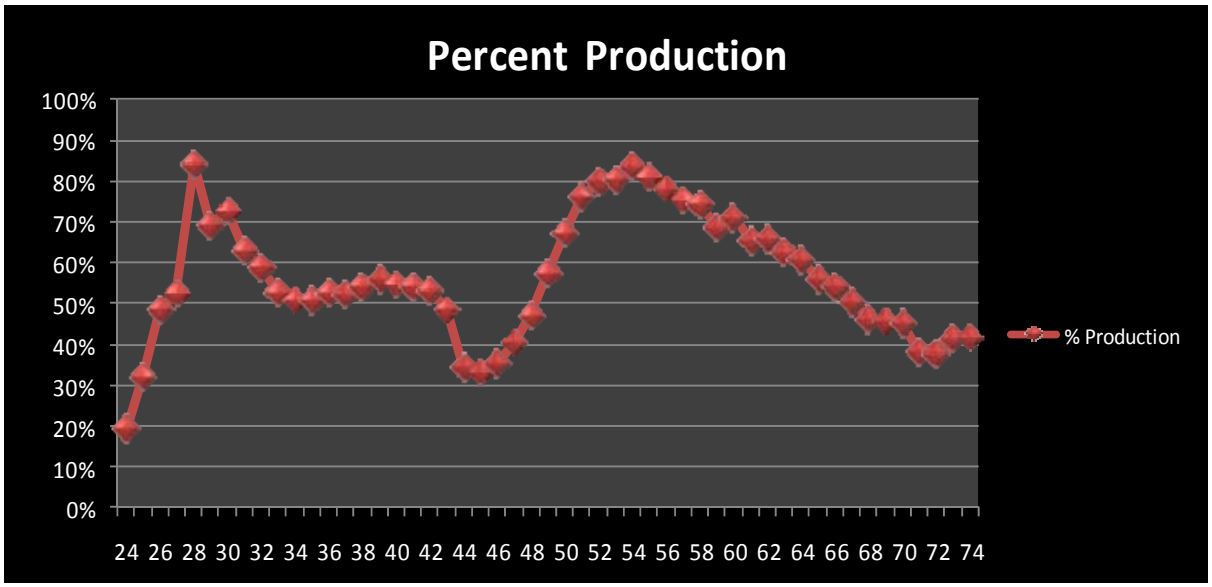
Notes : Samples 164 duck

The mean weight of eggs obtained in this study 63.88 ± 3.30 grams. The results obtained is greater than the average Alabio egg weight investigated by Gunawan (1987) on Alabio (AA), Alabio Khampbell Khaki cross (KA X AK), Khaki Khambell (KK), and Alabio Tegal cross (AT), aged 16-72 weeks were: 62.9, 61.8, 62.8, 57.3 and 65.0 grams respectively. Compared with the results of Hardjosworo, (1985) for six months, the average body weight of duck egg production and Alabio respectively 65.00 ± 8.94 grams and 68.57 ± 6.07 grams from overall data obtained. The average body weight of Alabio, is 73.4 grams of egg maximum and minimum of 55.0 grams. Differences in the amount of egg weight on the research results caused by the different age: mature sex, strain, protein levels in diet, the way of maintenance, environmental temperature and age of birds (Stadelman and Coterill, 1977). Too quickly of sexual maturity tend a small size egg and high mortality. A large body weight will produce a large egg weight, while the birds are lighter body weight will produce eggs that are light weight also (Chrismast, et al 1979). Egg weight is influenced by environmental temperature, the ambient temperature increased to 20^0C caused a decrease in egg weight by 0.2% (Lillie

Ota, et al, 1976). Egg weight was reached at a temperature of 12.89^0 C (El Boushy and Morle, 1978). Egg weight will affected by age, the older age of the poultry, the greater egg weight and would be relatively constant after 2-3 months since first nesting birds (Izat, Gardner and Mellor, 1985).

Production intensity (percent of egg production)

Alabio production intensity of research results for one year of production was 56.66 percent with 84.33% peak production, that is lower than the results of Chavez and Lasmini (1978), who obtained duck production intensity, Alabio and Bali for six months of production respectively: 74.7, 80.7 and 53.6 percent with a peak production: 79.7, 87.6 and 60.9 %. Alabio egg production percentage for one year in the next chart.



From the chart can be seen that within one year production from the age of 22 weeks to 48 weeks and from 49 weeks to 72 weeks will reach peak production in week seven (84.33 %), while Rahmat (1989) reported that Alabio parent peak production reached at the age of 36 weeks or at week twelve that is equal to 71.67 %. The occurrence of two peaks of production in one year due to variation of Alabio population, so in this case from the chart above, there should be a selection of ducks populations, so can get the normal curve for Alabio egg production percentage.

Response Selection

Performance is the result of interaction between genotype and environment. Performance generated in animals reared in an environment in which the uniform is an expression of genetic (Martojo, 1995). In this research, the results that can be used as selection criteria among others; the number of eggs, egg weight and some other properties. For the calculated prediction of the effectiveness of selection or response selection based on the number of eggs and egg weight as well as responses per year.

Calculation results of response from selection based on the total population is maintained (20 % of the total population), obtained by selection responses number of eggs by 11.30 points and the response of grain annually for 20.07, this is due to the parent generation interval is one half years. Results obtained greater than Gunawan (1987) who obtained a

response selection Alabio number of eggs during the year is 14 points. The differences is caused by differences in heritability values used. Heretability values used in this study is based on heritability of Rahmat (1989), 0.509 ± 0.241, while Gunawan (1987) using the number of eggs heretability by 0.22. Selection response was obtained for egg weight and response of 1.85 grams grams annually 1.23 grams.

CONCLUSION

From this research we can conclude that: the average number of Alabio eggs that are accompanied by a good egg weight. The diversity of the average number of eggs are quite large (CV> 20). Response Selection the number of eggs obtained for one year and predicted a good response selection. From the results obtained showed that the Alabio potential good enough to be developed, especially when seen from the average number of eggs and egg weight are quite good, but when seen from the percent of egg production, the production of less than 60%, so it is not profitable. Therefore, if seen from its diversity as the number of eggs (CV> 20 percent), then for the other properties are encouraged to do the selection.

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TERATOLOGICAL AND PATHOLOGICAL STUDIES OF CEFOPERAZONE IN FEMALE ALBINO RATS

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ABSTRACT

Cefoperazone was given intramuscularly to female albino rats at a dose of 180 and 360 mg/ Kg body weight over a period of 6-15 days of pregnancy during which organogenesis of feti occur .The dams were killed on day- twenty of gestation and their feti were subjected to morphological , visceral and skeletal examinations. The drug was significantly decreased the number of viable feti and significantly increased the number of resorbed feti. No dead feti and induced retardation in growth of viable feti, some visceral and skeletal defects in these feti were seen. These effects were dose dependant .Histopathological changes of female organs were seen in liver, kidney, skeletal, muscle, placenta, heart, spleen and brain. Conclusively, cefoperazone caused some fetal defects and abnormalities as well as some histopathological changes in female organs. Accordingly, it is advisable to avoid uses of this drug during pregnancy.

INTRODUCTION

Cefoperazone is broad spectrum semi synthetic 3rd generation cephalosporin antibiotic .Cefoperazon acts on, *Enterobacter-acae , Pseudomonas aeruginosa*, *Cocci, Bacilli* including (B- lactematase producing *Staphylococci* and some bacteria even ampicillin-resistant *E. Coli*) and carbenicillin and gentamycin- resistant *P.aeruginosa* **(Mitsuhashi, 1980 and Czeizel *et al.*, 2001)**. Cefoperazone acts as bactericidal by penetration of the outer layers of the cell wall of bacteria **(Zimmermann, 1979)**. **Matsubara,** (**1980)** mentioned that the antimicrobial activity of cephaperazone was attributed to its affinity for penicillin binding proteins. It has a high affinity for penicillin binding protein 1B, 1A and 2, which are involved in the initiation of cell wall elongation. Third generation cephalosporins selectively inhibit the synthesis of mucopolypeptides in bacterial cell wall. They are unqiue in their ability to remain stable in the presence of beta-lactemase enzyme (**Burke and Ristuccia, 1982)**. All cephalosporins are expected to cross the placenta and are probably excreted into breast milk **(Gerald, 2004)**. The potential adverse drug reaction in young is greater than in adults **(Thomas *et al.*, 2002)**.

This study was designed to explore the effect of cefoperazone on fetal development and histopathological changes in female rat organs.

MATERIAL AND METHODS

A. Drug

Cefoperazone is sodium (6R, 7R) – 7 (® - 2- (4- ethyl -) 2, 3 dioxo – 1 – piperazine carboxamido – 2P- hydroxyl phenyl) acetamidol 3- (methyk -1-H tetrazol – 5YL) Thiomethyl 1-8- oxo-5thio tazobicyclo (4-2-0) octo 2-one 2 carboxyl ate, ($C_{25}H_{26}$ N9 NaO $8S_2$). It was obtained as sterile powder for injection of 1 gram vial from Epico Company under trade name cefazon®. It is widely used parentally and has a wide spectrum of effects. It is administered by deep intramuscular or slow intravenous injection.

B- Experimental animals:

Sixty mature healthy female Albino rats were obtained from the laboratory animal colonies, Ministry of Public Health, Helwan, Egypt. Animal were kept under hygenic conditions and fed on balanced ration and water *ad libitum*. Female rats were examined periodically using vaginal smear test to ensure that they were always in regular oestrus cycle **(Chean, 1966 and Hassert *et al*., 1973).** Each female in the oestrous phase was caged over night with fertile male. The presence of sperms in the vagina next morning was considered as the first day of pregnancy **(Barcellona *et al*., 1977).** Pregnancy was confirmed by persistence of distrous state for 5 days after mating.

C–Experimental design:

1) Effects on fetal developments:

Thirty pregnant dams were divided into three groups each of 10 rats. Rats within the 1st group were kept as a control, while rats within groups 2 and 3 were injected intramuscularly once daily with cefoperazone at rate of 180 (therapeutic dose) and 360 (double therapeutic) mg/ Kg body weight per day, respectively. The drug was given from 6th to 15th days of gestation during period of fetal organogenesis (**Cook and Fairweather , 1968).** All females were killed on the 20th day of pregnancy and their uteri were dissected in order to record the position and number of viable, resorbed or dead feti. The surviving feti were weighed and the length from crown to ramp was measured and examined for any external gross malformations, while others were stained by alizarin red for skeletal examination **(Hays *et al*., 1988)**.

2) Histopathological examination:

Thirty pregnant dams were divided into three groups each of 10 rats. Rats within the 1st group were kept as a control, while rats within groups 2 and 3 were injected intramuscularly once daily with cefoperazone at rate of 180 (therapeutic dose) and 360 (double therapeutic) mg/ Kg body weight per day, respectively. The drug was given from 6th to 15th days of gestation during period of fetal organogenesis (**Cook and Fair-weather, 1968).** All females were killed on the 20th day of pregnancy and specimens were collected from brain, heart, lung, spleen, liver, uterus, kidney and placenta from each scarified mother and fixed directly in 10% formalin for histological examination. They were embedded in paraffin; serial sections of 5-7 microns thickness were stained with haematoxylin and eosin (**Drury and Wallington, 1980**).

RESULTS

Intramuscular injections of cefoperazone in therapeutic and double therapeutic doses (180 and 360 mg/ Kg b.wt) to pregnant female rats from 6th to 15th days of pregnancy induced changes in number of viable, dead, resorbed feti and fetal body weight & crown – rump length (Table 1 and Fig 1- 3).

Visceral- abnormalities of feti were recorded (Table 2 and Figs. 4 -11). While skeletal examination of alizarin red stained feti obtained from dams given intramuscular injection of cefoperazone in doses of 180 and 360 mg /Kg.b.wt. from 6th to 15th days of gestation showed different abnormalities (Table 3 and Figs 12 - 18).

Histopathological examination of female organs (liver, kidney, brain, spleen, lung , heart , intestine ,skeletal muscle ,and placenta) showed insignificant changes in female organs after administration of dose 180mg/Kg.b.Wt. But significant changes were recorded in organs of female administered dose of 360mg/Kg.b.Wt.

Table (1):Effect of cefoperazone on feti obtained from pregnant female rats after repeated intramuscular administrations of 180 and 360 mg/Kg.b.wt from 6th to 15th days of pregnancy once daily (n=10) .

Parameters	Control group		Treated group			
			180 mg/Kg. b.wt		360mg/Kg.b.wt	
Number of female rats	10		10		10	
Number of viable feti	No	%	No	%	No	%
	92	100	60	76.9	35	50.72
Number of dead feti	-	-	-	-	-	-
Number of resorbed feti	-	-	18	23.07	28	40.58
Fetal body weight (gm)	4.47±0.1118	-	3.21±0.1612	-	2.5±0.164	-
Fetal crown- rump lengthcm (cm)	4.25±0.06635	-	3.46±0.1638	-	3.11±0.2643	-

Table (2): Visceral abnormalities in feti obtained from pregnant female rats after repeated intramuscular administration of 180 and 360 mg cefoperazon /Kg.b.wt from 6th to 15th days of pregnancy once daily (n= 10).

Groups	Dose	Number of examined feti	Abnormalities													
			Brain diverticulum		Thymus hypoplasia		Lung hhypoplasia		Heart enlargment		Liver enlargment		Kidney hypoplasia		Supra renal gland enlargmentrnal	
			No	%	No	%	No	%	No	%	No	%	No	%	No	%
Group 1	Control	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group 2	180 mg/kgb.w	10	10	34.48	7	24.14	15	51.72	14	48.27	12	41.38	19	65.51	9	31.03
Group 3	360 mg/kgb.w	10	9	47.36	7	36.84	12	63.15	10	57.89	13	68.42	15	78.94	8	42

% Percent of total abnormalities in relation to the number of examined feti.

Table (3): Skeletal abnormalities in feti obtained from pregnant female rats after repeated intramuscular administration of 180 and 360 mg cefoperazon/ Kg.b.wt from 6th to 15th days of pregnancy in female rats once daily (n = 10).

Groups	Dose	Number of examined feti	Abnormalities													
			Brain diverticulum		Thymus hypoplasia		Lung hhypoplasia		Heart enlargment		Liver enlargment		Kidney hypoplasia		Supra renal gland enlargmentrnal	
			No	%	No	%	No	%	No	%	No	%	No	%	No	%
Group 1	Control	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group 2	180 mg/kgb.w	10	8	44.44	3	15.78	11	61.11	10	52.63	7	36.84	5	26.31	6	31.57
Group 3	360 mg/kgb.w	10	9	69.23	4	30.76	10	76.92	11	84.61	7	53.84	5	38.46	6	46.15

% Percent of total abnormalities in relation to the number of examined feti.

DISCUSSION

Intramuscular administrations of cefoperazone in therapeutic (180 mg / Kg. b. Wt) and double therapeutic (360 mg/Kg.b.wt) doses during the period of organogenesis induced significant decrease in the number of feti as well as viable feti per mother. These results are agree with those recorded by **Kurebe *et al*. (1984)** who found that administration of cephamycin in a dose of 40mg/Kg.b.wt increased the number of resorbed feti and decreased number of viable feti. In the same direction, administration of ceftiofur to rats was retarded the embryonic development resulted in embryonic death so decrease the number of viable fetus **(Joette *et a.*, 1995)**. The decrease in number of feti per mother might be attributed to the lack of oval production or of the basic cell constituent as a result of drug administration **(Tuchmann, 1975)**. Decrease in number of viable feti might be explained on the basis of incomplete formation of the placenta and degeneration of the trophoblast and decidual cell, which play an important role in the transmission of nutrients to the embryo **(Kurebe *et a*l., 1984)**. The histopathological finding in the present study confirm this finding which appear as severe congestion with recent thrombosis which led to degeneration and focal area of necrosis in a dose dependant manner.

Tuchman (1975) attributed the numerical increase in the number of resorbed feti to the interference of administered drug to transfer of some essential nutrient (lucin and magnesium) responsible for development of embryo. These results were agree with that obtained by **Holyoak *et al*. (1998)** who found that when fertilized oocytes were exposed to concentrations more than or equal to 50ug / ml of ceftiofur soduim during culture, which significantly retarded embryo development (e.g the numbers of ova developing to the marula and blastocyst stages were reduced, and alarge proportion of embryes were bloked at the 8-cell stage). Also, **Joette *et al*. (1995)** found that cephalosporin when used during the first 7 days after conception could affect embryonic development resulted in embryonic death. **Nathanson *et al.* (2000)** suggested that the beta- lactam antibiotics cross placenta during pregnancy**. Gerald (2004)** reported that cephalosporins had a molecular weight below 1000 dalton , so were expected to cross the placenta to the embryo or fetus and are probably excreted into breast milk .The increase in the fetal resorption rate in dams in the present study might be due to some chemicals or xenobiotics interfere with the implantation of the embryo or destroy the chromosomes of the embryo and resulted in early embryonic death and consequently its resorption during critical period of intrauterine development . Also, **Shaheen *et al.* (2000)** documented that the treatment of healthy female rats with ceftiofur sodium show increase in fetal death and resorption. **Haschek and Rousseau.(1993)** investigated that chromosomal aberration (ringing, sticky, delation, centromeric, attenuation, hypoloidy, polypoidy) cause early embryonic death.

Administration of cefoperazone to female pregnant rats during the period of organogenesis produced significant decrease in both weight and length of fetuses. These results were consistent with those previously reported by **Sugiyama *et al*.** (**1990)** following administration of cefpirome sulfat in rats. They attributed to deficiency of nutritional supply from dam to fetus as female administered cefoperazone exhibited imbalance of intestinal micro flora**.** Also, our results were supported by results recorded by**Shaheen *et al.*** (**2000)** who recorded that ceftiofur evoked a very highly significant decrease in both body weigh and length (2.29 ± 40.06 gm, 3.61± 0.04 cm) that due to chemicals or the endotoxine secreted by this antibiotic may destroy cellular active DNA , So reduced biosynthesis of essential compound like protein and energy sources (ATP, NAD, and NADP). **Haschek and Rausseaux, (1993)** attributed the decreased of Fetal body weight and length to the chromosomal

abnormalities. **Caballe et *al.* (2003)** suggested that cephalosporins might impair the uptake of monosaccharide from intestine; a factor essential for fetal growth.

Repeated Intramuscular administrations of 180 and 360 mg/Kg.b.wt of cefoperazone to pregnant female rats during the period of organogenesis induced many fetal visceral abnormalities as diverticuluim dilatation which might be attributed to the lake of placental transfusion of amino acid arginine metabolism in fetus **(Tuchmann 1975)** or due to some antibacterials had neurotoxic effect as levofloxacin and ciprofloxacin which easily cross-blood brain barrier and compete with gamma- amino butyric acid receptor **(Akahan *et al*., 1993).** Cefoperazone induced thymus hypoplasia of the examined feti. **Yoneda *et al*. (1980)** showed atrophy of the thymus after administration of cefoperazone to beagle dogs recived up to 250 mg/Kg/day. This result might be attributed to cytotoxicity of drug which promote decrease of cell growth or cell death according to the dose by converting top isomerase to cellular poisons. Repeated administrations of cefoperazone induced pulmonary hypoplasia. This result agree with **Czeizel *et al. (*2001)** who showed pulmonary hypoplasia following administration of cephalosporins to women, which attributed to the stenosis of pulmonary artery and thrombosis of pulmonary blood vessels.

The drug induced cardiac hyperplsia with increased the dose. This result agreed with that recorded by **Czeizel *et al*. (2001)** who reported cardiovascular anomalies in fetus after administration of cephalosporin (cephalexin, cefamandol, cefoperazone) these abnormalities might be attributed to ability of antibiotics to block cardiac channel(H E K G) which lead to Q-T interval prolonged with cardiac arrhythmia and consequently cardiac hyperplasia (**Anderson *et al*., 2001)**

Cefoperazone administration induced hypoplasia or atrophy of one or both kidneys with unilateral or bilateral dilatation of renal pelvis. these results agreed with those reported by **Wold *et al.* (1978)** following administration of cefamandole nafate to rats and rabbits. **Nathanson *et al.* (2000)** suggested that administration of ceftraixone to rats impaired nephrogenesis except at dose of 1000 Mg/ml which block kidney development completely. These abnormalities attributed to lipid peroxidation which would be a possible toxicological mechanism of acute renal damage **(Yamada 1995). Rush *et al*. (1992)** reported that cephaloridine induced mitochondrial alteration leading to ATP depletion, cell injury and necrosis end by cell atrophy. Administration of cefoperazone induced hepatomegaly of fetus. This result agreed with **Yoneda *et al.*, (1980)** after administration of cefoperazone to beagle dogs who found severe anemia which led to extramedullary hematopoiesis in the liver .Our results were reinforced with those obtained by **Shaheen *et al.*((2000)** who found that ceftiofur sodium induced teratogenicity in the visceral organs. A general mechanism of teratogenicity might be due to chromosomal damage. **Sugiyama *et al*. (1990)** studied that cefoperazone sulfate induced some visceral abnormalities in rats.

Cefoperazone dose of 360mg/Kg.b.wt produced some fetal skeletal malformations as impaired ossification of skull, absence of sternabre, absence of caudal vertebrae, absence of digit's bone of fore and hind limbs and absence of some metatarsal and metacarpal bones. These results agreed with those reported by **Delar *et al. (*1988)** after administration of cefazedone to dogs who reported that cephalosporins induced changes in ultrastucture of canine bone due to mitochondrial damage in haemobiotic and non haemobiotic cells, thickening of endosteal bone lining layers, increased adventitial coverage of vascular sinuses,and an increased number of active macrophages. Skeletal malformations also agreed with that recorded by **Shaheen *et al. (* 2000)** who mentioned that the administration of ceftiofur intramuscularly for five successive days from 6^{th} to 10^{th} daysof gestation evoked an incomplete ossification of cranial bones, redimentary or absence of strenbrae & sternum,

absence of phalanges of both fore and hind limbs, and coccygeal verebrae of examined foeti. **Cablle *et al.,* (2003)** investigated that cephalosporins impaired monosaccharide intestinal uptake which essential for bone development.

The present investigation revealed many histopathological lesions in different organs as brain, spleen, liver, kidney, muscle, intestine, placenta and uterus which agreed with those reported by **Yoneda *et al.*,(1980)** who reported the signs of pain after injection of cephalosporins, focal area of necrosis, hemorrhage, cell infiltration, fibrosis of muscle at site of injection which found microscopically in dogs received dose of 500 and 250 mg/kg.b.wt . **Yilmaze *et al.* (1999)** reported that histopathologic examination indicated hepato toxicity of cefezolin **.Prochazka *et al*. (2003)** reported that cefoperazone had good penetration to liver and bile and its concentration in bile and liver was hundred times as that of serum ;factor led to toxic effect in liver .**Czeizel *et al.* (2001)** studied that uses of cephalosporins during second and third months of pregnancy caused cardiovascular anomalies. **Rush *et al.* (1992)** studied the nephrotoxic effects of cephaloridin in female rabbits due to lipid peroxidation and mitochondrial ultration and leading to ATP deplation and cell injury. **Hassert *et al.* (1973**) studied the acute and subacute toxicity of cephradine in mice, rats and dogs who found that hepatic and renal damage in mice ,but in dog and rat marked degeneration of muscle with diffuse hemorrhage and moderate edema. **Caballe *et al.* (2003)** discussed that cephalosporins impaired monosaccharide intestinal uptake which affect on brain, liver and kidney.

From these observations, it is suggested that administration of cefoperazone in therapeutic and double therapeutic doses causes some visceral and skeletal abnormalities which supported by histopathological changes in mother organs. These results proved that cefoperazone in both therapeutic and double therapeutic doses produced some teratogenic effects of feti, so take in consideration when used this drug during pregnancy.

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Fig. (1): Gravid rat uterus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone/Kg.b.wt from 6th to 15th days of pregnancy showing early uterine resorption.

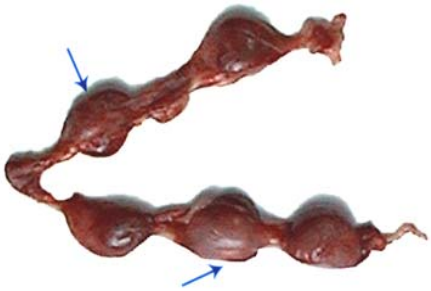


Fig. (2): Gravid rat uterus *obtained* from mother after repeated intramuscular administrations of 360 mg cefoperazone/ Kg.b.wt from 6th to 15th days of pregnancy showing late uterine resorption

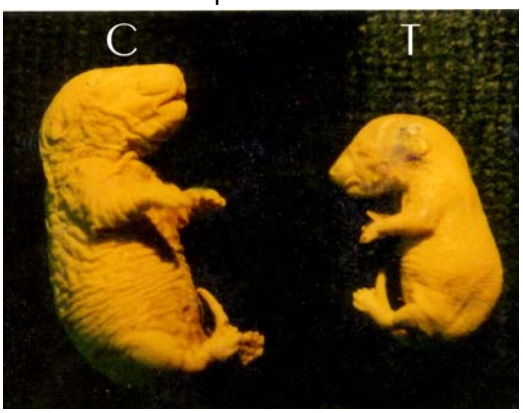


Fig. (3): Retardation of growth of a fetus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone/ Kg.b.wt from 6th to 15th days of pregnancy

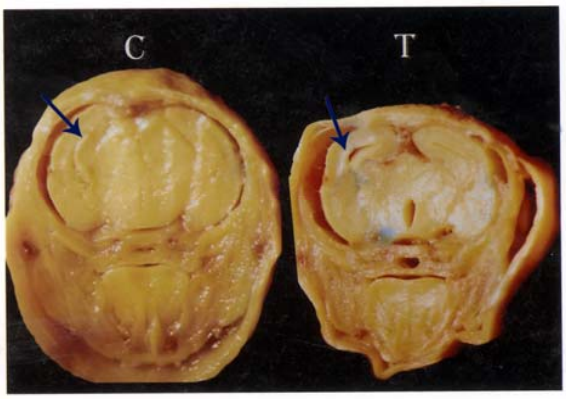


Fig. (4): Diverticulum dilatation of a fetus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone / Kg.b.wt from 6th to 15th days of pregnancy

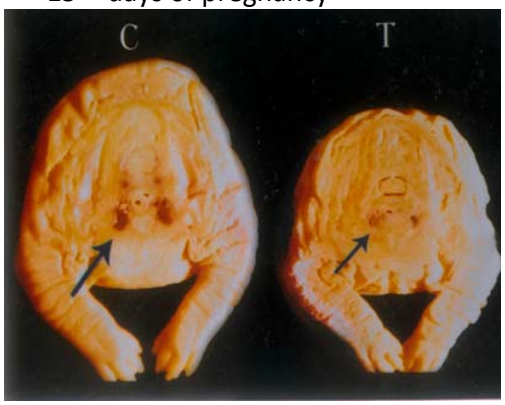


Fig. (5): Thymus hypoplasia of a fetus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazon/ Kg.b.wt from6th to 15th days of pregnancy

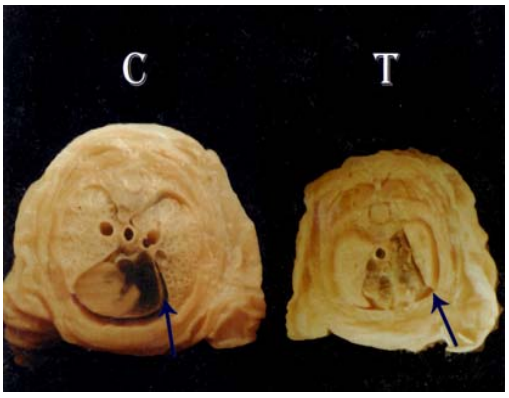


Fig. (6):Pulmonary hypoplasia with cardiac enlargement of a fetus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone / Kg.b.wt from6th to 15th days of pregnancy

Fig. (1): Gravid rat uterus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone/Kg.b.wt from 6th to 15th days of pregnancy showing early uterine resorption.

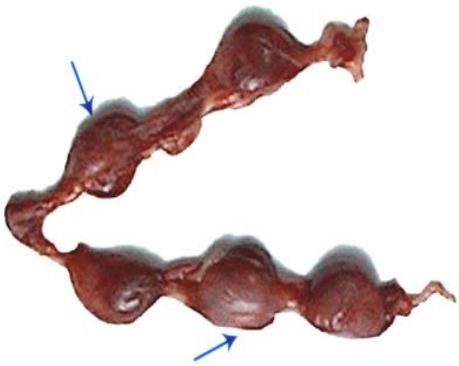


Fig. (2): Gravid rat uterus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone/ Kg.b.wt from 6th to 15th days of pregnancy showing late uterine resorption

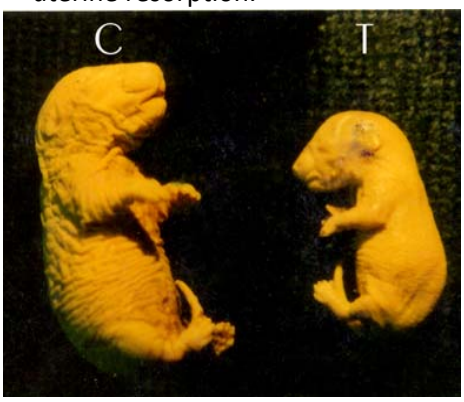
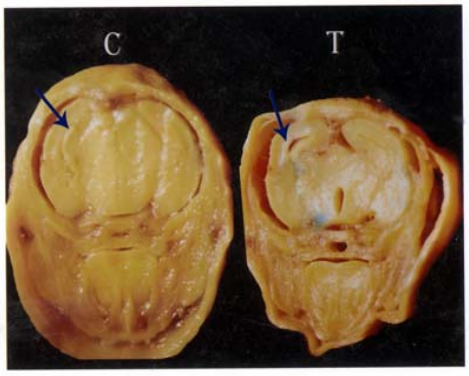


Fig. (3): Retardation of growth of a fetus obtained from mother after repeated intramuscular administrations of 360 mg cefoperazone/ Kg.b.wt from 6th to 15th days of pregnancy



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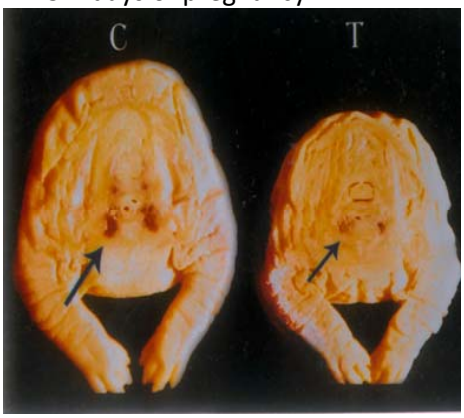

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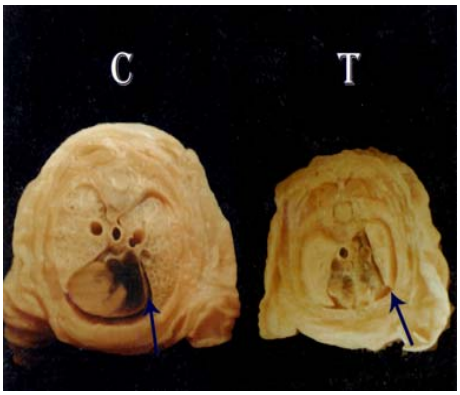

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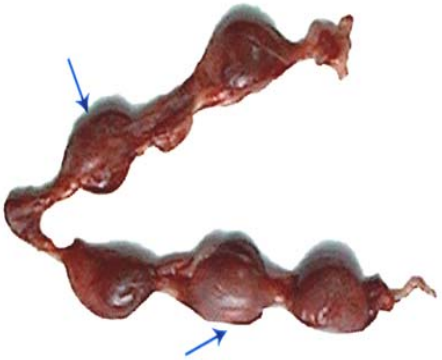

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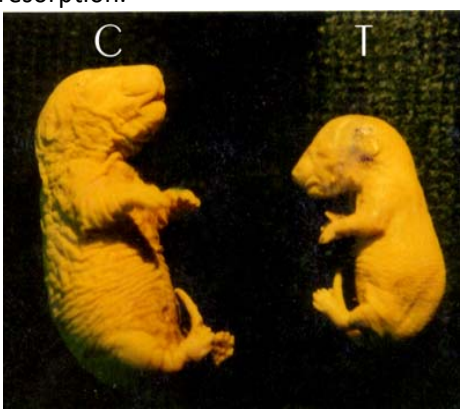

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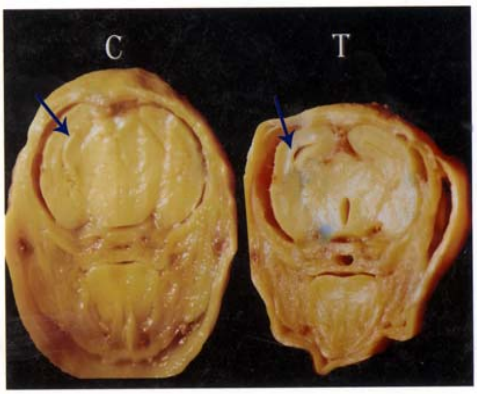

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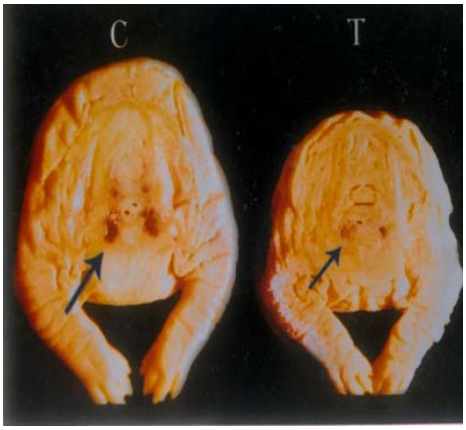

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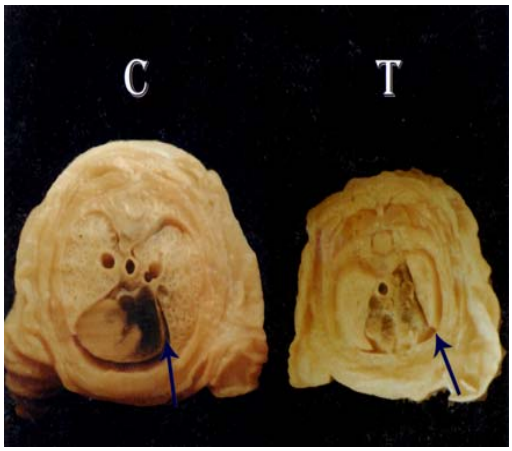

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A NEW METHOD FOR ANALYZING THE *AGOUTI* LOCUS INVOLVED IN THE COAT COLOUR OF HORSES

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Abstract
It is proposed that the black hair pattern characteristics in horses are due to the ASIP gene. PCR analysis revealed an 11 bp deletion in the ASIP gene associated with the black color. The mutation occurs in the second exon of the ASIP gene and can be detected with a simple PCR test. Our objective is to develop a straightforward method to identify this mutation and then to examine the Agouti locus in horse populations. In our experiment the set of designed primers amplify only a 96 bp fragment from the ASIP gene which may contain the 11 bp deletion. The forward primer was labelled with 6-FAM dye. The PCR products will be analyzed using ABI Prism 310 DNA Genetic Analyzer (AppliedBiosystems). The number of allele peaks depends on whether the individual tested is a heterozygote (carrier, Aa) or homozygote (normal, AA or affected, aa). Using this technique, we established an easy and efficient method that can be used to screen the Agouti locus in horse populations.
Key words: horse, *Agouti* locus, coat colour, mutation, PCR.

INTRODUCTION

The basic set of horse colours, black, bay, chestnut and grey, can be described by the actions of alleles of the genes placed on *Agouti*, *Extension* and *Grey* loci. The collection of colours is extended in some breeds with colour dilution genes (*Cream*, *Dun*, *Champagne,* and *Silver*) and with white pattern genes (*White*, *Roan*, *Tobiano*, *Overo,* and *Leopard Spotting*).
In horses, melanin is the most important coat colour pigment. Coat colour variation is produced by the genes that alter the basic pigment type in melanocytes, or the presence, shape, number or arrangement of pigment granules. Melanin occurs in two related forms: eumelanin - black or brown - and phaeomelanin - red or yellow - (Bowling, A. T., Ruvinsky, A., The Genetics of the Horses, 2000). The genes for controlling the eumelanin/phaeomelanin switch producing the colours chestnut and bay/black are placed on the *Extension* and *Agouti* loci.
Horse breeds usually display a huge variety of distinct coat color patterns. Nevertheless, some of them are known for their particular coat color, indicating homozygozity for this character. Friesian horses, for example, are thought to be all black, except for a low frequency of the chestnut allele (*Ee*), resulting occasionally in chestnut-colored horses when homozygous. Solid black is quite a rare coat color in most horse breeds and seems to be essentially recessive (Rieder *et al.,* 2001).
Melanocortin-1-receptor (MC1R), encoded by the *Extension* locus, and its peptide antagonist agouti-signaling-protein (ASIP), encoded by the *Agouti* locus, control the relative amounts of melanin pigments in mammals (Lu *et al*. 1994; Siracusa, 1994). ASIP acts as an antagonist of MC1R by nullifying the action of a-melanocyte-stimulating hormone (a-MSH). Loss-of-function of MC1R results in the yellow pigment (pheomelanin), whereas gain-of-function of MC1R or loss-of-function of ASIP seems to result in the production of the black pigment (eumelanin).

Rieder *et al.,* 2001 show that black horses are homozygous for a deletion in the *Agouti* locus. Our main objective was to develop an easy method to identify this mutation and then to examine the *Agouti* locus in horse populations.

MATERIALS AND METHODS

A group of 50 Hucul horses from Lucina Stud was analysed. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).
For PCR we used one set of primers amplifying a fragment from the *ASIP* gene. The forward primer was labelled with 6-FAM dye. PCR conditions were optimized by varying the annealing temperature (51–59^{o}C) on a gradient thermocycler IQCycler (BioRad).
PCR was done using a GeneAmp 9700 PCR System (AppliedBiosystems). The reactions were carried out in 25 µl final volume containing PCR Buffer, $MgCl_2$, 200 µM of each dNTP, diluted DNA, 0.5 µM of each primer, 0.5 units of AmpliTaq Gold DNA Polymerase and nuclease-free water. PCR amplifications were performed in 0.2 ml tubes by 40 cycles with denaturation at 95ºC (30 s), annealing at 57°C (30 s) and extension at 72°C (60 s). The first denaturation step was of 10 min at 95ºC and the last extension was of 10 min at 72°C. PCR products were loaded with the GeneScan-500 ROX Internal Size Standard (AppliedBiosystems) into one of the ABI Prism 310 DNA Genetic Analyzer (AppliedBiosystems).
The results were analyzed with the GeneScan 3.1.2. Software (AppliedBiosystems) which assigns a base pair size for each signal. GeneScan data can then be exported directly to Genotyper 2.5.2. Software (AppliedBiosystems) for automated genotyping.

RESULTS AND DISCUSSION

Black hair pattern characteristics in horses are proposed to be due to *ASIP* gene. The action of the dominant allele (*A*) in horses causes the distribution of hair with eumelanin to be restricted to the points. The recessive allele (*a*) does not restrict the distribution of black hair and produces a uniformly black horse.
The appearance of a recessive alelle is due to an 11 bp deletion in the second exon of the gene coding for *ASIP* protein. The deletion in *ASIP* exon 2 alters the aminoacid sequence and is believed to extend the regular termination signal by 210 bp to 612 bp. The frameshift initiated by the deletion results in a novel modified Agouti-Signaling-Protein (Rieder *et al.,* 2001).
In our experiment the set of designed primers amplify only a 96 bp fragment from the *ASIP* gene which may contain the 11 bp deletion. Conditions for PCR were selected in such maner to permit the amplification of the DNA from homozygous and heterozygous horses. PCR conditions were optimized by varying the annealing temperature on a gradient thermocycler as shown in Figure 1.
Successful amplification yields one or two allele peaks with an expected size of 85 and (or) 96 bp. The number of allele peaks depends on whether the individual tested is a heterozygote (carrier, Aa) or homozygote (normal, AA or affected, aa). For (AA) genotype we must obtain just one peak at 96 bp; for (aa) genotype we also obtain just one peak, but at 85 bp. If the horse is a heterozygous (Aa) we must obtain two peaks at 96 and 85 bp because one allele is normal and the other one contains the deletion. In our study, the analyzed horses present two kinds of genotypes (Figure 2).

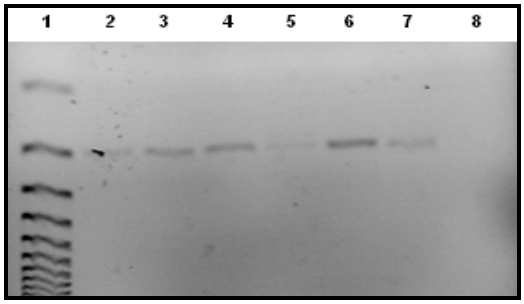

Figure 1: PCR in temperature gradient for analysis of *Agouti* locus: 1 – molecular size marker 50 bp; 2 – 51°C; 3 – 51.7°C; 4 – 52.9°C; 5 – 54.5°C; 6 – 56.8°C; 7 – 58.5°C; 8 – negative control.

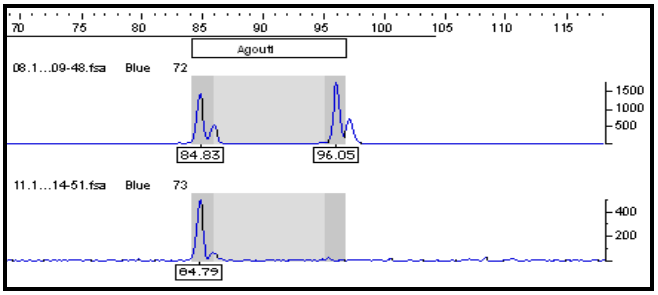

Figure 2: Genotyper software analysis of PCR amplification product for a heterozygous carrier (Aa) and for an affected homozygous (aa) horse.

Using this molecular technique, we established for the first time in Romania, an easy and efficient method that can be used to determine the normal or recessive genotypes for the *Agouti* locus. Therefore, this new method increases the panel of molecular tools available to horse breeders for improving horse identification and artificial selection. Results suggest that the genetic test will be useful in identifying horses which are heterozygous or recessive for this trait.

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FELINE INFECTIOUS PERITONITIS – CASE PRESENTATION

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Feline infectious peritonitis is a fatal cat disease, which affect specially animals who live in communities. The disease is produced by a coronavirus, group I, which is a mutant of the benign enteric coronavirus. The wet for of the disease is characterized by ascitic fluid accumulation in body cavitites, in function of the affected blood vassel.
Our case has been represented by a common breed cat, two years old, brought at the doctor because of the loss of appetite, weight loss (from 6 to 4 kg), diarrhea, dehydration. Following necropsy, it was observed a large amount of ascitic fluid (200-300 ml) the abdominal cavity, partly in the form of jelly deposits. Organs of the abdominal cavity and mesentery were covered with fibrin deposits. Hematological tests showed leukocytosis with neutrophilia and lymphopenia. Following RT-PCR feline coronavirus was present both in the ascitic fluid and in faeces.

***Key words**: coronavirus, cat, fibrin, ascitic fluid*

Feline infectious peritonitis (FIP) is a viral disease of cats produced by a coronavirus, that can affect many systems of the body. It is a progressive disease and almost always fatal (5). It is found worldwide and affects not only domestic cats, but many wild ones as well, including cougars, bobcats, lynx, lions, and cheetahs (2, 5).

The cats most likely to develop FIP are those with the weakest immune systems, including kittens, cats infected with feline leukemia virus (FeLV) and geriatric cats. The largest number of FIP cases occurs in young cats. Rarely cats between 3 and 10 years of age are seen. However, starting at 10-12 years of age, the immune systems of these older cats apparently decline, making them more susceptible (1, 5).

There are two types of feline coronavirus (FCoV) which cannot be distinguished from each other in laboratory tests. One is avirulent (does not cause disease or only mildly virulent) called feline enteric coronavirus (FECV), the other type is virulent, being is the cause of FIP, and is called feline infectious peritonitis virus (FIPV). It is believed that FIP occurs when FECV mutates to FIPV in the cat and starts to replicate in the cat's cells. What causes this mutation is unknown (2, 3, 5).

Studies have shown that approximately 25-40% of household cats, and up to 95% of cats in catteries are or have been infected with FCoV. The development of fatal FIP occurs rarely in households with one or two cats. In multi-cat households and catteries 5% of cats die from FIP (1, 6).

FCoV can be found in the saliva and feces of infected cats. Therefore, cat-to-cat contact and exposure to feces in litter boxes are the most common modes of infection. Contaminated food or water dishes, bedding, and personal clothing may also serve as sources of infection (4).

Although we separate FIP into 2 forms, wet and dry, there is really a gradient between the two forms, and we may often see signs of both forms.

In the wet form, early in the disease we can see similar signs to the dry form including weight loss, fever, loss of appetite, and lethargy. Anemia with resultant pale mucous membranes (e.g., gums) is often seen. Constipation and diarrhea can also occur. The wet form

of the disease progresses rapidly and soon the cat may appear pot-bellied in appearance because of the fluid accumulation in the abdomen. Generally, the cat shows no signs of abdominal pain. Fluid may also accumulate in the chest causing respiratory difficulties. Most cats with the wet form of FIP die within 2 months of showing signs of disease (5).

MATERIAL AND METHOD

The case has been represented by a common breed cat, two years old. It was brought at the doctor because of the loss of appetite, weight loss (from 6 to 4 kg), diarrhea, dehydration. On examination it was found that the cat had fever (39,8^{0}C), pale conjunctival mucosa. Also, it was observed the collection of ascitic fluid with the perception of a wave sensation in the abdomen.

There were collected faecal samples using buffer throat swabs, blood and ascitic fluid in EDTA tubes, for virological examination to identify feline coronavirus. The cat died within 24 hours and was subjected to necropsy.

For virological examination, pathological materials were processed as follows: extraction was performed with viral RNA kit *QIAamp Viral RNA Mini Kit, Qiagen*. For amplification by RT-PCR *Qiagen One Step* kit and couple of primers P205 / P211 were used.

p205* : GGCAACCCGATGTTTAAAACTGG

p211* : CACTAGATCCAGACGTTAGCTC

This couple of primers allowe the amplification of most well conserved region of coronaviruses genome, common in all coronaviruses from group I.

The entire RT-PCR lasts 40 cycles in 4.30 hours, with several steps at specific temperatures: reverse transcription (30 minutes at 50°C), activation of the polymerase (15 minutes at 95°C), denaturation of the DNA strand (1 minute 94°C), hybridization of primers to DNA (1 minute at 48°C), polymerization (1 minute at 72°C), final extension (10 minutes at 72°C).

To investigate the presence of feline coronavirus, the primers used are the one described in the literature (21), allowing amplification of the 3‘ region of the viral genome, a region less variable among a group of coronaviruses. The size of the amplified fragment is 223 base pairs (bp).

Amplified DNA fragments were separated by electrophoresis in 2% agarose gel, with *GelPilot DNA Loading Dye*, at 200mA, 80V for one hour. There was used as molecular weight marker *DNA Molecular Weight Markers GelPilot*.

The materials used for virological examination were procured through financing by CNCSIS of the project IDEI 1129/2008.

RESULTS AND DISCUSSIONS

Because the clinical symptoms are not specific, there should always be combined with results of laboratory tests for confirmation. Sometimes only certain signs can be found according to stage of disease development, but these signs may encounter in other diseses: cancer, systemic mycosis, liver and kidney disease, toxoplasmosis, etc.

Since before necropsy, we observed that the animal was in good shape, not very skinny (during necropsy it was observed the subcutaneous fat deposit quite well represented). Conjunctival and oral mucous surfaces were very pale. Following necropsy, it was observed a large amount of ascitic fluid (200-300 ml) the abdominal cavity, partly in the form of jelly deposits (*fig. 1*).

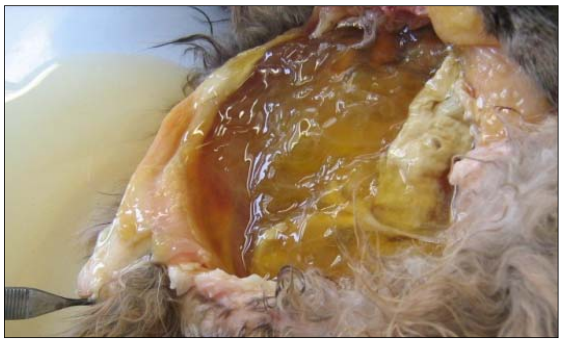

Fig. 1 - *Ascitic fuid with jelly appearence*

Organs of the abdominal cavity and mesentery were covered with fibrin deposits (*fig. 2, 3*).

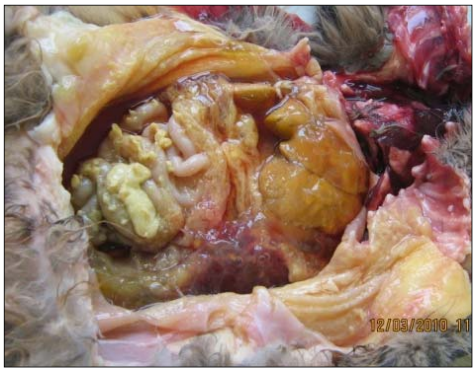

Fig. 2 – *Fibrin deposits on the organs from abdominal cavity*

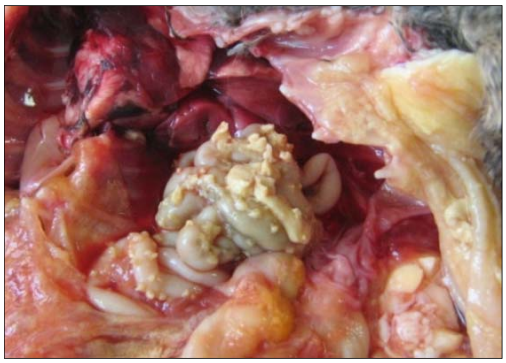

Fig. 3 - *Fibrin deposits on the organs from abdominal cavity*

Also we noted an increase in volume of mesenteric lymph node and spleen. Liver was increased in volume, pale yellow – mustard, with fatty aspect (*fig. 4*), besides enlarged spleen and fibrin deposits on the surface (*fig. 5*).

Fig. 4 – *Fatty aspect liver*

Fig. 5 – *Enlarged spleen, with fibrin deposits on the surfave*

During necropsy samples of pathological material were collected for virological examination (ascitic fluid, faeces, blood). Since the body was frozen immediately after animal death at-20^{0}C, we could not take samples for histopathology. Samples collected were processed by methods described above.

Corroborating history, physical examination and data collected at necropsy, we suspected the evolution of acute forms of feline infectious peritonitis, wet form. Confirmation was done by identifying the coronavirus in the collected pathological material.

In our study hematological and biochemical examination was performed, before knowing the results of virological exam and found leukocytosis with neutrophilia and lymphopenia. Blood chemistry showed specific organ damage. Antigen-antibody complexes deposited in the kidney may cause pyogranulomatosis glomerulonephritis showed by azotaemia. Decreased proteinuria and urinary specific density may induce a wrong diagnosis being indicative of primary renal disease. Unfortunately, the changes are not pathognomonic for FIP.

Following RT-PCR feline coronavirus was present both in the ascitic fluid and in faeces (*fig. 6*).

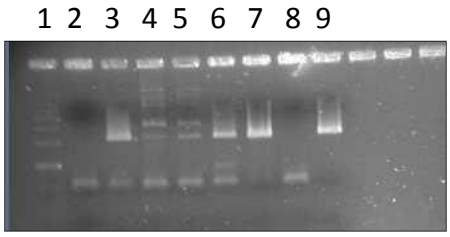

Fig. 6 – *Electroforesis of samples*

1- DNA molecular weight marker
3 – feces
7 – ascitic fluid
8 – negativ control
9 – positive control

Although it is known that laboratory tests can not distinguish between feline enteric coronavirus, other coronaviruses from group I and feline infectious peritonitis virus, the presence of virus in ascitic fluid made the difference from normal coronavirus present only in feces.

Appearance of ascitic fluid, gelling aspect observed opening the corpse, due to high protein content (8,9 g/dl), fibrin deposits on nearly all organs in the abdominal cavity were other defining elements.

Although the certainty diagnosis of feline infectious peritonitis is difficult to determine by the usual methods we used for this all results, clinical examination, laboratory tests and, last but not least, history. Linking all these aspects, we certified the diagnosis.

CONCLUSIONS

1. The case has been represented by a common breed cat, two years old, brought at the doctor because of the loss of appetite, weight loss (from 6 to 4 kg), diarrhea, dehydration.
2. Following necropsy, it was observed a large amount of ascitic fluid (200-300 ml) the abdominal cavity, partly in the form of jelly deposits.
3. Organs of the abdominal cavity and mesentery were covered with fibrin deposits.
4. Hematological tests showed leukocytosis with neutrophilia and lymphopenia.
5. Following RT-PCR feline coronavirus was present both in the ascitic fluid and in faeces.

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THE SPIDER LAMB SYNDROME ABSENCE IN FIVE ROMANIAN SHEEP BREEDS

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Abstract

Spider lamb syndrome (SLS) or ovine hereditary chondrodysplasia is a semi-lethal congenital disorder of the musculo-skeletal system that causes important economical losses in the sheep industry. The aim of this study was to determine the possible carriers of SLS in five Romanian sheep breeds. We have analyzed 120 samples and amplified the polymorphic region of the ovine FGFR3 gene. The T→A transversion at nucleotide 69 of the ovine FGFR3 exon 17 determines the non-conservative substitution valine→glutamate at the residue 700 in the second tyrosine kinase domain of the receptor. The obtained results highlighted the absence of SLS in five Romanian sheep breeds.

Keywords: sheep, SLS, carrier, PCR-RFLP, Romanian.

INTRODUCTION

The reduction of viable lambs per ewe can have a major effect on reproduction systems (Wang & Dickerson, 1991). One disease that can affect negatively the production efficiency is represented by the Spider Lamb Syndrome (SLS). One way to increase the productivity is to determine the SLS carriers and to eliminate them from the mating process. Certain anomalies located at the skeletal level, like long, disproportional feet shaped like a spider, facial deformations, the absence of body fat and muscular atrophy are associated with SLS (Cockett et al., 1999). Spider Lamb Syndrome or ovine chondrodysplasia is a genetic disorder characterized by severe skeletal abnormalities.

The gene responsible for SLS is inherited in an autosomal recessive mode. The causative mutation was identified after positional cloning of the ovine fibroblast growth factor receptor 3 (FGFR3) gene on sheep chromosome 6 (Cockett et al., 1999). The fibroblast growth factor receptor 3 (FGFR3) is a negative regulator of bone growth as it is responsible of chondrocyte proliferation and differentiation during endochondral ossification (Deng et al., 1996). In SLS, the FGFR3 mutation induces elongation of bones formed by endochondral ossification by removing the FGFR3-induced inhibition of chondrocyte proliferation (Beever et al., 2006).

The nucleotide mutation T→A from position 69 of the ovine FGFR3 exon 17 determines the non-conservative substitution valine → glutamate at residue 700 in the second tyrosine kinase domain of the receptor. In case of homozygotes, the mutation determines the loss of receptor function and thus a poorly controlled chondrocyte differentiation (Drogmuller et al., 2005). The aim of this study is to identify possible carriers in five Romanian sheep breeds.

MATERIALS AND METHODS

Sampling and DNA extraction

Blood samples were collected in EDTA-treated plastic vacutainers from five breeds: Botosani Karakul, Romanian Blackhead Ruda, Palas Milk Line, Palas Meat Line and Palas Merino. For each breed we analyzed 24 animals. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

PCR reaction

In order to amplify a 432 bp fragment we have used a pair of primers (forward: 5'-CCTTGTTTGACCGCGTCTAC-3'; reverse: 5'- ATGTACCTGGGGGACATGC -3') corresponding to positions 472–908 from exon 16 to intron 17 of FGFR3 (GenBank AY737276).

The PCR conditions were optimized in order to determine the best annealing temperature for the two primers, between 51-60˚C on a gradient thermocycler IQCycler (BioRad).

After determining the optimum temperature of 58˚C, the amplification reactions were carried out in 25 µL final volume and consisted of 1X PCR Buffer, $MgCl_2$ (35nM), 200µM of each dNTPs, DNA template (50ng), 0.5 units of AmpliTaq Gold DNA Polymerase, 10mM of each primer and nuclease free water. PCR amplifications were performed in 0.2 ml tubes using a program with 40 cycles. Denaturation was performed at 95˚C for 30 seconds, annealing at 58˚C for 30 seconds and extension at 72˚C for 1 minute. The first denaturation step was of 10 minutes at 95˚C and the final extension was of 10 minutes at 72˚C.

The PCR products obtained were digested with 1U of *BtgI* restriction endonuclease (Promega) for 3 hours at 37˚C. The restriction fragments were directly analyzed by electrophoresis in 3% agarose gels in 1X TAE buffer, stained with ethidium bromide, and visualized under UV light. The genotypes of the analyzed individuals were established using the restriction fragments observed in the gel.

Sequencing

The obtained PCR products were subjected to the sequencing reaction. In order to undergo this reaction, the amplicons were initially purified using the Wizard PCR Preps DNA Purification System Kit (Promega) according to the manufacturer's instructions. The next step was to mix them with ABI Prism ® BigDye Terminator Cycle Sequencing Ready Reaction Kit. The purification of the amplified products was done using the BidDye XTerminator ® Purification kit. The products were analysed on a ABI Prism 3130 Genetic Analyzer and the nucleotide sequences were aligned with the BioEdit program.

Results and Discussions

The aim of this study was to develop a cost-effective and rapid method to identify possible SLS carriers in five Romanian sheep breeds. The PCR products obtained were separated on 2% agarose gel and the results showed we have obtained a fragment of 432bp that was consistent with the target one and had good specificity (Figure 1).

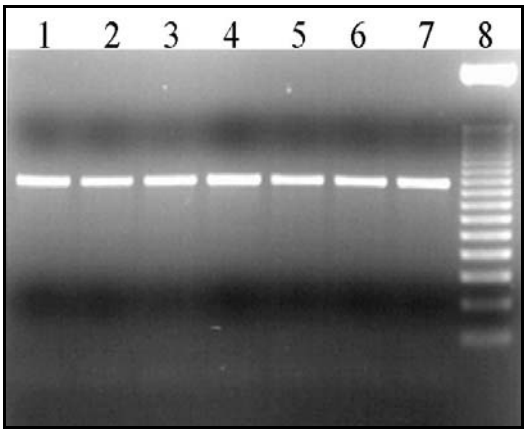

Figure 1: PCR reaction optimization for a 432bp fragment from the ovine FGFR3 gene. 1: 51°C; 2: 51.9°C; 3: 54.6°C; 4: 55.4°C; 5: 56.3°C; 6: 58.1°C; 7: 60°C; 8: 50bp molecular weight marker

In this study we have analysed 120 animals from five Romanian sheep breeds. In order to evaluate the presence or absence of SLS carriers, the amplified products were subjected to enzymatic digestion using the *BtgI* restriction enzyme. The possible polymorphism will modify the restriction site of this enzyme and thus we will be able to diferentiate between carriers and non-carriers. After PCR amplification, enzymatic digestion with *BtgI* and agarose gel electrophoresis, the T allele (wild type, non-carriers) yields three fragments of 63, 166, and 203 bp, the A allele (mutated) yields two fragments of 63 and 369bp, while the heterozygotes have all four fragments.

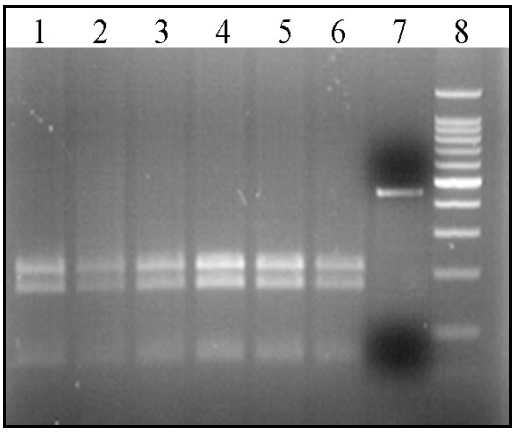

Figure 2. Results after *BtgI* digestion of a 432pb fragment FGFR3 gene; 3% gel electrophoresis. 1-2: Blackhead Ruda; 3: Botosani Karakul; 4: Palas Merino; 5: Palas Milk Line; 6:Palas Meat Line; 7: uncut fragment; 8: 50bp molecular weight marker.

The results after enzymatic digestion on a 3% gel electrophoresis are represented in Figure 2. After the digestion with *BtgI* restriction enzyme, we have obtained in all five sheep breeds only the wild allele which represents the non-carrier animals.

In order to confirm the obtained results using the PCR-RFLP technique we have sequenced the amplicons. After aligning the obtained sequences with the reference sequence from GenBank database we have obtained a homology of 99% (Figure 3). The observed polymorphism is situated at the level of intron 17, thus the protein sequence is not affected.

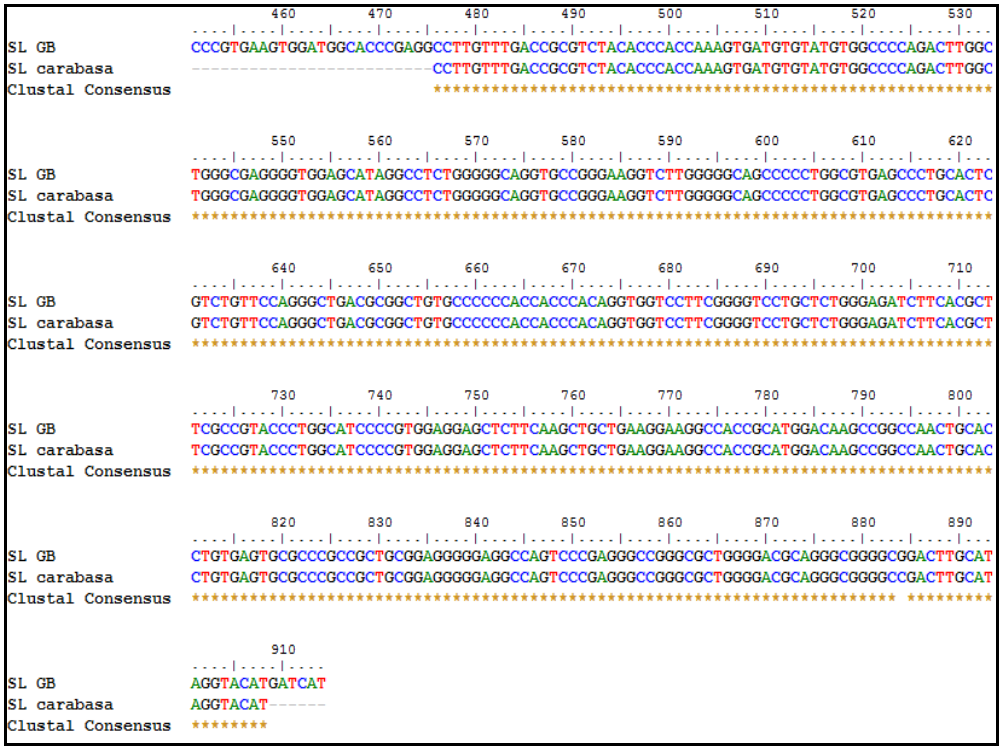

Figure 3. BioEdit alignment between a reference sequence (Genbank database accesion number AY737275) and the sequence we obtained for Blackhead Ruda.

During the 1980s there were many reported cases of SLS animals (Cockett *et al.,*1999). Although many breeders tried to eradicate possible carriers, the frequency of this syndrome was still high. The old and traditional breeding methods, as well as testing the males used for mating can reduce the frequency of SLS, but these are time and money consuming. An alternative to these methods is represented by DNA testing. A genetic test for SLS based on T→A polymorphism in exon 17 of ovine FGFR3 gene was introduced. After the introduction of this test the incidence of this disease decreased drastically in New Zeeland (Jolly et al., 2004) and the United States of America (Beever et al., 2006).

In the five analyzed Romanian sheep breeds we have identified no SLS carrier. The Palas Merino, Palas Milk Line and Palas Meat Line were obtained from Palas Research Institute from Constanta, the Blackhead Ruda from INCDBNA Balotesti and Botosani Karakul from Research and Development Station from Popouti. Although for these breeds we have not identified any carrier or affected animal, this does not exclude the possibility that this disease exists in other parts of the country.

CONCLUSIONS

Congenital diseases like Spider Lamb Syndrome can have a negative effect on ovine production. The eradication of SLS carriers from different populations can positively affect the ovine industry. For this reason we wanted to use a modern, efficient and economic method in order to identify the animals carrying the valine→glutamate substitution in position 69 of exon 17 of the ovine FGFR3 gene. The results obtained highlighted the absence of SLS carriers in five Romanian sheep breeds.

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IMPLICATION EFFECT OF PROBIOTIC BACTERIA TO YOGHURT QUALITY AND ENZYME ACTIVITIES

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The aims of this research is to understand the implication effect of probiotic bacteria as starters to yoghurt quality and the activities of enzyme in yoghurt. Results indicated that the probiotic bacteria combination as starters were the highest enzyme activities, even for yoghurt quality there is no significancy as starters.The activities of enzyme lipase and protease, from the probiotic starters, has highest activities than the controle starters, respectively for lipase and protease are 0.45 and 1.70 unit/ml; 0.19 and 1.65 unit/ml; then 0.27 and 1.62 unit/ml; 0.18 and 1.45 unit/ml from Bifidobacterium spp and Lactobacillus acidophilus; Lactobacillus bulgaricus and Streptococcus thermophilus and Lactobacillus acidophilus; Lactobacillus bulgaricus and Streptococcus thermophilus and Bifidobacterium spp; then Lactobacillus bulgaricus and Streptococcus thermophilus.

Keywords: enzyme activities, Bifidobacterium spp, Lactobacillus acidophilus, Lactobacillus bulgaricus, Streptococcus thermophilus.

The lactic acid bacteria have long been used in fermentation to preserve the nutritive qualities of various foods. Other than major function of a starter culture as lactic acid producer, it will act a source of proteolytic enzyme during growth in milk, finally contributing to the preservation of the fermented product as a consequence of a number of inhibitory metabolites produced by the lactic cultures (O'Keeffe and Hill, 1999). Lactic acid bacteria are among the most important probiotic microorganism typically associated with the human gastrointestinal tract (Holzapfel, et al, 2001). *Bifidobacterium* spp and *Lactobacillus acidophilus* as yoghurt starter produced lower lactic acid and acetic acid than *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Lengkey and Adriani, 2009). Health benefits associated with fermented milk products can be provided by the bacterial starter culture or by dietary adjuncts added after the product is fermented (Nighswonger, et al, 2007). Probiotics control intestinal pathogens through the production of antibacterial compounds, including lactic and acetic acid and antibiotic-like substances, competition for nutrients and adhesion sites, increased and decreased enzyme activity, increased antibody levels. (Hose and Sozzi, 1991). According to Adriani, et al (2009), lipase activities in yoghurt with *Bifidobacterium* spp. and *Lactobacillus acidophilus* starters is higher (0.45 unit/ml) than *Lactobacillus bulgaricus* and *Streptococcus thermophilus* starters (0.18 unit/ml).

MATERIAL AND METHODS

The milk, from farm animals at the faculty.
The bacteria, are pure cultivated *Bifidobacterium spp, Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus.* The identification of the cultures was based on the characteristics of the lactobacilli and streptococci as described in Bergey's Manual of Determinative Bacteriology (Holt, et al, 1994)
Lactobacillus bulgaricus and *Streptococcus thermophilus* are the controle starters.

Yoghurt quality, was tested by preference test.
Enzyme activities, in unit/ml.

RESULTS AND DISCUSSION

Preference test.
For preference test, we're using 25 well trained tester, for testing the aroma, colour, consistency, taste and texture. In Table 1, presents the results of the preference test. The preference test is used for determine yoghurt quality.

Table 1. Preference test

Yoghurt starters			Consumer Preferency			
	Aroma	Colour	Consistency	Taste	Texture	Average
B-La	3.30	3.35	2.95	2.80	3.00	3.08
Lb-St-B	3.45	3.30	3.25	3.15	3.45	3.32
Lb-St-La	3.25	3.36	3.31	2.70	3.01	3.12
Lb-St	2.70	3.25	3.20	3.05	3.15	3.07

Notes:
B-La = *Bifidobacterium* spp*. : Lactobacillus acidophilus* = 1 : 1
Lb-St = *Lactobacillus bulgaricus : Streptococcus thermophilus* = 1 : 1
Lb-St-B = *Lactobacillus bulgaricus : Streptococcus thermophilus : Bifidobacterium* spp = 1 : 1 : 1
Lb-St-La = *Lactobacillus bulgaricus : Streptococcus thermophilus : Lactobacillus acidophilus* = 1 : 1 : 1
Scale : 1 = dislike extremely
2 = dislike moderately
3 = moderately
4 = like moderately
5 = like extremely

From Table 1, for yoghurt quality the highest average for preference test is yoghurt from combination starters *Lactobacillus bulgaricus, Streptococcus thermophilus* and *Bifidobacterium* spp; and then *Lactobacillus bulgaricus, Streptococcus thermophilus* and *Lactobacillus acidophilus*; and *Bifidobacterium* spp, *Lactobacillus acidophilus* and the lowest is from *Lactobacillus bulgaricus* and *Streptococcus thermophilus* starters; even there is no significancy for yoghurt quality between all yoghurt. Probiotic bacteria starters (*Bifidobacterium* spp.and *Lactobacillus acidophilus*) has higher results if added to the controle starters, or without controle starters.
Lactobacillus bulgaricus and *Streptococcus thermophilus* are the controle starters.

Enzyme activities.

Table 2. Enzyme activities

Yoghurt starters	Enzyme activities (unit/ml)	
	Protease	Lipase
B-La	1.70	0.45
Lb-St-B	1.62	0.27
Lb-St-La	1.65	0.19
Lb-St	1.45	0.18

Notes:
B-La = *Bifidobacterium* spp. : *Lactobacillus acidophilus* = 1 : 1
Lb-St = *Lactobacillus bulgaricus : Streptococcus thermophilus* = 1 : 1
Lb-St-La = *Lactobacillus bulgaricus : Streptococcus thermophilus : Lactobacillus acidophilus* = 1 : 1 : 1
Lb-St-B = *Lactobacillus bulgaricus : Streptococcus thermophilus : Bifidobacterium* spp. = 1 : 1 : 1

From Table 2, the enzyme activities of Lactobacillus bulgaricus -St reptococcus thermophilus starters has the lowest activities for both enzyme (protease and lipase), but if we add *Lactobacillus acidophilus* or *Bifidobacterium* spp. as starter, the enzyme activities will raised. The highest enzyme activities is the yoghurt with probiotic bacteria starters only (*Bifidobacterium* spp and *Lactobacillus acidophilus*). This is to be in accord with Hose and Sozzi, 1991; that probiotics will increase or decrease the enzyme activities.

CONCLUSIONS

Yoghurt quality for all starters are no significance, but yoghurt with starters of *Lactobacillus bulgaricus : Streptococcus thermophilus : Lactobacillus acidophilus* got the highest points (3.12 points). Enzyme activities of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* starters has the lowest enzyme activities (protease and lipase), but as we add *Bifidobacterium* spp or *Lactobacillus acidophilus* starters, enzyme activities will raised; but the highest enzyme activities are in yoghurt with combination probiotic starters (*Bifidobacterium* spp and *Lactobacillus acidophilus*).

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PROBIOTIC BACTERIA AS YOGHURT STARTER AND ITS IMPLICATION EFFECT TO THE PATHOGENIC AND NON PATHOGENIC BACTERIA IN MICE GASTROINTESTINAL

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The purpose of the research was to study the effect of bacteria consortium of Lactobacillus bulgaricus, Streptococcus thermophillus, Lactobacillus acidophilus and Bifidobacteria on the ecosystem of gastrointestinal in mice.The aim of this study was to explore the differences between yoghurt content from different consortium, with 1,25% dosage; in mice during three until five weeks. Also, the effect on the number of population of non pathogenic bacteria (Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacteria) and the total pathogenic bacteria in the segment of the mice gut (jejunum, ilium and colon). Results indicated that the bacteria mixture have a good implementation in microbial intestine of mice, which increased the population of non pathogenic and decreased of pathogenic bacteria.

Keywords*: total numbers of bacteria, pathogenic bacteria, and non-pathogenic bacteria.*

According to the previous studies, some genera of lactic acid bacteria and bifidobacteria make an extremely important group of probiotic bacteria. Microflora of the gastrointestinal tract of human or animal, they offer considerable potensial as probiotic because of their history of save use and the general body of evidence that supports their positives role (Björkstén, et al, 2001, Guarner and Malagelada, 2003a, Sears,2005 and Steinhoff , 2005). Namely probiotics are microorganism which had been included in food without any adverse effects and which were present in the gastrointestinal tract for health. At present, these microorganism, called probiotics, have been selected from mostly lactic acid bacteria, e.g. Lactobacillus acidophilus. Bifidobacteria is a part of the normal intestinal microflora of human, since the microorganism are indigenous to the colon. The importance of an indigenous microflora in the gastrointestinal tract as a natural resistance factor against potential pathogenic microorganism was already recognised by Metchnicoff. Probiotic strain can be used only , if the microorganism active in the body of the host if the fulfill a large number of criteria. On the other side, lactic acid and acetic acid caused intestine acidity and can prevented the growth of pathogenic bacteria. Those acids reduce absorbsion of ammonia and amine since the large number of ammonia and amine can rise blood pressure, cholesterol,and cancer because of nitrosamine.

Lactobacillus is a group of gram-positive anaerobic colonic bacteria that produce lactic acid. Supplementation with *Lactobacillus* and the resultant increased colonic levels of this organism has maintained the health of subjects with several intestinal disorders including diarrhea, ulcerative colitis (Bettelheim, et al, 1974 and Tap, et al, 2009). Research has shown that *Lactobacillus* effectively competes with pathogenic bacteria in the colon for binding to the epithelial cells that line the intestines (Schwiertz, 2003). It also inhibits pathogenic bacteria by producing lactic acid and increasing epithelial mucous production.

MATERIALS AND METHODS

Raw milk used for making yoghurt, from farm animal at the faculty. The bacteria are pure cultivated *Bifidobacterium, Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The identification of the cultures was based on the characteristics of lactobacilli and streptococci as described in Bergey's Manual of Determinative Bacteriology (Holt et al, 1994).
120 mice, from a commercial hatchery.
The experimental design were Completely Randomized Design (CRD), with six treatment and four replication. Every cages was filled with five mice.

RESULTS AND DISCUSSION

Total non pathogenic bacteria in each segment of 6 weeks Mice Gastro Intestinal (cfu/ml).

In Table 1, presents the total non pathogenic bacteria in each segment of 6 weeks mice gastro intestinal (cfu/ml).

In Table 1 , the total of non pathogenic bacteria in the colon are higher than the total bacteria in the stomach, jejunum and ileum, especially with ration + Bifidobacteria starter (R3, R4 and R5). Helpful bacteria prevent the growth of pathogenic species by competing for nutrition and attachment sites to the epithelium of the colon. Symbiotic bacteria are more at home in this ecological niche and are thus more successful in the competition. Indigenous gut floras also produce bacteriocins which are proteinacious toxins that inhibit growth of similar bacterial strains, substances which kill harmful microbes and the levels of which can be regulated by enzymes produced by the host (Guarner and Malagelada, 2003a). The resident gut microflora positively control the intestinal epithelial cell differentiation and proliferation through the production of short-chain fatty acids. They also mediate other metabolic effects such as the syntheses of vitamins like biotin and folate as well as absorption of ions including Magnesium, Calcium and Iron (O'Hara and Shanahan , 2006).

Tabel 1. Total non pathogenic bacteria in each segment of 6 weeks Mice Gastro Intestinal (cfu/ml).

Dosages	Gastro Intestinal Segments	Non Patogenic Bacteria	Treatments R0	R1	R2	R3	R4	R5
		Lb	$1.32x10^{4}$	$1.00x10^{5}$	$7.08x10^{4}$	$1.29x10^{2}$	$1.00x10^{0}$	$4.47x10^{4}$
		St	$1.00x10^{1}$	$1.00x10^{0}$	$1.00x10^{0}$	$1.00x10^{0}$	$3.09x10^{2}$	$1.00x10^{0}$
		La	$3.47x10^{2}$	$7.08x10^{1}$	$1.00x10^{0}$	$2.69x10^{4}$	$1.38x10^{4}$	$7.76x10^{1}$
	Stomach	B	$7.24x10^{7}$	$1.00x10^{0}$	$5.37x10^{7}$	$8.91x10^{7}$	$1.05x10^{8}$	$4.27x10^{7}$
		Total	$7.25x10^{7}$	$1.00x10^{5}$	$5.38x10^{7}$	$8.92x10^{7}$	$1.05x10^{8}$	$4.27x10^{7}$
		Lb	$1.02x10^{4}$	$5.50x10^{2}$	$1.05x10^{2}$	$1.95x10^{3}$	$1.00x10^{0}$	$3.63x10^{3}$
		St	$1.00x10^{0}$	$1.00x10^{0}$	$1.35x10^{2}$	$1.00x10^{0}$	$1.00x10^{0}$	$1.35x10^{2}$
		La	$3.63x10^{0}$	$5.01x10^{3}$	$3.72x10^{1}$	$8.91x10^{1}$	$1.20x10^{4}$	$3.72x10^{1}$
	Jejunum	B	$1.23x10^{4}$	$2.69x10^{7}$	$2.82x10^{7}$	$8.13x10^{7}$	$4.17x10^{7}$	$2.00x10^{7}$
		Total	$2.25x10^{4}$	$2.69x10^{7}$	$2.82x10^{7}$	$8.13x10^{7}$	$4.17x10^{7}$	$2.00x10^{7}$
		Lb	$1.74x10^{2}$	$5.62x10^{2}$	$1.62x10^{3}$	$2.88x10^{2}$	$3.63x10^{2}$	$1.62x10^{3}$
		St	$1.00x10^{0}$	$1.00x10^{0}$	$9.55x10^{2}$	$6.46x10^{1}$	$2.69x10^{2}$	$6.17x10^{4}$
		La	$3.80x10^{4}$	$3.89x10^{3}$	$3.31x10^{5}$	$1.00x10^{0}$	$1.00x10^{0}$	$5.50x10^{2}$
	Ileum	B	$6.92x10^{5}$	$1.66x10^{6}$	$1.32x10^{8}$	$3.31x10^{7}$	$6.76x10^{7}$	$9.77x10^{7}$
		Total	$7.30x10^{5}$	$1.66x10^{6}$	$1.32x10^{8}$	$3.31x10^{7}$	$6.76x10^{7}$	$9.78x10^{7}$
		Lb	$8.32x10^{2}$	$1.00x10^{0}$	$2.19x10^{3}$	$2.19x10^{3}$	$2.51x10^{2}$	$2.19x10^{6}$
		St	$1.00x10^{0}$	$8.32x10^{2}$	$2.82x10^{2}$	$2.45x10^{2}$	$8.91x10^{1}$	$6.92x10^{4}$
		La	$1.41x10^{3}$	$1.40x10^{3}$	$1.02x10^{6}$	$1.00x10^{0}$	$245 x10^{6}$	$1.07x10^{3}$
1,25%	Colon	B	$6.76x10^{4}$	$1.10x10^{6}$	$2.88x10^{7}$	$8.32x10^{7}$	$6.31x10^{7}$	$6.76x10^{7}$
		Total	$6.99x10^{4}$	$1.67x10^{6}$	$2.99x10^{7}$	$8.48x10^{7}$	$6.56x10^{7}$	$6.99x10^{7}$
	TOTAL 1,25%		$7.33x10^{7}$	$3.09x10^{7}$	$2.44x10^{8}$	$2.89x10^{8}$	$2.80x10^{8}$	$2.30x10^{8}$

Notes :
Lb = Lactobacillus bulgaricus, St = Streptococcus thermophilus,
La = Lactobacillus acidophilus B = Bifidobacterium spp.
R0 = Controle ration (without yoghurt) R1 = Controle ration + Lb and St starter
R2 = Controle ration + Lb, St and La starter R3 = Controle ration + Lb, St and B starter
R4 = Controle ration + Lb, St, La and B starter R5 = Controle ration + La and B starter

Total pathogenic bacteria in each segment of 6 weeks Mice Gastro Intestinal (cfu/ml).

In Table 2, presents the total pathogenic bacteria in each segment of 6 weeks mice gastro intestinal (cfu/ml).

Tabel 2. Total pathogenic bacteria in each segment of 6 weeks Mice Gastro Intestinal (cfu/ml).

Dosage	Gastro intestinal segment	Patogenic bacteria	Treatments					
			R0	R1	R2	R3	R4	R5
1,25 %	Stomach	Stp						
		Mcc	$9,6x10^4$	$6x10^2$	$6x10^2$			
		B						
	Jejunum	Stp	$2,1x10^3$				$1.3x10^3$	
		Mcc	$3,4x10^3$		$2,4x10^2$			$1.0x10^2$
		B						
	Ileum	Stp	$3,5x10^3$				$5x10^2$	
		Mcc	$1,2x10^5$		$6,3x10^2$	$1,7x10^3$		
		B						
	Colon	Stp	$2,1x10^3$		$1,7x10^3$	$1,9x10^3$		
		Mcc	$5,0x10^3$	$6,6x10^2$	$6,0x10^2$			
		B	$5,5x10^5$					

Notes :
B = Bacillus, Mcc = Micrococci, Stp = Staphylococcus aureus.
R0 = Controle ration (without yoghurt) R1 = Controle ration + Lb and St starter
R2 = Controle ration + Lb, St and La starter R3 = Controle ration + Lb, St and B starter
R4 = Controle ration + Lb, St, La and B starter R5 = Controle ration + La and B starter

From the data from Table 2, the mice that have been feed with R5, has reducing the pathogenic bacteria especially in the colon. This fact are agree with Beaugerie and Petit (2004) that fermentation process, since it produces lactic acid and different fatty acids, also serves to lower the pH in the colon, preventing the proliferation of harmful species of bacteria and facilitating that of helpful species. The pH may also enhance the excretion of carcinogens.

Metabolic function

The gut flora plays a major role in metabolizing dietary carcinogens (Junjie Qin; *et al* (2009); the microcomponents and the macrocomponents. The microcomponents are genotoxic and the major focus is on recent advances in heterocyclic amines (HCAs) which are produced by cooking proteinaceous food such as meat and fish which can then induce tumors in organs like breast, colon and prostate. HCAs are naturally occurring therefore the complete avoidance of them is impractical which is why the metabolic function of gut flora of such components is of

great importance to our body as this would help in prevention of such tumors that are difficult to avoid. The macrocomponents consists of the excessive intake of fat and sodium chloride which can later promote tumors such as in breasts and colons from fat and gastric carcinogenesis from sodium chloride(Guarner and Malagelada , 2003b)

CONCLUSION

The yoghurt with consortium starters with Lactobacillus acidophilus and Bifidobacterium spp. with 1.25% dosage of mice body weight have been raised the non pathogenic population and decreased the pathogenic bacteria in colon.

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STUDY OF AN OUTBREAK OF FOWL TYPHOID IN PHEASANTS

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*Fowl typhoid is produced by an immobile salmonella causing significant economic losses when appear and it is evolving in birds. The present study is addressed to salmonella infection caused by **Salmonella gallinarum-pullorum**. Following an epidemiological survey conducted on a flock of 7914 pheasants, 71 cases were investigated by laboratory tests, of which 22 died and 49 slaughtered. The necropsy, histology and bacteriology confirmed etiological diagnosis rate of 86% to dead birds and 35% to slaughtered birds. From 36 positive bacteriological cultures, 33 were identified as **Salmonella gallinarum** type "**S**".*

Keywords*:* fowl typhoid*, S. gallinarum-pullorum,* pheasant

Infections with bacteria of the genus *Salmonella* are responsible for a range of acute and chronic diseases in different species of animals and birds.

Many species of *Salmonella* are not involved in the morbid processes, their presence in the body is only expressing epifitism states. The problem occurs when salmonella infections produces clinical manifestations in both humans and animals as well as significant economic losses.It is estimated that 80% of the mammalian pathogen *Salmonella* serotypes were isolated from birds (2,5).

This study is addressed to salmonella infection caused by *Salmonella gallinarum pullorum* in pheasants.Fowl typhoid is a contagious infectious disease of birds characterized by digestive disorders and high mortality in chicken embryos and by laying anemia and disturbances in adults (2,4,5).Avian salmonellosis specific lesion is inflammation that is evolving in lungs, liver and myocardium -"fowl typhoid nodules"; in the liver of birds with salmonellosis occur frequently in the secondary lesions of mucoid and fibrinoid deposits and retentions gall to print a brown-green body – "liver brown" (3,4).

Fowl typhoid constitutes an important problem and in some parts of the world determined considerable economic losses trough the extensive eradication programs (2).

MATERIAL AND METHOD

During the period 12/08/2008 to 03/30/2009 in the study were 71 pheasants, of which 49 were slaughtered and 22 died. Investigation carried out allowed the knowing of farm location, analysis of growth system, the species affected, the age and the number of affected subjects.

Examinations conducted in this study were realised in laboratories of pathology and microbiology of DSVSA Braila Microbiology Laboratory, the FMV Microbiology Laboratory Science, the FMV Pathology Laboratory Science, the Department of Bacteriology-Romanian National Laboratory for Salmonella and Pets and "Cantacuzino" Institute Bucharest.

As the necropsy method we used the method of apparatus for organic systems, and preparations made from the organs with pathological lesions were stained by HEA method (Hematoxiline-eosin-methylene blue). For the isolation of *Salmonella* strains and confirmation of

suspected salmonella infection was performed bacteriological examination of long bone unopened, liver, gall bladder, intestine content, spleen and heart.

Following the classical scheme of isolation of Salmonella were used commonly culture media and special culture media: agar-lactose and bile salts (Mac Conckey), medium-eosin methylene blue (Levin), medium with xylose dezoxicolat-lysine (XLD), Salmonella-Shigella agar (S-S) (1, 6, 7).

RESULTS AND DISCUSSIONS

Subjects under study are represented by 7914 pheasants, aged between 140-150 days, which come from the farm Lacul Sărat city, county Brăila, being reared in semi-intensive system.

The epidemiological investigation transverse in all areas to increase pheasants, respectively winter gardens, storage spaces, halls of growth and have been reported multiple galleries rats. Growth system with semi - liberty pheasants, winter gardens and feeding mode, caregivers who move from one shelter to another, allowed dust, fluff, feathers resulting from congestion to serve as the disease sent to all existing farm sheds. Disease are predisposing factors in downgrading and environmental factors - cold rains of autumn-winter period, and adding protective devices on the beak (8, 9).

From 49 birds slaughtered and pathological examinated, 22 had macroscopic and histological lesions specific chronic *Salmonella* infection. The macroscopic examination of the heart noted in some cases the presence of small nodules under-epicardial, gray-whitish color (fig 1). Histopalogic examination revealed proliferation of heart lymphocytes (lymphocytic myocarditis) and waxy Zenker necrosis (fig. 2) and granular degeneration of myocardic cells (fig. 3).

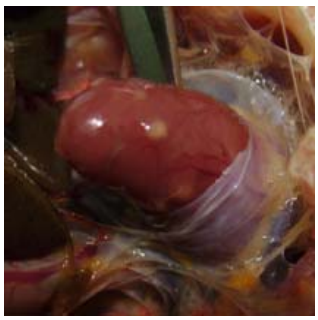

Fig.1 Lymphocytic myocarditis, small nodules under-epicardial Heart of pheasant

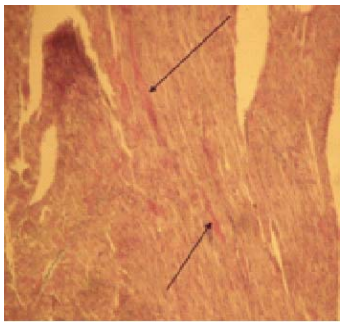

Fig.2 Waxy Zenker necrosis. Cord pheasant. Col. HEA, x 100

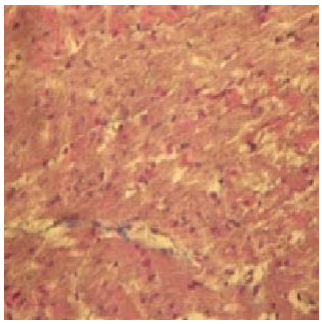

Fig.3 Degeneration of myocardic cells. Heart of pheasant. Col. HEA, x 200

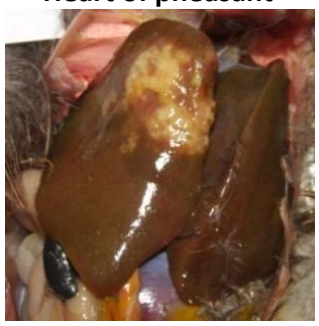

Fig.4 Liver hypertrophy, steatosis, liver tan, foci of necrosis. Pheasant liver

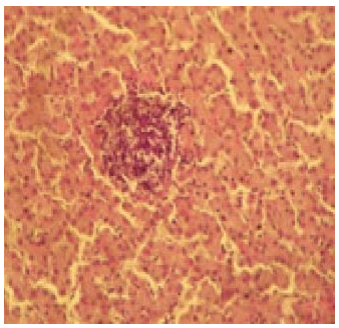

Fig.5 Intralobular lymphocityc proliferation. Pheasant liver. Col. HEA, x 200

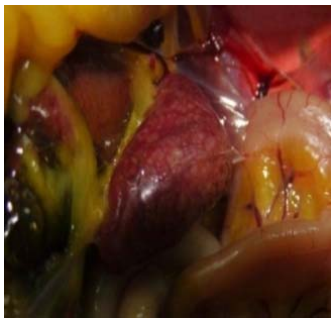

Fig.6 The presence of splenomegaly with follicular drawing very well expressed. Pheasant spleen

The liver, macroscopic and histological examination revealed the presence of pathological processes of disease-specific state: stasis, steatosis but also characteristic lesions of fowl typhoid: tan liver (fig. 4), dried coagulation necrosis foci and intralobular proliferation of lymphocytes (fig. 5).

Spleen volume was increased in all subjects examined, histopathology showing a lyphocytic proliferation what was the basis of splenomegaly (fig. 6). The macroscopic examination of the 22 bodies the specific lesions of fowl typhoid were present in 100% cases. Bacteriological and serological diagnosis are the only ones who can determine certainty the sallmonelosis infection. (3)

Following microbiological examination conducted for the 71 birds, of which 49 slaughtered and 22 bodies, 17 (34.69%) of those sacrificed were positive and 19 (86.36%) from cadavers to infection with Salmonella (fig 9). Besides typhous infection were also identified other bacteria: *E. coli*, *Citrobacter* and *Proteus*.

Specific colonies of *Salmonella gallinarum - pulorum*, translucent yellow-looking, smooth type, grew in abundance on moderately selective environment Mac Conkey (fig.7), for 14 of the 49 subjects slaughtered. On moderately selective medium XLD *Salmonella gallinarum-pulorum* expressed H_2S production (Fig. 8). Samples from birds sowings on XLD medium gave positive results are in total 15 of the 49 sacrificed. Salmonella Agar - Sighella (S-S) are shown in figure 9, where the average positivity occurred in 8 of birds slaughtered.

Given the complexity of tests required to identify biochemical and serological *Sallmonela gallinarum* strains isolated from 33 bacterial cultures samples were examined at the Bacteriology Department - LNR for Salmonella animals, so the diagnosis was *Salmonella gallinarum* type 'S' for all 33 samples. For five bacterial cultures isolated from pheasants were indetified like 2a lizotype, a result confirmed by the "Cantacuzino" Institute Bucharest.

Fig.7 Colonies of Salmonella gallinarum - pulorum on Mac Conkey medium

Fig.8 Salmonella colonies on XLD medium with expression of H2S

Fig.9 Salmonella aspect on the cultural medium S-S

CONCLUSIONS

1. At necropsy of the 49 pheasants slaughteret, 22 of them presented lesions specific to chronic infection salmonella: myocardial dystrophy, hypertrophic liver, tan liver and splenomegaly.
2. At the necropsy of cadavers, all of them had fowl typhoid specific lesions.
3. Following microbiological examination on a sample of 71 birds, of which 49 killed and 22 bodies, was diagnosed infection with Salmonella gallinarum from poultry slaughtered 17 (34.69%) and 19 cadavers (86.36%).
4. From the 36 positive cultures, 33 were identified as Salmonella gallinarum type "S".

5. Although the fowl typhoid is not transmisibile to humans, it causes significant economically losses, as confirmed by the entire liquidation proceedings of the house owner, represented by a number of pheasants 7914, youth from 150 days as the sole method control.

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CONSIDERATIONS ON THE ASSOCIATION OF PERIODONTAL DISEASE WITH OTHER ORGANIC DISEASES IN DOGS AND CATS

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Abstract
Relatively high share of stomatitis associated with other organic diseases draws attention to the oral health assessment in dogs and cats.
Untreated gingivitis leads to periodontitis often associated with tooth exfoliation, vascular barrier crossing bacteria causing, sometimes, bacteremia, a possible cause of distance organic infection.
Following investigations it was found that 53 cases of 61 cats with periodontitis were dianosticated with periodontitis associated with various organic diseases: 25 (47,5%) cases with urogenital infections, 16 (30%) with bacterial dermatitis, 8 (15%) associated with otitis, 3 (5%) showed heart syndrome and 1 (1,8%) with liver syndrome.
107 cases of 133 dogs with periodontitis presented other organic disorders: 42 (39,25%) cases of urogenital disease, 16 (30%) cases associated with skin disease, 23 (21,5%) associated with otitis, 1 (0,9%) heart syndrom and 2 (1,8%) cases diagnosed with liver syndrome.

Key words: periodontal disease, organic disease, dogs, cats

Datas from literature and from practicing veterinarians often signals the presence of oral diseases in dogs and cats. Of these, periodontal disease is most common, regardless of the geographical areas of the world. The frequency of this disease is linked by most authors with age. (3,13)

Periodontal disease is the result of existing plaque bacteria action that lead to inflammation of the gums, followed by destruction of periodontal tissues with important implications in oral pathology. This presents two oral manifestations: gingivitis - an early and reversible form of the disease and periodontitis - a chronic inflammation of gum tissue, with negative impact locally, but also, generally.(3,5,10,11)

In theory it is possible transmitting, progressivelly, local infections, to other organs. Oral infections and particularly periodontal ones are associated, in animal and human pathology, with the existing bacterial microflora in the mouth and, in particular, in plaque. (1,2,4,7,10)

By the anatomical structure, periodontal tissues are irrigated with heavy blood flow and potentially pathogenic bacteria may facilitate bacteraemia and systemic spread of bacterial components and immune complexes. The possibility of dissemination of these microorganisms by oral starting point has long been the subject of numerous studies. Gray (1923) refers to a number of secondary complications of periodontal disease in various organs and tissues.(13)

Penman and Harvey (1990), Bennett and Pollard (1993), Lonsdale (1993) attempt to elucidate the presence of kidney, liver and heart disorders in dogs and cats with periodontitis. Dębowa (1993) expands secondary complications of periodontitis listing further conditions

that: bacterial endocarditis, polyarthritis, polyvasculitis, ankylosing, endotoxiemia and pulmonary diseases.(13)

In human pathology were reported links between periodontal disease and systemic disorders such as diabetes and cardiovascular disease. Similar consequences were highlighted in a study in which dogs with periodontitis was frequently diagnosed diabetes (Hamlin, 1991). The exact mechanism of this association has not been fully elucidated. (11,13)

Recent advances in classification and identification of oral microorganisms and the particularity of certain microorganisms to colonize only mouth opened ways to assess the importance of focal infection in the body. The possibility of spreading oral infections to distance, affecting other organs or leading to systemic infection, sometimes distraught doctor in establishing cause-effect correlation.(3, 4,5,11,13)

The purposes of this research were to highlight periodontal disease in dogs and cats as a factor involved in the emergence of systemic problems, to seize and list of organic conditions associated with periodontal disease in dogs and cats.

MATERIAL AND METHOD

During our research 194 pets were diagnosed with periodontitis, of which 131 were dogs and 63 cats of various breeds and ages (2-15 years). Investigated animal body weight varied between 3-15 kg. The cases were presented for various investigations in veterinary private clinics and clinics of the Faculty of Veterinary Medicine.

Attendance at initial consultation aimed oral diseases located in the two species. Subsequently, after further clinical assessment other organic diseases were identified. There have also been other cases when diagnose periodontitis was a surprise, since the animals were examined and clearly suffering from other causes. Medical conduct adopted for use in the study was: history, clinical examination and laboratory tests.

Oral examination consisted in identifying plaque, the plaque and gingival inflammation status. There were followed tooth mobility (by digital pressure), apical abscess and gingival ulceronecrosis.

General clinical examination consisted in functions evaluation: cardiovascular (heart rate, pulse, mucous membranes, temperature) and respiratory (respiratory rate and its type).

Urogenital examination was to assess urinary transit and biochemical and microbiological examination of urine. To confirm the diagnosis of urinary disorders ultrasound ALOKA prosound 2 was used.

Otic evaluation consisted in otoscopie and microbiological examination of otic exudate.

Dermatologic examination consisted of taking superinfected skin scrape and its microbiological investigation.

Biological samples were collected from periodontal lesions in dogs and cats with skin abscesses in various locations, urine samples from suspected cases of urinary infections and otic exudates. Specimens were subjected to microbiological investigations, conduct following the steps of classic diagnostic.

There were made bacterioscopic exams directly from pathological material, stained by Gram method and the actual bacteriological examination consisted of sowing the usual culture media (broth and nutrient agar supplemented with horse blood serum 10%) and other special mediums. After that followed the examination of cultural characters, morphology and biochemistry of isolated bacteria.

Microbiological results obtained from each case were compared to identify a common bacterial etiology of periodontitis and various organic diseases.

RESULTS AND DISCUSSION

Researches showed a noticeable incidence of periodontitis associated with other organic diseases. (***table 1***).

Investigations identified 133 dogs and 61 cats with periodontitis. Of these, 107 dogs and 53 cats were diagnosed with other organic diseases.

Microbiological results of the collected pathological material have shown Gram-positive and Gram-negative bacteria from different genres.

Table 1

Presentation of cases with periodontal disease associated with various oral diseases in dogs and cats

Species	Total cases			Urogenital disease		Skin disease		Otic disease		Cardio-vascular disease		Liver disease	
	Periodontal disease	Associated periodontal disease											
	nr	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%
Dogs	133	107	100	42	39,25	39	36,45	23	21,5	1	0,9	2	1,8
Cats	61	53	100	25	47,5	16	30	8	15	3	5	1	1,88

Urogenital diseases were diagnosed by performing renal ultrasound and urine microbiological examination (***fig. 1***).

Of 107 dogs and 53 cats associated with periodontitis, 42 dogs (39,25%) and 25 cats (47,5%) showed various urogenital diseases. These varied from case to case and consisted of uremic syndrome, renal colic, pyelonephritis and changes of transparency color of urine. (***table 2, fig. 2***)

Table 2

Urogenital clinical aspects identified in cats and dogs with periodontitis

Species	Nr. cases with periodontitis	Number of cases with clinical manifestations localized urogenital					
		Pyelo nephritis	Renal abscess	Uremic syndrome	Renal colic	Urine changes	Preputial lesions
Dogs	42	27	2	4	29	37	4
Cats	25	21	3	6	10	23	2

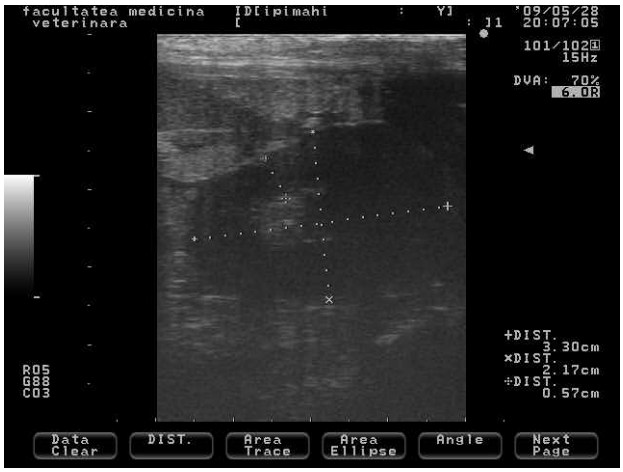

Fig.1 - *Ultrasound image of a 5 years old cat with kidney failure, renal edema, thickening of the renal cortex*

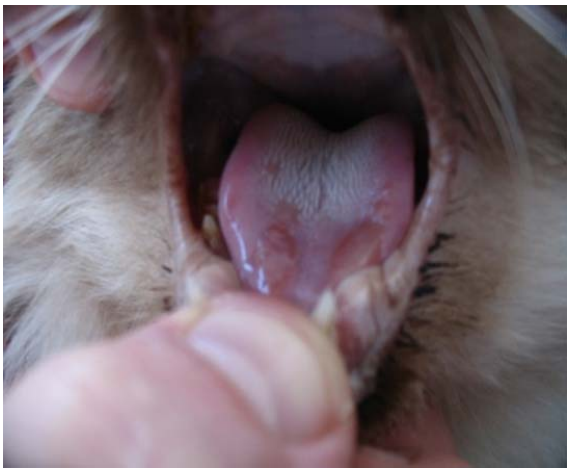

Fig.2 Lingual ulcerations in a 5 years old cat with kidney failure

It is important to note that following microbiological analysis of urine, harvested from dogs and cats with kidney disease, have identified different bacterial species (***table 3***).

Comparing the results of microbiological exam of urine and periodontal samples, was found that in the etiology of these diseases *Staphylococcus aureus* was identified in 8 dogs (21,6%) and 9 cats (39%), *Streptococcus ssp*. was isolated from 2 dogs (5,40%) and *Escherichia coli* in 7 dogs (19%) and 6 cats (27%).

Table 3

Microflora and periodontal bacteria identified in urine specimens

Species	Number of identified cases							
	No. of cases		Identified bacterial species					
			Staphylococcus aureus		*Streptococcus ssp.*		*Escherichia coli*	
	nr	%	nr	%	nr	%	nr	%
Dogs	37	100	8	21,62	2	5,40	7	19
Cats	23	100	9	39	-	-	6	27

Identification of bacterial strains in oral infections and kidney, from the same animal, can be considered a causal factor, without being able to demonstrate precisely the exact mechanism of transmission. Specialized studies mentioned oral bacteriemia starting point, representing the main cause in the emergence of renal disease in dogs and cats (9,12).

Dermatitis with bacterial etiology was identified in 39 dogs (36.45%) and 16 cats (30%) with periodontitis.

Microbiological investigations identified similar bacteria in periodontal specimens and skin scrape (***table 4***).

Table 4

Microbiological rezults to pardontale and skin samples

Animal species	Bacterial etiology of periodontitis and dermatitis									
	Cases		Bacterial species identified							
			Staphylococcus aureus		*Staphylococcus intermedius*		*Streptococcus spp.*		*Pasteurella multocida*	
	nr	%	nr	%	nr	%	nr	%	nr	%
Dogs	39	100	6	15,4	-	-	4	10,3	3	7,7
Cats	16	100	4	25	3	18,75	2	12,5	6	37,5

Strains of *Staphylococcus aureus* were identified in 6 dogs (15,4%) and 4 cats (25%), *Staphylococcus intermedius* in 3 cats (18,7%), *Streptococcus spp*. (nehaemolyticis) in 4 dogs (25%) and 2 cats (12,5*%), Pasteurella multocida* 3 dogs (7,7%) and 6 cats (37,5%).

Presence of bacterial strains involved in both periodontal and skin lesions can be easily justified by the behavior of licking the wounds. This action can disseminate some potentially pathogenic bacteria in the mouth of dogs and cats.(3,5)

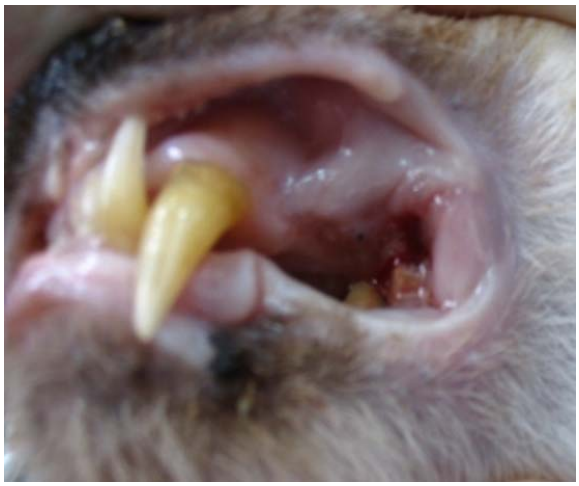

Fig. 3 - ***Periodontitis in a 6 years old cat***

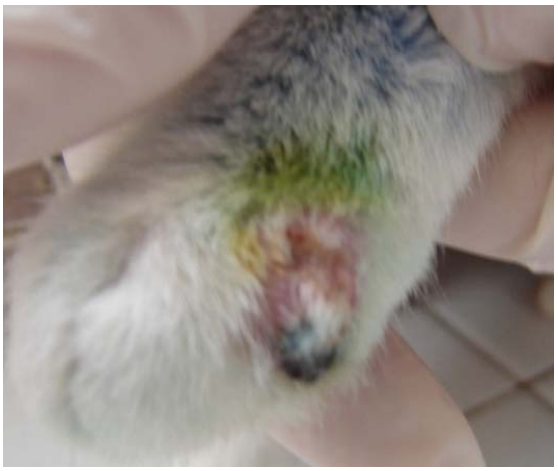

Fig. 4 - ***Infected cutaneous wound in a 6 years old cat***

Otoscopic exam showed some changes of color and consistency of otic secretion in 23 dogs (21.5%) and 8 cats (15%) diagnosed with periodontitis

Otitis secretion was yellowish in early stage disease or viscous-looking brick, as the disease process was advanced. In some cases, otitis were accompanied by erosion and ulceration in the pinna. Etiology of otitis was polimicrobiane. Assessment on each case has revealed the involvement of common microorganisms in otitis and periodontitis. There was one exception to a cat with periodontitis, whose development led to the formation of fistulas urosinusală external opening near the right ear (***fig.5, fig.6***).

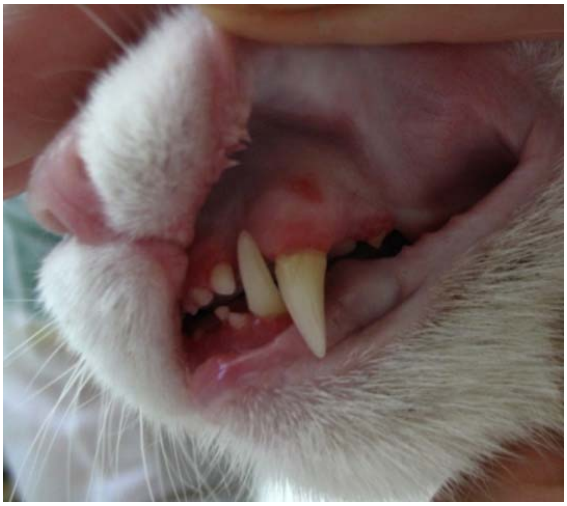

Fig. 5 - ***Periodontitis, apical abscess, in 4 years old cat***

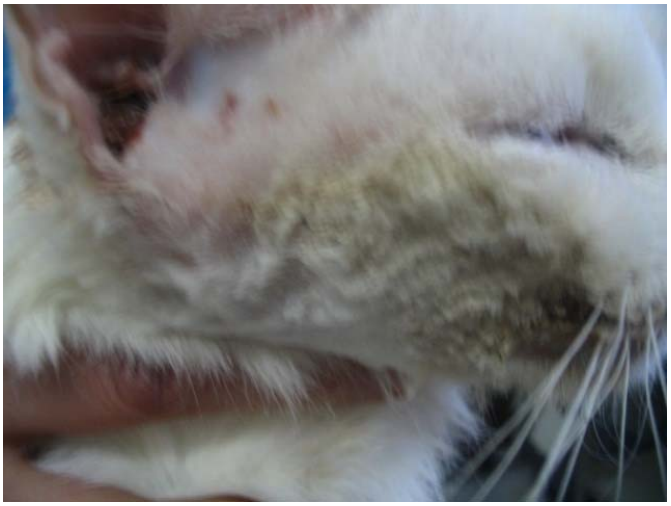

Fig. 5 - ***Ear infection, orosinusal fistula in a 4 years old cat***

Cardiac function examination results revealed changes in heart rhythm and cardiomyopathy, endocarditis and hypertension in 3 cats (5%) and 1 dog (0,9%) with chronic periodontitis. Although it can not be proven in this case, correlations between the two conditions, however some specialized studies consider a possible mechanism for oral chronic inflammation of the release of potentially pathogenic oral bacteria.

In human medicine, patients with periodontal disease are at increased risk for systemic diseases, especially heart disease, the most commonly encountered being infectious endocarditis.(1,2,10)

Periodontal diseases are able to predispose individuals to cardiovascular disease because of the multitude of gram-negative species involved, the higher levels of proinflammatory cytokines, immune and inflammatory infiltration and by increased levels of fibrinogen and WBC.(7,8_

Extrapolation to human studies and numerous research conducted on dogs and cats, confirmed the risk of cardiovascular disease in animals with periodontitis.(1,2,6,7,8,10)

Identification of liver diseases in dogs and cats with periodontitis was based on ultrasound results and laboratory analysis. Thus in 2 dogs (1,8%) and 1 cat (1,8%) was found liver echogenity change and doubling of normal liver enzymes: ALT (Alanine aminotransferase), AST (aspartataminotransferaza) and GGT (Gamaglutamil transferase).

Periodontitis, as a major oral infection, can cause complications by contiguity (retropharyngeal infections, pleuropulmonary infections, etc.) or by marrow dissemination (urogenital infections, heart infections, etc.). (3,4,5,7)

Microorganisms that penetrate the blood, circulating through the body, can cause a transient bacteremia, sometimes accompanied by a slight increase in body temperature. If microorganisms find favorable conditions in the body, they can multiply. Onset of infection may alter the functions of kidneys, lungs, liver or heart and can be seriously compromised, sometimes with irreversible consequences.

Summarized data emphasize the high frequency of periodontitis associated with the cases studied. Although it is difficult to involve oral microorganisms in triggering systemic infection, the simultaneous presence of these conditions remains an open medical research.

CONCLUSIONS

1. Of 133 dogs with periodontitis, 107 cases were associated with other conditions, as follows: 42 (39,25%) cases suffering from urogenital diseases, 39 (36,45%) cases exhibited skin diseases, 23 (21,5%) cases were associated with otitis, 1 (0,9%) case this heart syndrome and 2 (1,8%) cases showed liver syndrome
2. Of 61 cats with periodontitis, 53 cases were diagnosed with periodontitis associated with various organic disease: 25 (47,5%) cases with urogenital infection, 16 (30%) cases showed bacterial dermatitis, 8 (15%) cases were associated with ear infections, 3 (5%) cases exhibited cardiac syndromes and 1 (1,8%) cases with liver syndrome.
3. Comparing the results with the microbiological exam of urine and periodontal specimens it was found that in the etiology of the disease *Staphylococcus aureus* was identified in 8 dogs (21,6%) and 9 cats (39%), *Streptococcus ssp.* was isolated from 2 dogs (5,40%) and *Escherichia coli* in 7 dogs (19%) and 6 cats (27%).
4. Comparing the results with the microbiological skin scrape specimens, obtained from periodontal isolates, were identified *Staphylococcus aureus* in 6 dogs (15,4%) and 4 cats (25%), *Staphylococcus intermedius* in 3 cats (18,7%), *Streptococcus spp* (nehaemolytics) in 4 dogs (25%) and 2 cats (12,5%) and strains of *Pasteurella multocida* in 3 dogs (7.7%) and 6 cats (37,5%).
5. Assessment on each case has revealed the involvement of common microorganisms in otitis and periodontitis.

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THE INFLUENCE OF SEASONAL CHANGE TOWARDS THE NUMBER OF OUTBREAKS AND POULTRY DEATH RATE CAUSED BY AVIAN INFLUENZA AT BANDUNG DISTRICT

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Community Based of Integrated Research on Influenza

Abstract
Generally, change of season always followed by disease emergence which connected with poultry stamina reduction that could lower body reaction towards disease especially Avian Influenza Viruses, which at the end poultry will easily get sick and died. Fluctuating weather conditions or frequent changes in a short time will reduce poultry stamina, even poultry productivity. The global outbreak of Avian Influenza is seasonal in recent four years, mainly from the end of dry to the beginning of rainy season, and from the end of rain to the beginning of dry season.
This survey was conducted to determine how number of rainy days and fluctuating weather condition influence outbreak and poultry death rate which caused by Avian Influenza Viruses. This research uses survey methods with household who kept poultry and household where sick or died poultry found as unit of analysis. Data were taken from the outbreaks which took place in Bandung District.
Result showed that February and June 2009 give the highest number of Avian Influenza rapid test positive with highest poultry death rate. February with 26 rainy days (RD), found four outbreaks with the highest poultry death rates (21.66%) which caused by the fluctuation of rainy days. However, At the end of the outbreak series, found three outbreaks on June (15 RD) with poultry death rate increase until 17.52%. This situation probably caused by transition from monsoon to dry which all of that causing uncertain weather fluctuation.

Keywords: ***avian influenza, rainy days, weather fluctuation, outbreak, poultry death rate***

INTRODUCTION

Since 2003, Bird flu has emerged as a potentially lethal threat to humans. Avian influenza has become a common threat to health in worldwide range. The deadly H5N1 virus had claimed over 216 lives globally from 349 confirmed cases between 2003 until April 2008. Human cases of avian influenza were reported in 15 countries during the period 2003-2009; the highest number of cases was reported in 2006. Indonesia was the most affected country, with 115 deaths out of 141 cases during the period 2005-2009 (World Health Organization/WHO, 2009).

West Java especially Bandung District was an Indonesian District with highest incidence and prevalence of human cases of avian influenza. This situation will persist for the near future, since steps to control its spread have been met with ignorance and resistance. Bandung's people have not raised sufficient public awareness of the serious danger. Beside that, the topography of the area was quite possible that a highly pathogenic mutant Avian Influenza Virus (AIV) will arise in Bandung District, where the next pandemic may possibly start.

Bandung district area were influenced by tropical monsoon with rainfall average 1500 up to 4000 mm per year, temperature average 19° to 24° C and the relative humidity average were 78% on rainy season and 70% on dry season (Social services of West Java Province, Not dated). This condition allows the AIV to survive outside the host especially on feces or an eggshell. Avian influenza virus can survive outside the host for certain periods of time depending on the environmental conditions. However, AIV is susceptible to heat and dryness and is easily destroyed by strong acid or alkaline conditions and by disinfectant (Chumpolbanchorn, 2006). In Thailand, Songserm, *et al*. (2005) studied that AIV (H5N1) can live for only 30 minutes on allantoic fluid or fresh feces at 33-35°C and it has a longer survival time if kept at room temperature, which is up to 10 days. Dryness was also a factor that affects AIV survival time, thus high number of rainy days will allow AIV to survive longer and increased the prevalence of bird flu outbreaks in this area.

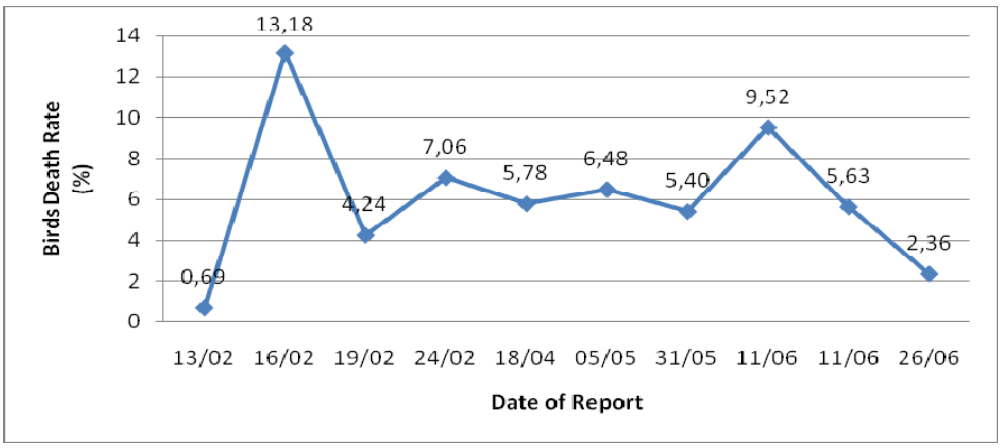
The fastigium of avian influenza usually emerges when the season changes or number of rainy days fluctuates greatly. The global outbreak of avian influenza is seasonal in recent four years, mainly from the end of dry to the beginning of rainy season (October to November), and from the end of rain to the beginning of dry season (February to April). February to April is just at the end of rain and beginning of dry season, at this time, number of rainy days fluctuates greatly, and epidemic influenza prevails easily, so number of rainy days is an important factor to induce the outbreak of avian influenza.

METHODS

The method of the research was survey method with household who kept poultry and household where sick or died poultry found as unit of analysis. This survey was held on October 2008 - June 2009 at Bandung District. The number of poultry death rate were collected from ten outbreak area (Cangkuang, Panyirapan, Nanjung, Jagabaya, Sayati, Pamubusan, Soreang, Cibangkonol and Cimekar) based on Livestock Services Information with AIV positive tested using Rapid Test and the number of rainy days data were taken from The Meteorology, Climatology and Geophysics Agency Bandung Chapter.

RESULT AND DISCUSSION

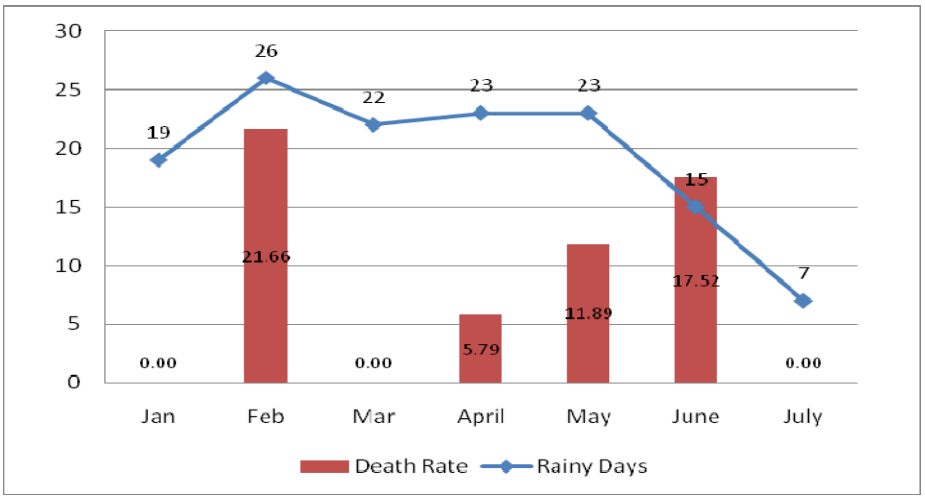
Avian influenza outbreak was occurring from February until June 2009, ten reports of sick or dead poultry which AI rapid test positive found in Bandung District. Graphic 1 showed that on the beginning poultry death rates were highly increase from 0.69 percent to 13.19 percent, and then decline until 4.24 percent. It is also happen at the end of the outbreak series start on 7^{th} outbreak with 5.4 percent poultry death rate increase to 9.54 percent on 8^{th} outbreak and then decrease to 5.63 percent on 9^{th} outbreak until 2.35 percent poultry death rate on 10^{th} outbreak. This situation was caused by changing season from rainy to dry that causing uncertain weather fluctuation.



Graphic 1. Avian Influenza Outbreak Timeline

As shown in Graphic 2, great fluctuating number of rainy days trigger high poultry death rate which caused by AIV infection. Fluctuating number of rainy days on the beginning of January until March 2008 gives the highest poultry death rate on February 2008 which is 21.66% poultry death rate. The highest poultry death rate was taken from four outbreaks which is the highest number of outbreak number in a month.

High number of rainy days causes wet environment that can make AIV survive longer. The condition accordance with what was found during the three outbreaks on February (16, 19, 24 Feb). The poultry death series occurred more than one day on every outbreak. This also due to avian influenza virus could live for 4 days in water at 22°C and up to 30 days at 0°C (Webster, *et al.*, 1978), furthermore AIV (H5N2) in feces that kept at 20°C could be recovered until 7 days (Beard, *et al.*, 1984). Lu, *et al*., (2003), found the virus (H7N2) died on chicken feces within one week at 15-20°C.



Graphic 2. Correlation Betweeen The Number of Rainy Days and Poultry Death Rate

Fresh fecal droppings which have at least 60% moisture, the AIV can survive for 4 days in fresh fecal droppings (Songserm, *et al*., 2005). The moisture content of the environment surrounding the virus is an important factor for the survival period of the virus. Hence the increase of neighborhood moisture that caused by high number of rainy days will increase the survival period of the virus.

May until July 2009 was the end of rainy season, the number of rainy days were decrease drastically. The number of rainy days which decrease drastically also caused the second highest number of poultry death rate (17.53%) from three outbreaks on June 2009. Rainy days were still found during the three months period, however the duration and rainfalls was uncertain. Change of season or weather patterns will affect the poultry life cycle. Erratic weather and unpredictable climate patterns will result poultry difficult to adapt. Moreover poultry preserve what we now require the provision of comfortable conditions (*comfort zone*) to be able to develop the genetic potential optimally (Medion, 2009).

CONCLUSIONS

February and June 2009 give the highest number poultry death rate. The fluctuation of rainy days number during January until March 2009 gives the highest poultry death rates (21.66%) on February 2009. High number of rainy days increase the environment moisture that can make AIV survive longer. However, at the end of the outbreak series, drastically decreased of rainy days numbers from May until July 2009, caused three outbreaks on June 2009 (15 RD) with poultry death rate increase until 17.52%. This situation also caused by uncertain weather fluctuation that can make poultry difficult to survive.

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EPIDEMIOLOGICAL INVESTIGATIONS IN THE EAST OF ROMANIA REGARDING THE SEROPREVALENCE OF INFLUENTZA A TYPE VIRUSES IN DIFFERENT SPECIES OF ANIMALS

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Influentza A virus infection in human and animals is an actual subject in Romania because the "new flu" or "swine flu" represents a real threat for the public health. The type of flu caused by H1N1 virus is consider to be a disease with a zoonotic character, knowing that the introduction in the human population of the influenza type A virus specific for pigs and poultry can be an intermediate step in the pandemic transmission of virus.

We consider the study of influentza A viruses in different species of animals relevant because the virus has the capability to recombine in various species of and to determine the apparition of new viral subtypes. Pigs have been considered the most adequate, for the role of intermediary because these animals may serve as hosts for productive infections of both avian and human viruses and, in addition, the evidence strongly suggests that pigs have been involved in interspecies transmission of influenza viruses, particularly the spread of H1N1 viruses to humans.

In the cadre of a research project we have done a serosurvey of Influentza A antibodies in the horse, pig and bird population from 7 districts placed in the east and south-east of Romania (the inferior area of Danube). Specific antibodies anti –influentza A virus were detected using a blocking-ELISA (Pourquier Institute) in different percents depending of the species tested and place of the sampling.

The investigations are continuing.

Key words: influenza, type A, seroprevalence, multispecies

INTRODUCTION

Influenza is a highly contagious, acute illness which has afflicted humans and animals since ancient times. Influenza viruses are part of the Orthomyxoviridae family and are grouped into types A, B and C according to antigenic characteristics of the core proteins. Influenza A viruses infect a large variety of animal species, including humans, pigs, horses, sea mammals and birds, occasionally producing devastating pandemics in humans (Alexander D.J., 2000)

Influentza A virus infection in human and animals is an actual subject in Romania because the "new flu" or "swine flu" represents a real threat for the public health. The type of flu caused by H1N1 virus is consider to be a disease with a zoonotic character, knowing that the introduction in the human population of the influenza type A virus specific for pigs and poultry can be an intermediate step in the pandemic transmission of virus (Brookes S.M., 2010)

We consider the study of influentza A viruses in different species of animals relevant because the virus has the capability to recombine in various species of and to determine the apparition of new viral subtypes. Pigs have been considered the most adequate, for the role of intermediary because these animals may serve as hosts for productive infections of both avian and human viruses and, in addition, the evidence strongly suggests that pigs have been

involved in interspecies transmission of influenza viruses, particularly the spread of H1N1 viruses to humans(Yassine H.M.,2009).

MATERIALS AND METHODS

In the cadre of a research project we have done a serosurvey of Influentza A antibodies in the horse, pig and bird population from 7 districts placed in the east and south-east of Romania. In the inferior area of Danube (Tulcea and Brăila) the area of study was chosen due to previous development of influenza viruses epidemics (Ward M.P., 2007), and the existence of wild birds migration route. The epidemiological survey was expanded in order to have an overview of the extension of the infections caused by type A influenza viruses in east and south east area of Romania. Samples were collected from swine, equine and poultry, mainly from households. The serological surveillance of influenza viruses from migratory and domestic birds has also been made on an extensive area in some selected counties to establish epidemiological risk areas.

A total of 392 samples were collected from the counties of Bacau, Braila, Botosani, Neamt, Suceava, Tulcea, Vaslui (tab.1).

Table 1.

Study areas and total number of samples

No.	County	Species			Total number of samples
		equine	swine	poultry	
1	Bacău	9	5	7	21
2	Brăila	48	-	-	48
3	Botoşani	10	10	10	30
4	Neamț	10	78	16	104
5	Suceava	22	9	20	51
6	Tulcea	15	65	29	109
7	Vaslui	9	10	10	29
Total		123	177	92	392

Of the 392 samples collected, a total of 363 samples of which 90 samples from poultry, 173 samples taken from pigs and 90 samples from horses, were analyzed for the detection of Specific antibodies anti –influentza A virus using a blocking-ELISA (ELISA Blocking serum screening influenza monocupule version P00555/01, produced by the institute Pourquier, Montpellier France). The use of blocking ELISA has the advantage of being more sensitive than the immunodiffusion test.

RESULTS AND DISCUTION

The serosurvey of type A influenza virus in swine, horse and poultry populations in the seven counties showed different percentages of the global seroprevalence. Of 90 birds serologically tested: 82 birds responded negative (91.11%), 7 birds responded doubtfull (7.78%) and we obtained a positive response from a single bird (1.11%).

Of 173 pigs serological tested: 80 pigs were negative (47%), six samples were apreciated as doubtfull (3%) and 87 pigs responded positive (50%).

A number of 92 swine samples tested have been collected from two industrial systems of growth were we have appreciated a global seroprevalence of 72.83%.

From 100 horses serologically evaluated: 66 horses have negative response (66%), 7 responded dubious (7%) and 27 horses were positive, representing a rate of 22,22%.

Regarding the distribution of serologically positive animals tested in the seven counties studied we have obtained different percent of seropositivity depending of the species tested and place of the sampling.

Fig. 1 Seroprevalence rate in the study areas

In Suceava county we have tested 32 sera all with a negative response for the *Influentza A viruses* antibodies, regardless of the species tested.

In Bacău county were tested 21 animals (seven grouse, five pigs and nine horses).The serologically positive samples were registered only in horses 3 samples from 9 tested (33,3%).
In Botoşani county 30 samples were tested, ten from each species.The serologically positive samples were registered only in 4 horses (40 %).

In Tulcea county the most seropositive results were registred in Cetalchioi district. In this case from 25 animals tested (eight grouse, nine horses and eight pigs), 13 animals were serologically negative and 10 animals responded positively, including a grouse, seven pigs and two horses, the rest two samples were doubtfull (a grouse and a pig). The other samples tested (no.82 -19 birds, 6 horses and 57 pigs) were positive (3 samples from 57), in a 5,26% percent only for pigs from two farms.

In Neamț county, in 19 animals tested from households (three horses and 16 grouse) we have only negative signals, but in two swine farms all the animals sampled (77 samples) were positive-a 100% seroprevalence for specific antibodies anti –influentza A virus.

In Vaslui county from a total of 39 samples tested (ten from grouse, ten from horses and 9 from pigs) the seroprevalence was 50% for grouse, 1% for horses and 0% in swine.

The sera tested *in Brăila* county were sampled only from horses (Chişcani, no.38) and Gropeni (no.10) and the registered global seroprevalence rate was 37,5% respectively (28,95% and 70%).

CONCLUSIONS

Taking into account the hight levels of seroprevalence in pigs from herds with intensive farming, may be taken on the issues of the import and purchase of animals that were previously infected or come from infected mothers.

A percentage of 22.22% of the animals tested serologically for equine influenza type A is significant and important having in mind the long lifetime of these animals, thus confirming the existence of a long-lasting immunity H3 (human, pig, poultry, horses), H7 (human, seal, poultry, horses), N7 (humans, seals, poultry, horses), N8 (humans, poultry, horses), H3N8 beeing a strain circulating in the equine population since 1988 and currently present (H3 and N8 subtypes) in the human population.

In the context of the existing situation consecutive to the outbreaks of avian influenza which reemerged in the last years and the high risk of pandemic „swine flu", the signals of equine influenza which has been found should not be minimized

Equine influenza can have implications in terms of its transmission among domestic carnivores, cycle in which the man could be easily co-opted by different genetic mechanisms different from previous patterns. The life of birds and pigs due to consumption of meat is low. Compared with these two horses are species longevity, and serological surveillance designed to monitor long-term immunity (cell) could cover both pre and post enzootic periods.

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PERFORMANS GENETIC QUALITATIVE AND QUANTITATIVE OF THIN TAIL SHEEP AND PRIANGAN SHEEP

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ABSTRACT
This study aimed to identify genetic differences in the nature of qualitative and quantitative as well as a series of blood protein bands on the Thin Tail Sheep and Priangan Sheep. This study uses 48 head of Thin Tail Sheep and 34 head of Priangan Sheep, Rams and Ewes aged 2 years in the same environment. Results of color pattern on Thin Tail Sheep, 31% plain white, 43% black and white color combination-and 15% brown and white combination. Color pattern on Priangan Sheep, is 40% plain black, 6% plain white and 41% black and white. Brown color is a color found on Thin Tail Sheep. The ear shape is generally 89% "rumpung" (small) in Priangan sheep and 88% "rubak" (wide) in Thin Tail Sheep . Priangan Sheep have thick tails and Thin Tail Sheep have thin tails; in accordance with its name. Body-size differs significantly (P <0.05) between males and females. Skull length and height, length and width of the ear was also significantly difference (P <0.05). Five types of protein band (post transferring-1, post-transferrin-1, post-albumin and albumin) are all homogenous in Priangan Sheep 9 bands while in Thin Tail Local Sheep heterogenous between 7 to 10 bands (average of 8 bands).

Keywords: ***Thin Tail Local Sheep, Priangan Sheep, Qualitative and Quantitative Factors, Blood Proteins.***

Sheep farming in Indonesia in general is still a business with a pattern of maintenance of his nature as economic savings in mix farming systems, in order to support the economic sustainability of households.

Sheep which spread in Indonesia is still very diverse, so too about his origins are still little known. Nevertheless there are sheep that can be grouped into three categories: Thin Tail Sheep, Fat Tail Sheep and Priangan Sheep also known as Garut Sheep (Mulyaningsih.N, 1990). Diversity sheep there is a rich source of germplasm, and needs to be preserved, especially the existence of local livestock.

Thin Tail Sheep is a sheep with small body size: Height shoulder ewe average 57 cm and 60 cm in ram, ewe body weight of 25-35 kg while the ram 40-60 kg. Coat color is generally white with black patches around the eyes and nose, short-horned male horns while the female does not, the size of the ears and the tail of the medium there was no deposition of fat (Mason, 1980; Devendra and McLeroy, 1982). Thin Tail Sheep have a high fertility rate, the first litter at the age of 18 months and the average birth of two tails, mortality until weaning age of approximately 33% (Subandriyo, et al., 1981).

Priangan Sheep is the result of three breeds sheep crosses: Merino - Australia, Kaapstad from South Africa and Fat Tails Java in Indonesia. Javanese Fat Tail Sheep preexisting long ago as a kind of local sheep, Merino sheep brought by Dutch traders to Indonesia, while Kaapstad Sheep,the Arab traders brought to the land of Java around the 19th century. Priangan sheep is a type of tropical sheep are lambing proliflic that it can be more

than 2 (two) tails in a cycle of birth. Priangan sheep have a short lambing interval with 170% lamb production per year. These sheep have an average weight loss on the other Indonesian local sheep. Ram can weigh about 60-80 kg and some even can reach more than 100 kg. While ewes weighed between 30-50 kg. Physical characteristics of a horned male Priangan Sheep, big-necked and strong, with shades of white, black, brown or mixture of all three. The dominant feature of ewes is not horned, but even if a small horn with a similar color pattern of the ram (Merkens and Soemirat 1926; Devendra and McLeroy, 1982).

The aim of the study is to determine the characters of genetic differences that arise both qualitatively and quantitatively and blood protein polymorphism in a series of bands Thin Tail Sheep and Priangan Sheep. The benefits of this research is expected to be known for certain clarity of the two breeds sheep by electrophoresis in an effort to collect germplasm with the aim of maintaining purity and breed further.

MATERIAL AND METHODS

This research conducted over three months using the 48 Thin Tail Sheep and 34 Priangan Sheep, ewes with rams aged about two years. Parameters observed consisted of a qualitative nature based on the properties visible from the outside ie: hair color patterns, ear shape and tail shape (Diwyanto.K, 1982) and quantitative traits such as body measurements are: Weight loss (kg), body length (cm), shoulder height (cm), chest circumference (cm), the chest (cm), skull length (cm), skull width (cm), ear length cm), ear width (cm), tail length (cm) and tail width (cm) (Amano, et al, 1982). The blood samples were taken of each of 10 blood samples for Thin Tail Sheep and Priangan Sheep.

The blood samples analyzed in the laboratory observed using electrophoresis techniques, from the blood serum protein bands were observed that occurred as a post-transferrin-2-one post-transferrin, transferrin, post-albumin and albumin (Johansson and Rendel, 1966; Warwik, et al ., 1983, and Nicholas, 1987).

The research method used is a case study.

To examine the qualitative nature of the observed relative frequency of use calculations for the properties tested in groups of sheep using the formula:

$$\boldsymbol{Character\ frequency\ A} = \frac{\sum \boldsymbol{character\ A}}{\boldsymbol{N}} \times \mathbf{100}\%$$

Character A : One of the properties observed
N : Number of samples in one group of sheep

To test differences in quantitative traits using Student's t-test (Steel and Torrie, 1989) with the following formula:

$$\boldsymbol{t} = \frac{\overline{\boldsymbol{X_1}} - \overline{\boldsymbol{X_2}}}{\boldsymbol{S_{X_1 - X_2}}}$$

t : the mean value
$\overline{X_1}$: average sheep X_1
$\overline{X_2}$ *: average sheep* X_2
$S_{X_1 - X_2}$ *: standard deviation* $X_1 - X_2$

RESULTS AND DISCUSSION

CHARACTERS OF QUALITATIVE

PATTERN COAT COLOR

Results of this research about coat color pattern on Thin Tail Sheep and Priangan Sheep, shown in Table 1.

Table 1. Color Pattern on Thin Tail Sheep and Priangan Sheep

Color Pattern	Thin Tail		Priangan	
	Frequency	(%)	Frequency	(%)
Plain Black	0.00	0.00	0.40	40.00
Plain White	0.31	31.00	0.06	6.00
Plain Brown	0.03	3.00	0.00	0.00
Color Phenotype	**0.34**	**34.00**	**0.46**	**46.00**
Black-White	0.03	3.00	0.41	41.00
Black-Brown	0.05	5.00	0.03	3.00
White-Brown	0.15	15.00	0.05	5.00
Double Color Phenotype	**0.23**	**23.00**	**0.49**	**49.00**
Black-White-Brown (Phenotype Mix)	0.43	43.00	0.05	5.00
Total	**1.00**	**100.00**	**1.00**	**100.00**

Note : The color is called first, then more dominant than the so-called next. Thin tail n = 48 and Priangan n = 34

In Table 1, it appears that the pattern of hair color Thin Tail Sheep in a plain white for the frequency of 0.31 (31%) while the plain black only 0.03 (3%) and the largest variation is a mixture of black and white-chocolate for 0 , 43 (43rd%). These results are in line with the opinion of Mason (1980); Devendra and McLeroy (1982),Thin Tail Sheep belonging to a small body sheep, the performances are generally white hair with black patches around the eyes and nose. Suspected white coat color is determined by one or more dominant gene (Johansson and Rendel, 1966). On Priangan Sheep for a plain black coat color greatest frequency of 0.40 (40%), while the plain white 0.08 (8%), mixed black-white color-brown 0.05 (5%) and the most is black-white 0.41 (41%). Basic color pattern of black coat color is more dominant than the other Priangan Sheep. The results showed the differences in hair patterns Thin Tail Sheep which vary from plain white, brown and mix white-black-brown with variations. Hair patterns Priangan Sheep with plain black, plain white, a combination of black and white and very little mixture of three colors-black and white -brown, and may not find a plain brown color.

EARS

Based on their opinions Siregar (1981) and Diwyanto (1982), ear shapes are classified into three categories, namely: (1) Small ears (Rumpung), rounded with length <5 cm. (2) Ear medium (Daun Hiris) with 5-8 cm long ears. (3) Large ears (Rubak) widened perfect, ear length> 8 cm. Observations form the ears Thin Tail Sheep and Priangan Sheep, shown in Table 2.

Table 2. Ear Shape Thin Tail Sheep and Priangan Sheep

Ear Shape	Size (cm)	Thin Tail		Priangan	
		Frequency	(%)	Frequency	(%)
Rumpung	<5	0.00	0.00	0.89	89.00
Hiris Leaves	5-8	0.12	12.00	0.11	11.00
Rubak	>8	0.88	88.0	0.00	0.00
Total		**1.00**	**100.00**	**1.00**	**100.00**

Note : Thin Tail n=48
Priangan n=34

In Table 2, it appears that Thin Tail Sheep have a big ear shape (Rubak) 0.88 (88%) and ear shapes are (Daun Hiris) 0.12 (12%), while in Priangan Sheep form small ears (Rumpung) 0 , 89 (89%) and ear shapes are (Daun Hiris) 0.11 (11%). Form of small ears (Rumpung) on Priangan Sheep, probably due to selection factors that made for a long time, directed as sheep type Thin Tail Sheep complaints while the selection is not done. Small ear shape is assumed to be determined by a pair of recessive genes, while the long ears so that the dominant gene on the condition of the ear shape is heterozigotmemberikan gene (Leaf Hiris), in accordance with the opinion of Bogart (1959).

TAILS

Long and short tails in sheep varied from a normal condition until the condition in which the proportion of the tail bone is lost or there is none (Lasley, 1978). The observation of tails on Thin Tail Sheep and Priangan Sheep, shown in Table 3.

Table 3. Form of Thin Tail Sheep and Priangan Sheep

Tail Shape	Size (cm)	Thin Tail		Priangan	
		Frequency	(%)	Frequency	(%)
Thin	5	0.96	96.00	0.19	19.00
Medium	5-8	0.04	4.00	0.81	81.00
Thick	8	0.00	0.00	0.00	0.00
Total		**1.00**	**100.00**	**1.00**	**100.00**

Note : Thin Tail n=48
Priangan n=34

In Table 3, it appears that a characteristic of the local Thin Tail sheep have thin tails form as much as 0.96 (96%) and the shape of the tail was only 0.04 (4%). In Priangan Sheep have tails form is as much as 0.81 (81%) and forms a thin tail of 0.19 (19%). The results showed that the typical local lamb is to have a thin tail (Sumoprastowo, 1987 and Rangkuti, et al., 1989), whereas Priangan Sheep has a fat tail as a form of blood donations from fat-tailed sheep Kaapstad (Merkens and Soemirat, 1926; Atmadilaga, 1959).

CHARACTER OF QUANTITATIVE

The observation of body parts were classified into three parts: Part of body, the skull / head and tail sections are measured, shown in Tables 4 and 5.

Table 4. Body measurements and Student's t-test results on Rams of Thin Tail Sheep and Priangan Sheep at Age Two Years.

Body Size	Thin Tail	Priangan
Body Part :		
Body weight (kg)	$17,0 \pm 2,0^a$	$39,3 \pm 5,5^b$
Body Length (cm)	$47.5 \pm 1,9^a$	$65,7 \pm 3,2^b$
Height (cm)	$54,0 \pm 2,0^a$	$73,3 \pm 1,6^b$
Chest circumference (cm)	$62,5 \pm 4,5^a$	$82,7 \pm 2,3^b$
Chest Width (cm)	$11,0 \pm 0,6^a$	$16,0 \pm 1,0^b$
In The Chest (cm)	$25,3 \pm 2,6^a$	$36,7 \pm 1,2^b$
Head Part :		
Skull Length (cm)	$19.8 \pm 1,3^a$	$22,2 \pm 1,4^a$
Skull Width (cm)	$8,7 \pm 0,6^a$	$10,5 \pm 0,4^b$
Skull Height (cm)	$13,1 \pm 0,8^a$	$14,7 \pm 1,2^a$
Ear Length (cm)	$9,4 \pm 1,9^a$	$4,9 \pm 2,3^b$
Ear Width (cm)	$4,8 \pm 0,3^a$	$3,1 \pm 0,8^b$
Tail Part :		
Tail Length (cm)	$13,7 \pm 1,2^a$	$14,3 \pm 1,2^a$
Tail Width (cm)	$3,8 \pm 0,9^a$	$5,4 \pm 0,5^b$

Note: The same letter on the same line showed no significant difference

Table 5. Body measurements and Student's t-test results on Ewes of Thin Tail Sheep and Priangan Sheep at Age Two Years.

Body Size	Thin Tail	Priangan
Body Part :		
Body weight (kg)	$21,1 \pm 5,8^a$	$23,9 \pm 5,7^a$
Body Length (cm)	$51,4 \pm 5,7^a$	$55,2 \pm 3,8^b$
Height (cm)	$57,3 \pm 5,2^a$	$62,6 \pm 1,9^b$
Chest circumference (cm)	$67,6 \pm 5,8^a$	$68,1 \pm 2,8^a$
Chest Width (cm)	$12,6 \pm 2,4^a$	$12,7 \pm 0,7^a$
In The Chest (cm)	$27,5 \pm 2,8^a$	$29,1 \pm 1,6^a$
Head Part :		
Skull Length (cm)	$18,4 \pm 1,5^a$	$19,1 \pm 0,7^a$
Skull Width (cm)	$7,9 \pm 0,4^a$	$7,9 \pm 0,4^a$
Skull Height (cm)	$13,1 \pm 0,8^a$	$14,3 \pm 0,7^b$
Ear Length (cm)	$10,6 \pm 1,4^a$	$2,5 \pm 0,4^b$
Ear Width (cm)	$4,9 \pm 0,4^a$	$1,6 \pm 0,2^b$
Tail Part :		
Tail Length (cm)	$12,2 \pm 1,9^a$	$13,6 \pm 1,0^b$
Tail Width (cm)	$3,2 \pm 0,6^a$	$4,6 \pm 0,4^b$

Note: The same letter on the same line showed no significant difference

In Table 4 and 5, shows the difference all the body measurements (weight, length, height, chest circumference, chest width, and in the chest), both rams and ewes between Thin Tail Sheep in Priangan Sheep. Results of testing entirely on rams were significantly different ($P < 0.05$), while testing at a significantly different ewes only body length and height. Body measurements are usually used on livestock to make the selection. Section heads (Table 5),

showing cranial height, ear length and width of the ears all significantly different between Thin Tail Sheep in Priangan Sheep, this is probably due to homogeneity of a recessive gene on Priangan Sheep. Priangan Sheep show has been happening indirect selection of fighting sheep type, whereas in Thin Tail Sheep is not the case (Triwulaningsih, et al., 1981).

BLOOD PROTEIN POLYMORPHISM

Blood protein polymorphism is a way to determine phylogenetic lineage or relationship of a nation of cattle based on analysis of biological material such as blood proteins. Results of analysis of blood plasma Thin Tail Sheep Sheep and Priangan Sheep, shown in Figure 1 and 2.

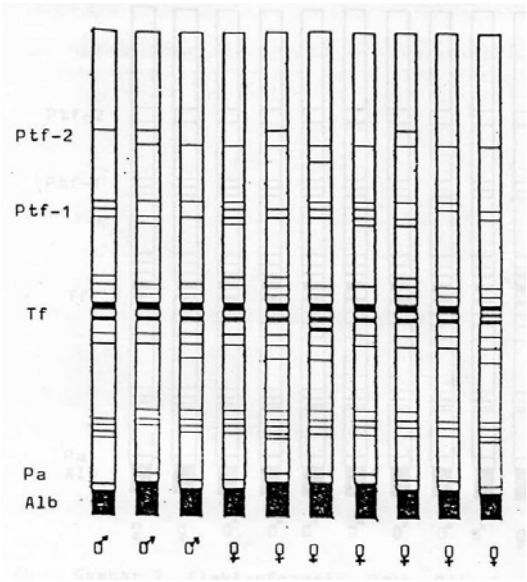

Figure1. Protein Electrophoresis Patterns of Plasma Blood Band Thin Tail Sheep

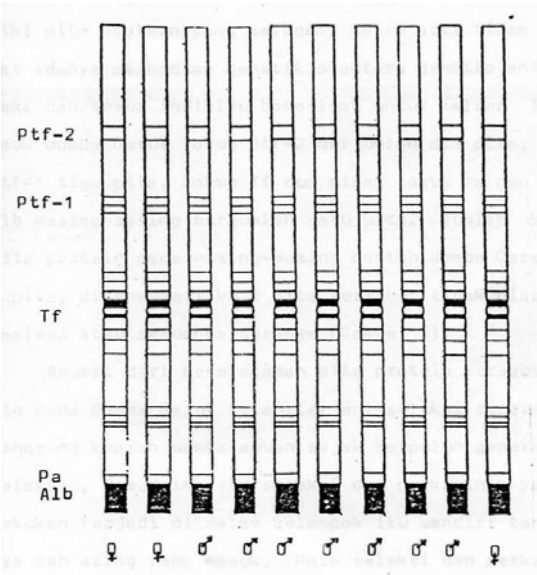

Figure 2. Protein Electrophoresis Patterns of Plasma Blood Band Priangan Sheep

The analysis of Thin Tail Sheep showed the diversity of five types of proteins that were identified and not found of individuals that are homozygous at all five loci. Regarding the number of protein bands on Thin Tail Sheep between 70-10 bands (average) 8 ribbons). Ribbons is shown in Figure 1, the locus of PTF-2 amounted to 1-2 bands (average of a tapes), locus 2-4 PTF numbered -1 (average of three bands), 2-3 loci Tf bands (average Average 2 ribbons), Alb locus locus Pa and numbered each one protein band. Protein bands on Priangan Sheep that there is no diversity in identification or in this case the protein bands Priangan Sheep have a uniform, meaning there are no genetic differences among genotypes between themselves and all individuals homozygous for the five loci. Assumption of uniformity protein bands are thought to have occurred decades indirect selection generations back. Selection and mating patterns in a limited environment as well as the males are used, will more easily obtain uniformity, both qualitatively and or quantitatively. Clarity of what appears is on the qualitative character fixed by one or two pairs of genes and not much influenced environment factor, unlike a lot of quantitative trait variation due to different factors of maintenance.

CONCLUSION

Thin Tail Sheep and Sheep Priangan have varying color patterns of black, white and brown. Thin Tail Sheep more colors-black and white mix-chocolate (43%) and plain white color (31%).Priangan Sheep more plain black color (40%) and black and white (41%). The ears shape is Rubak (88%) and Daun Hiris (12%) for Thin Tail Sheep, whereas Priangan Sheep have Rumpung (89%) and Daun Hiris (11%). Significantly different body size in both sheep is shoulder length and height. For all male sex were significantly different sizes. The size of the head which is the width of the skull was significantly different in males, the females are high skull. Of the five types of protein band Thin Tail sheep tested in real time varied between 70-10 protein bands (average of 8 bands), while in Priangan Sheep homogeny of nine protein bands. From the variation of protein bands that existed at the Thin Tail Sheep gained some similarities with the protein band which marks Priangan Sheep contained Thin Tail Sheep blood.

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THE OUTBREAKS OF INFECTIOUS BRONCHITIS OF CHICKENS IN PRIVATE POULTRY FARM

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The article includes the results about case of infectious bronchitis of chickens in private poultry farm on Hînceşti district. The received dates include the epidemiological, clinical morfopatological and serological investigations. The obtained dates confirm that infectious bronchitis virus have a circulation on commercial and private poultry farms from republic and the used vaccines have a low level of immunizations. The losses of mortality were around 22%. More characteristic gross lesion was found in the bronchi of chickens (pneumonia) and kidneys: swollen and pale kidneys with the tubules and urethras often distended with urates and hemorrhages.
Key words: kidneys, hemorrhages, urates, mortality, vaccine.

Infectious bronchitis (IB) is an acute, highly contagious viral respiratory disease of chickens characterized by tracheal rales, coughing and sneezing.

The disease may affect kidneys and in laying flocks there is dropping egg production and there quality. Infectious bronchitis is a major economic importance which reflected true poor weight gain and feed efficiency. Usually the disease processed in association with mixed bacterial infection that produce airsacculitis that may result in condemnations at processing of chickens and declines egg-quality. The prevalence and economic importance of the diseases resulted in efforts of prevent IB in laying flocks. Other importance includes the establishment of the virus etiology. Infectious bronchitis virus sprees rapidly among chickens in a flock.

In Republic of Moldova for immunization of IB all poultry farmers used the different types of vaccine; live and inactivated which applied by the drenching water, spray method or intramuscular (for old laying flock).

MATERIAL AND METHODS

The investigations includes the material which was obtained from private poultry farm (district Hînceşti c.Ulmu) were was grow the case of infectious bronchitis on chickens. For diagnose confirmation was used the epidemiologically, clinically and patomorfologically dates and serologically tests (ELISA).In poultry farm there were 5000 chickens of 8 days age.

REZULTS AND DISCUSSIONS

The flock was vaccinated with life strain of vaccine H-120 at the age 5 days.

The suspicion of disease (IB) was established when the age of the chickens had 11 days. Usually the mortality till 11 days age compiling 10-15 chickens a day. Beginning the age of 11 days the mortality grow very quickly and complaining around 270-300 chickens a day.

Naturally occurring spread about 3 days. Mare characteristic clinical sings was: difficulties on respiration, gasping, coughing, sneezing, tracheal rales and nasal discharge. Occasional chick had swollen sinuses. The chicks appear depressed, seen huddled under a heat source. Feed consummation and weight going are significantly reduced.

The mortality was around 25%. On necropsy were established following gross lesions: infected chickens have serous, catarrhal or caseous exudates in trachea. Air sacs contain yellow caseous exudates, area of pneumonia in bronchi (Fig. 2).

Characteristic lesions were established in kidneys. (Fig. 1) which represents pale aspect, hypertrophies, with the tubules and urethras often distended with urates (Fig. 3). On fig. 3 we can observe the modification aspect of bursa of Fabricius which is liger on 2-3 times than normal volume of non affected bursa.

Mentioned gross lesions permit confirm that infectious bronchitis proceed in association with colibacilosis and Gambaro disease.

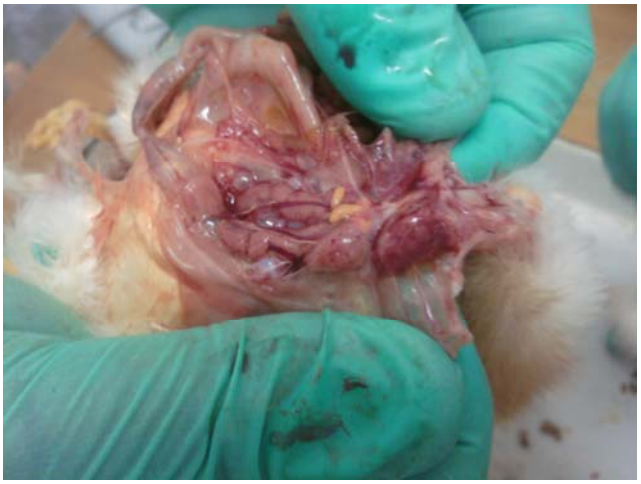

Fig.1 Kidneys lesions. Tubules and urethras distended with urates, (hypertrophies and hemorrhages).

There is no specific treatment for infectious bronchitis. In this situation for symptomatic treatment we used antibacterial (trmexin) in combination with vitamins (cictonic) with drinking water which reduced the losses from airsacculitis. In the feed for reducing losses of nephrites was used sodium and potassium. For next vaccination was remanded to use the vaccine strain „M5 Clone-30”.

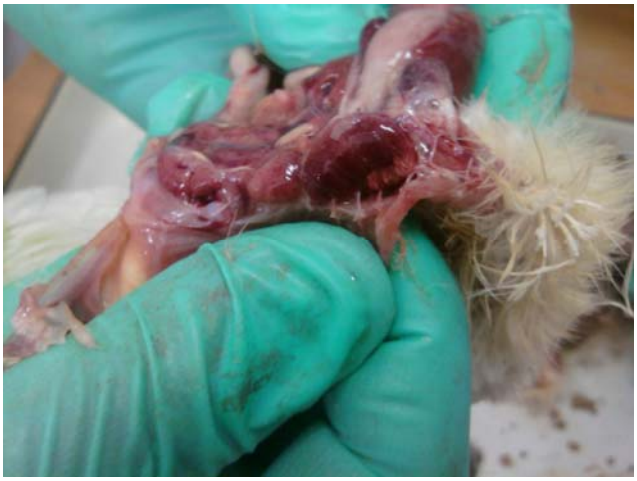

Fig.2 Areas of pneumonia.

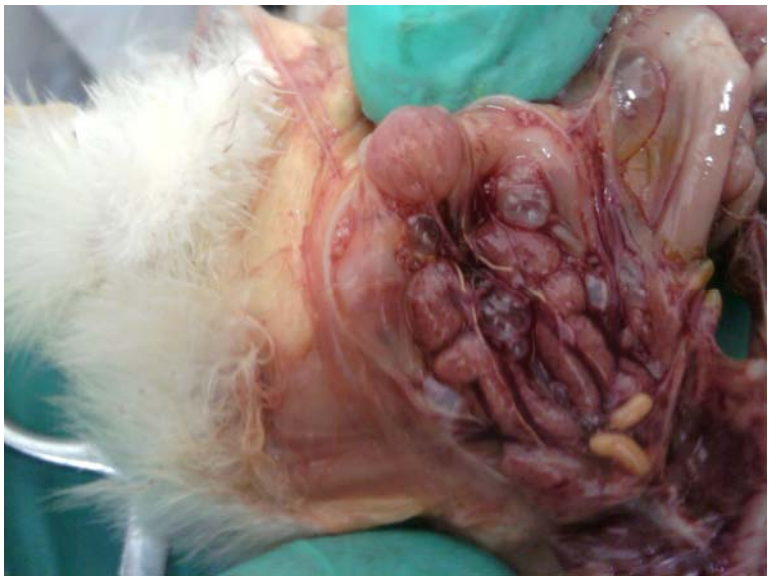

Fig.3 Kidney and bursa of Fabricius lesions.
Urates in urethras, kidney hypertrophies, increase in size, edema and hyperemia of cloacae bursa.

CONCLUSIONS

1. The used life vaccine strain H-120 in our situation demonstrated a low imunological effect which didnt protect chicken flocks.
2. Vaccine strain shoud be selected to represent the ontigenic spectrum of isolates in a particular countru or region.
3. For redusing the losses from secondary bacterially infectious is necessary use with drinking water antibacterials in combinations with vitamins (tromexin and cictonic).

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THE DETECTION OF ORF VIRUS BY PCR AT THE RUMINANS OF ROMANIA

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Differents viral species cause skin lesions in domestic ruminants, among all notifiable FMDV, BTV and poxviruses but also parapoxviruses (PPVs), Herpesvirus and Papillomavirus.The clinical aspect of skin disorders lead to frequent cases of misdiagnosis with possible severe consequences in terms of economic impact and zoonotic transmition. The possibility to identify the viral agents responsable of skin lesions with rapid direct method could help the definitive diagnosis. In the last years, in Romania the most common skin diseases affecting the domestic ruminanants are mainly those caused by PPVs ., Orf virus (OV), the prototype of PPV genus, affected the adult goats and the kids, with lesions localised in the mouth area, legs and on in the hudder. To identify the etiological agents causing cutaneos diseases in domestic ruminants from Romania, scab samples have been collected from affected goats .The animals were farmed in two different locations Iasi and Botosani. PCR has been performed on DNA extracted from either tissute or scabs, in particular for the identification of PPVs a fragment of the conserved gene B2L has been amplified using primers PPPI and IV . Our results showed that the skin lesions found in goats were caused by PPV and subsequent sequencing demonstrated Orf virus as the causative agent.. This study demonstrated that PCR can be affective for a rapid indentification of viral agents responsible of skin diseases of ruminants. This technique is useful as a starting point for genomic characterization of virus straints circulating in Romania.

Key word: Orf virus, contagious ectyma, PCR

MATERIALS AND METHODS

From 66 goats infected with virus ORF, based on clinical signs were collected tissue samples and scales from the mouth, from the podal, mamels, peribucal. Samples were frozen at -80 ° C until use, and then thawed in order to obtain the virus from tissues. Viral DNA extraction using NucleoSpin ® DNA kit (Macherey - Nagel), and to the protocol recommended by the manufacturer, after DNA extraction were preserved in the protocol recommended by the manufacturer, the extracted DNA was stored at - 20 ° C.

Mix was prepared for 11 samples, 9 obtained from DNA extracted from scabs, blank and positive control. Mix was prepared from: BEF 10x - 5µl (60 ml), solved Q - 10 ml (120 ml), dNTPs - 4 ml (48 ml), PPP1 - 1 ml (4 ml), PPP IV - 1 ml (4 ml), H2O - 23.75 ml (285 ml), Taq Polymerase - 0.25 ml (3µl), 5µl of DNA for each sample separately and the blank were added 5 ml of water. Primers used for the PCR are represented by PPPI - PPPIV and kit for amplification

using Taq Polymerase kit (Qiuagen). After realizing the mix, it has undergone the following cycle to termociclator: 94 ° C - 5min when there is distortion of the DNA 94 ° C - 30 seconds, 55 ° C - 30 seconds when there is alignment of DNA, 72 ° C - 30 seconds, 72 ° C - 7 minutes in its final extension occurs.

Detection of amplification products was achieved by using UV highlighting in 2% agarose gel bands. Gel was added 5µl ethidium bromide, and each amplified product was added bromine phenol blue 1µl, 5µl of each amplified product, resulting mixture was loaded on the gel with the positive control represented by a strain obtained after the extraction of crusts from sick animals in Cuba and a blank. Colored bands were highlighted by the image analyzer Fluorine - S Multimager (Bio-Rad).

RESULTS AND DISCUSSIONS

Gene sequence chosen for amplification provides such a diagnosis to detect certain virus ORF, since it remains constatnt of structurally from one strain to another. The choice is justified by sampling tissue tropism for the virus you epithelia. Irrespective of clinical form of disease can be extracted DNA from tissues such as scales and then subjected to amplification, which identifies individuals positive for ORFV (figura.1Ordinea evidence presented in photo are shown in the table below (Tabel.1).

Figura.1

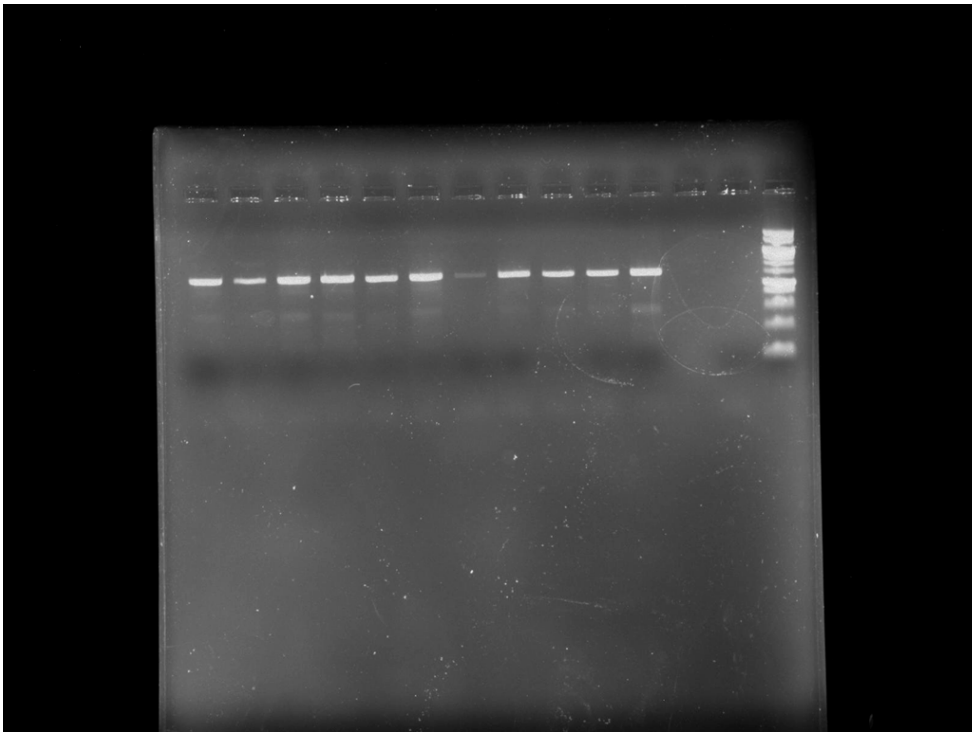

Table. 1

Nr. Probei de analizat	Rezultat PCR	Localitatea recoltării probelor
A1.	+	Lețcani
A2.	+	Bârnova
A3.	+	Botoșani
A4.	+	Botoșani
A5.	+	Botoșani
A6.	+	Lețcani
A7.	-	Lețcani
A8.	+	Lețcani
A9.	+	Lețcani
BLANC	-	-
K+	+	-

CONCLUSIONS

1) To highlight the ORF virus from scabs recolate to 66 sick animals infected with the virus, DNA extraction was performed easily in crusts, which were frozen at -20 ° C.
2) Mix was prepared for 11 samples obtained from DNA extracted from scabs, blank and positive control, thus putting on record hit after completion of PCR samples.
3) Whatever the clinical disease can be extracted DNA from tissues such as scales and then subjected to amplification, which identifies individuals positive for ORFV.
4) Detection of amplification products was achieved by using UV highlighting the agarose gel 2% bands.
5) Of the nine samples only eight of them were positive.

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DETECTION AND QUANTIFICATION OF *SALMONELLA SPP*. AND *CAMPYLOBACTER JEJUNI* ON POULTRY CARCASSES BY REAL-TIME PCR

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Abstract

Campylobacter jejuni and Salmonella spp. on poultry carcasses were detected and quantified using a Sybr Green Real-Time PCR system.
A number of 69 samples of poultry (39 for Salmonella spp. and 30 for C. jejuni) were analyzed in the 2009-2010 period. The samples were collected from the poultry carcasses and tested by classical bacteriological methods and by real-time PCR. Specific primers and Sybr Green I dye were used. The target genes were ttr gene for Salmonella spp and VS1gene for C. jejuni. The specificity of the test, was verified using seven Salmonella enterica strains (found in meat samples) and 14 non-Salmonella strains, respectively five C. jejuni strains and 6 non-C. jejuni strains. The assay correctly identified all the seven Salmonella and five C. jejuni strains. There was no cross amplification reaction. The selectivity of the test was high; there was no fals negative reaction. The detection limits of the test was 1 log CFU/ml for Salmonella spp (both in culture dilution and artificially inoculated samples) and 1 log CFU/ml for C. jejuni (in artificially inoculated samples).

***Key words**: Salmonella spp., Campylobacter jejuni, poultry carcasses, real-time PCR*

INTRODUCTION

Salmonella spp. and *Campylobacter spp*. are two of the main pathogenic bacteria in food responsible for numerous cases of foodborne illness [11, 16, 24]. In developed countries, investigations have shown that infections caused by *Campylobacter spp*. may be as serious as those by *Salmonella spp*., both in frequency and severity of symptoms [5].

Foods of animal origin, such as meat and poultry, have been reported as the vehicles associated with the transmission to humans of these pathogens [2, 13]. Poultry products are frequently implicated in foodborne outbreaks [13, 23]. To prevent chicken carcass contamination, it is important to control *Salmonella spp.* and *Campylobacter spp*. infections along the food production chain. But, in spite of improved hygiene at the farm and slaughterhouse levels, numerous poultry carcasses remain infected in retail shops[1, 13].

Nucleic acid-based detection methods such as PCR and real-time PCR have increasingly been used in recent years for detection of both pathogens from meat and meat products. *Salmonella spp*. and *C. jejuni* cells may enter the environment, including drinking water, through the feces of infected animals, birds, or humans. The infective dose of *Salmonella spp*. and *C. jejuni* cells is very small and it has been estimated that as few cell could cause human illness. This means that even a very small number of *Salmonella spp*. and *C. jejuni* cells in food present a potential health hazard [10, 17, 22].

Unfortunately, there are several problems concerning detection of pathogens in food using culture methods. The two most important difficulties are the small numbers and the slow growth rate of the organism. The traditional methods currently used are time-consuming and laborious, requiring prolonged incubation (1-2 days) and selective enrichment to reduce the growth of background flora, and biochemical identification [8, 9].

Currently, there is a requirement for rapid, quantitative and accurate methods for detection of pathogens in food because they possess inherent advantages such as shorter time to results, excellent detection limits, specificity and potential for automation [7, 19].

The aim of this study was the identification of *Salmonella spp.* and *C. jejuni* and the evaluation of contamination level in poultry carcasses by real-time PCR in comparison with classical microbiological methods.

MATERIALS AND METHODS

Poultry carcasses samples. The study was performed on 69 samples represented by neck skin, randomly collected after the evisceration stage. Each sample was placed in an individual sterile polyethylene bag and transported to laboratory in a cool box at 3-5ºC. Thirty of the samples were analyzed for *C. jejuni*, and 39 of the sample for *Salmonella spp.* The samples were first processed by classical microbiological methods, and then verified by real-time PCR.

Bacterial isolates and media. The 39 neck skin samples investigated for *Salmonella spp*. were aseptically trimmed to 25 g and homogenized for 1 min in 225 ml buffered peptone water (BPW) using a stomacher. Following overnight incubation at 37ºC, 0.1 ml were inoculated for enrichment in tubes containing 10 ml Rappaport–Vassiliadis broth (Oxoid LTD., Basingstoke, England) and incubated for 24 h at 42ºC. The selective medium used was XLD (Oxoid LTD., Basingstoke, England) and incubated 24 h at 37ºC. The suspect colonies were part translucent, pinkish color, with dark center (H_2S positive).

The 30 samples investigated for *C. jejuni* were enriched on Preston broth (Oxoid LTD., Basingstoke, England), and incubated 18 h at 42ºC, under microaerophilic conditions (85% nitrogen, 5% carbon dioxide and 10% oxygen).The selective medium used was Columbia agar, with 10% sheep blood (Oxoid LTD., Basingstoke, England) and incubated at 42ºC, for 72 h. The suspect colony were small, flattened, translucent, gray, α-hemolysis negative. The characteristic colonies was tested biochemically with API Campy (API-BioMerieux S.A., Marcy l'Etoile, France).

Eight strains of *Salmonella spp.* and the four strains of *C. jejuni,* were identified by bacteriological methods and were further verified by real-time PCR. The real-time PCR test performance parameters determinate were: specificity selectivity and sensitivity (detection limit).

DNA preparation for real-time PCR. The extraction of bacterial DNA was performed, the average enrichment with the PrepMan Ultra (Applied Biosystems, Foster City, USA).

One ml of enriched culture was transferred in the 2-mL microcentrifuge tubes. The samples were centrifuged for 3 minutes at room temperature in a microcentrifuge at 12000 rpm speed to pellet bacteria and residual food or other debris. Before use, the reagent was homogenized then, we transferred 100 µl sterile reagent into a 50 ml conical tube. From these tubes, we transferred 100 µl reagent into the samples tubes. The tubes were closed and mixed for resuspend the pellet, then placed heat block set at 100ºC for 10 minutes, the cooling was done at room temperature for 2 minute. We centrifuged the tubes at 12000 rpm for 2 minutes and we transferred 50 µl in other tubes. We used 0,2 µl for a reaction.

The primers for real-time PCR. The PCR nucleotide primers selected for detection of the two pathogens were adopted from previous reported real-time PCR methods, targeting a 94-bp amplicon from the ttr gene of *Salmonella spp.* [14] and a 358-bp amplicon from the VS1 gene of *C. jejuni* [6] (table 1). The oligonucleotides were synthesized and purchased from Applied Biosystems, Foster City, USA.

Table 1 - PCR primers for *Salmonella spp.* and *C. jejuni* detection

Species	Target gene	Primers
Salmonella spp.	Gene *ttr*	F- CTCACCAGGAGATTACAACATGG R- AGCTCAGACCAAAAGTGACCATC
C. jejuni	Gena *VS1*	F- GAATGAAATTTTAGAATGGGG R- GATATGTATGATTTTATCCTGC

The amplification was performed using iTaqTM Sybr Green Supermix with Rox (Bio-Rad Laboratories, Inc., USA).

Amplification reaction (50 µl) contains: 0.2 µl DNA sample, 25 µl mix (2X buffer, 0.4 mM ATP, 0.4 mM CTP, 0.4 mM GTP, 50U/ml iTaq DNA polymerase, 6 mM MgCl2,Sybr Green I dye, 1 µl ROX internal reference dye, stabilizers), 0.25 µl of each F and R primers, 22.5 µl RNase/DNase-free water. The following PCR conditions were used: heat denaturation 95ºC, 3 minutes, followed by 40 cycles: 95ºC 15 sec., 55ºC 45 sec. these steps were supplemented with a final heating at 95° C, then cooling to 60° C. Reactions and data analysis were performed in the Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, USA).

Detection limits of the real-time PCR. A poultry sample (25 g) was introduced in a sterile Stomacher bag and homogenized 1 min in 225 ml sterile buffered peptone water (BPW). Volumes of 900 µl of the homogenized liquid resulted, were inoculated with 100 µl of serially diluted *Salmonella* respectively *C. jejuni.* The final concentrations obtained range from 3.8 to 3.8 x 10^5 CFU/ml for *Salmonella spp* and from 5.2 to 5.2 x 10^5 for *C. jejuni*. The artificially inoculated samples and pure culture samples (the same concentration in BPW) were analyzed by the real-time PCR.

The specificity and selectivity of real-time PCR reaction. The test specificity for *Salmonella* was evaluated using seven strains of *Salmonella enterica* and 14 non-*Salmonella* strains respectively five strains of *C. jejuni* and six non-*C. jejuni* strains were used for specificity of *C. jejuni* (all from our laboratory collection).

The selectivity of the assay was tested on 6 samples composed by a mix (*Salmonella* DNA and non-*Salmonella* DNA) and 8 samples composed by a *C. jejuni* DNA and non -*C. jejuni* DNA mix.

RESULTS AND DISCUSSION

The real-time PCR method tested positive for all seven strains of *Salmonella enterica* (100%) and no positive reaction among the 14 non-*Salmonella* strains. The presence of any cross-amplification reaction was not detected (table 2)

The results obtained specificity confirmed the specificity of the test; no cross-amplifications reactions being detected. All the samples were positive, there was no false reaction obtained (table 3).

Table 2 - Salmonella and non-*Salmonella* strains used for specificity and selectivity tests

***Salmonella enterica* strains**	**Nr. strains**	**Results**	***Non - Salmonella* strains**	**Nr. strains**	**Results**
S. gallinarum	3	**+**	*Enterobacter spp.*	2	**-**
S. typhimurium	1	**+**	*E. coli*	3	**-**
S. arizonae	1	**+**	*Klebsiella spp.*	1	**-**
S. enteritidis	2	**+**	*Pseudomonas spp.*	2	**-**
			Staphylococcus spp.	4	**-**
			Pasteurella multocida	2	**-**

Table 3 - C.jejuni and non- C.jejuni strains used for specificity and selectivity tests

***C. jejuni* strains**	**Nr. strains**	**Results**	**Non – *C. jejuni* strains**	**Nr. strains**	**Results**
C. jejuni	5	+	*C. coli*	2	-
			C. lari	2	-
			E. coli	2	-

Detection limits of real-time PCR

In order to establish the PCR detection limits, triplicate reaction for each serial dilution prepared from bacterial culture and artificially inoculated samples. Standard curves of the template DNA, in units of cell number, were constructed to determine the detection limits of the real-time PCR assay.

In the range from 0.58 to 5.58 log CFU/ml the standard curves of *Salmonella spp.* showed a linear correlation between the values of CT (Cycle Threshold) and cell numbers (log CFU/ml). Samples inoculated with less than 10 CFU/ml of *Salmonella spp.* could not be quantified by quantitative real-time PCR (the CT values ≥ 40), both in spiked poultry samples and bacterial dilutions. This indicates that in our study the real-time PCR had a detection limit as low as 1 log CFU. The correlation coefficient (0.977) shows a good positive correlation between values of CT for two types of evaluated samples (fig. 1A).

The standard curves for *C. jejuni* showed a similar linear correlation between the CT value and cell numbers (log CFU/ml). Samples inoculated with less than 10 CFU/ml of *C. jejuni* could not be quantified by quantitative real-time PCR (the CT values > 40). In case bacterial dilutions, real time PCR tested positive at 0.72 log CFU/ml, the detection limits being higher than that of poultry samples.(fig. 1B).

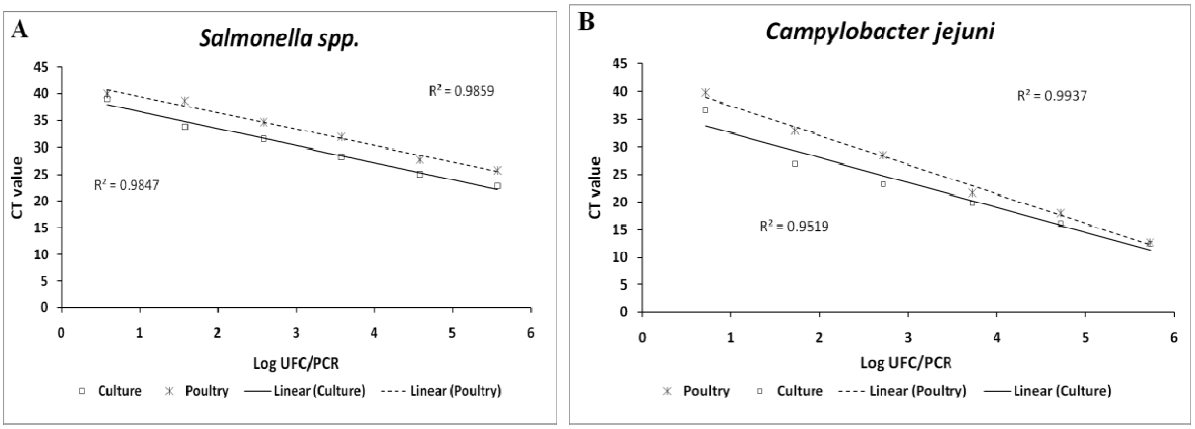

Fig. 1 A and B - Representative standard curves for the real-time PCR Assay performed on Salmonella spp. and Campylobacter jejuni in culture and spiked chicken samples

PCR real-time vs. classical assay

The examination of chicken samples by classical methods and real-time PCR shows similar results. All teh positive samples were detected by the both methods. Eight of 39 (20.5 %) of analized chicken samples tested positive for *Salmonella spp*. with both direct plating and the real-time PCR method. Our results are similar to the other references: BONARDI S. et all. (2008) obtained a prevalence of 18 % and MYINT M. S. (2004) found a 19 and 23 % by culture method and 18.3, 19.4 and 23.3 % by real-time PCR. Compared to the our study, many reference found a higher prevalence of *Salmonella* in other countries: 51.2 % in Argentina , 68.2 % in Ethiopia , and 72 % in Thailand, 16 % in Ireland , 22 % in the USA , 36.5% in Belgium and 55% in Spain [5].

In case of *C. jejuni*, four of the 30 samples tested positive with both methods. Other studies have reported a high proportion of chicken contamination with *C. jejuni*: from 30% [22], to 59% [5]. Other countries have also a high prevalence of *C. jejuni* (46 % in Germany, 46% in Japan, 73-100 % in USA) [5].

The positive samples show contamination levels varied from 1.4 to 3.2 log CFU/ml for *Salmonella spp*. and from 1.7 to 2.9 for *C. jejuni* (table 4).

Table 4 - The comparison of results obtained by direct plating and real time PCR

Bacteria	Samples	Positive		Level of contamination	CT value	Correlation coeficient
		DP*	RT PCR**			
Salmonella spp.	39	8	8	1.4 – 3.2	38.8 – 26.1	-0.97
Campylobacter jejuni	30	4	4	1.7 – 2.9	29.4 - 38.3	-0.949

* Direct plating

** Real-time PCR

Considering the reports of other authors [4], the contamination level for *C. jejuni* is low but is in line with that reported in other studies [20]. However, large variations of contamination level were noticed between different carcasses from 2.3 to 7.72 CFU per carcass [21].

The real-time PCR assay used in this study demonstrated a good specificity and selectivity both for *Salmonella* and *Campylobacter jejuni.* When this assay was applied, all isolates of *Salmonella enter*ica serotypes and all *C. jejuni* strains tested positive while all other non *Salmonella* strains and *Campylobacter spp*. other than *C. jejuni* provide negative results. This demonstrates the high specificity of this primer set.

A good linear relationship was obtained for the culture samples and artificially inoculated samples, which demonstrates the efficiency of the DNA isolation protocol in combination with the real-time PCR quantification.

The detection limit of the present PCR assay was estimated to be approximately 1 log CFU per PCR from bacterial culture. However, the sensitivity for detecting *Salmonella spp*. was between 0.58 and 1.58 log CFU/PCR and 0.72 and 1.72 CFU/PCR for *C. jejuni*. Higher values of CT in case of samples artificially inoculated indicate the possible presence of inhibitory substances in these samples.

The sensitivity obtained by this method is in the same range as those in earlier published papers of 10 CFU/ml and 20CFU/ml [3, 12].

In general, lower detection sensitivity was observed in artificially inoculated samples in comparison with tested culture samples.

CONCLUSIONS

1. Real- time PCR is an alternative method that can verify the presence or absence of *Salmonella spp* and *C. jejuni* in enriched samples.
2. The absence of post-amplification manipulation of the PCR product significantly reduces the risk of contamination due to amplicon carry-over
3. It is a selective method, detection of this pathogens is not influenced by the association microflora.
4. It is fast, specific and sensitive method that can be performed in 24 h.
5. The real-time PCR will become more prevalent in laboratories where rapid, sensitive, and high-throughput quantitative analysis is required.

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EPIDEMIOLOGICAL STUDY OF ECTOPARASITES IN STRAY DOGS IN KALUBYIA GOVERNORATE OF EGYPT WITH A SPECIAL REFERENCE TO ITS CONTROL IN PUPPIES BY DELTAMETHRIN AND IVERMECTIN

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Abstract
The present work aimed to determine and compare prevalence and intensity of ectoparasites infestation on dogs as well as their control by recent insecticides. For this purpose, a total of 94 stray dogs were examined for the presence of ectoparasites in Kalubyia Governorate during the period extended from March 2008 to Februray2009 . A total number of 2964 ectoparasites were found corresponding to 5 species in 54 out of 94 examined dogs (57.54%). The collected ectoparasites were identified as 27.43*% Ctenocephalides canis ,1.32% Ctenocephalides felis .26.79% Trichodectus canis , 44.47% Rhipecephilus sanguineous and Sarcoptes scabiei var canis* which infect dogs at 21/94 (22.34%), 5/94 (5.32%), 23/94(24.87%), 20/94(21.28%)and 3/94(3.19%) respectively. The mean parasitic intensity of ectoparasites was 45.38 and that of *R. sanguineous* was (57.3 tick/ animal) followed by *Tr. Canis* (39.7 lice / anima), *C. canis* (38.71 flea/ animal) ,while the lowest intensity was recorded in *C. felis* (7.8 flea/ animal) . The highest ectoparasitic infestation in dogs was recorded in spring (43.06%) followed by summer (33.33%) while the lowest percentages were recorded in autumn(13.89%) and winter (9.72%). Female dogs harbored higher infestation (74.19%) compared to males (49.21%). The ectoparasites infestation rate in puppies was 55.26 % and in young dogs 62.5% while in adult dogs (54.56%). Young dogs were infested by 4 parasitic sp. (*C. canis , Tri. Canis, R. sanguineous and S. scabiei var canis*) and puppies were infested by 3 sp. *(C. canis , C. felis and Tri. canis*) while adult dogs were infested by two sp. (*R. sanguineous and C. canis*). Superinfection by two ectoparasites was detected in 18 (33.33%) infested dogs. Both dipping of puppies in deltamethrin 0.025% or subcutaneous injection of ivermectin resulted in 100% reduction in the mean number of all fleas during the period extended from 48th hrs to 35th day post treatment . Deltamethrin also resulted in100% reduction in the mean number of *Tr. canis* from 48th hrs to 28th day post dipping.. Subcutaneous injection of ivermectin resulted in 5.3% reduction in the mean number of lice for 48th hrs post injection and 0.00 % after. It was concluded that, stray dogs specially puppies were infected by *C. canis, C. felis, Tr. canis , R. sanguineous and S. scabiei var canis* with higher infestation during spring . Fleas and lice were successfully treated by using deltamethrin dipping once in low concentration 0.025% which was more effective than ivermectin injection.
Key wards: *Ctenocephalides canis, C. felis, Trichodectus canis Rhipecephilus sanguineous and S. scabiei var canis Ivermectin, deltamethrin*

INTRODUCTION

Arthropod ectoparasites constitute a diverse and highly adapted group of animals that inhibit the external body surface and internal vital systems **(Soulaby 1986).** They may live permanently on their hosts or occupy the host ‘s nest and visit the host periodically. In the two cases ,there is a close dependency on the host for various life – sustaining resources (**Kettle 1985) .**

Some species of arthropods play an important role in causing clinical disorders in man and domestic animals as well as acting as vectors of important contiguous diseases. Among them, fleas have been known to infest man and animals and may cause life threating anemia in young and /or debilitated animals **(Araujo et al., 1998)** . Fleas are moderately host specific and the species *Ctenocephalides canis, Ctenocephalides felis* and*, Pulex irritans* are usually described in dogs **(Alcaino et al., 2002)**. These parasites usually associated with dermatitis and affect animals to different degrees according to nutrition status of the host , its immunological condition and to parasite intensity , and in extreme cases can lead to death (**Doganay, 1990, Guclu 2002 , Gonzalez, 2003**) **.** Fleas particularly the species of *Ctenocephalides* can cause considerable irritation to animals and humans and are responsible for cases of severe cases of allergic dermatitis **(Kalkofen and Greenberg 1974 , Amin 1976, Burgu 1985 Alcaino 1990).** Ectoparasites that affect the domestic dogs (fleas , lice and ticks) can also act as vectors of pathogenic agents , such as borreliasis , rickettsiasis and piroplasmosis and/or intermediate hosts of filarids and cestodes which cause serious diseases not only in dogs but also in humans **(Leriche 1988 , Gonzalez 2003)**. Various studies have found that *Ctenocephalides canis, Ctenocephalides felis, Pulex irritans* are the 3 most common flea species on dogs. However, the prevalence of these species varies in different geographic areas, *C.felis* is the most prevalent in **London B(eresford- Jones1981)**, **Egypt (Amin 1966)** and Denmark **(Kristensen 1978)**, while *C .canis* is the most dominant species in dogs in the rural parts in of the United Kingdom (**Chesney1995**) and Australia **(Coman1981)** . **P. irritans** was commonly found in dogs in Hawaii **(Haas 1967)** Rebublic of Korea **(Tippton 1972)** .

Deltamethrin is a synthetic chemical based on pyrethrin and acts on the insect nervous system to give a rapid knockdown . It is of greater stability and low mammalian toxicity . It is used for the control of ectoparasites of domesticated farm animals **(Wall and Shearer1997 , Curtis 2004**).

Ivermectin is a macro cyclic lactone interferes with the gamma amino butyric glutamate of arthropods and induces a flaccid paralysis leading to its death. Both injectable and pour-on formulations had been shown to control internal as well as external ectoparasites (Paradis and Villenenve 1988)).

The species of dog ectoparasites have been known for long time , but few studies have focused on the prevalence of their infestation in Kalubyia Governorate, Egypt although the stray dog populations is common. Consequently, The present work was conducted in order to determine and compare prevalence and intensity of ectoparasites infestation on dogs as well as their control by deltamethrin and ivermectin.

MATERIAL AND METHODS

I- Prevalence of different ectoparasites

Animals : A total of 94 stray dogs were collected in Kalubyia Governorate during the period extended from March 2008 to Februray2009 and examined for the presence of ectoparasites . Their sex and age were recorded. All investigated dogs in the present study did not receive control treatment .

Parasitological examination: The collection of ectoparasites was performed according to the protocol previously described by **Gonzalez et al., (2003**) and **Aldemir (2007)** as follow:

Each dog was thoroughly examined by using a fine comb in all areas of the body including head, pinna, ear canal, thoracic abdominal areas, elbow) for 10 minutes four times a day on each sampling date. The skin of dogs was rubbed with apiece of cotton soaked in ether in order to facilitate the extraction of ectoparasites by making them drowsy. The collected

ectoparasites were afterwards kept in 70% ethanol and identified according to the literature data given by **Whitlock (1960)** in addition to the keys provided **by Macy and Berntzen (1971)** and **Wall and Shearer 1997**) Areas of the skin with dermatologic lesions were moistened with mineral oil and scraped with a scalpel blade until capillary bleeding was visible . The scrapings were placed on glass slides with a drop of 10% KOH solution and were examined microscopically for the presence of mites . The detected mites were identified according to **Baker (1956).** The presence of 1 mite at any developmental stage of dog examined was considered to be a positive result **Aldemir (2007)**.

II- Experimental study on the effect of acricides on ectoparasites in puppies

A- Pesticides:

1-Deltamethrin: (Butox ®) (IR 3R). 3(2.3.dipromoving) 2-2-diethyl-lcyc lopropane carboxtlate of (5) - ά -cy-ano-3-phenoxy-benzyl. Produced by Hoechst Roussel. Vet.

2- Ivermectin : Nasromectin ® 1 % product of El nasr Pharmaceutical Chemicals Co. Abu-Zaabal.

B- Experimental animal groups : Eleven puppies showed mixed infection by *C. canis and Tr. cains* were captured , transferred t o laboratory , classified into 3 groups and kept in separate boxes in 3 separate rooms under strict hygienic measures and treated as follows :

Group A : consists of 4 puppies and was immersed once in 0.025 % Deltamethrin for 1 min. according to **Ramadan (2004)** .

Group B : consists of 4 puppies and was injected subcutaneously by Ivermectin 1% at a dose of 0.2 mg /kg. body weight. according to **Losson and Lonneux (1996**), **Payne and Ridley (1999)**

Group C : consists of 3 puppies , not treated and kept as control group.

Each dog group was thoroughly investigated just before, 48hrs , 7^{th} , 14^{th} 28^{th} and 35^{th} days post treatment for the presence of ectoparasite**s.**

The efficacy of insecticide was calculated as follow:

$$\textit{Efficacy \%} = \frac{\textit{number of parasite before treatment - number of parasite after treatment}}{\textit{number ofparasite before treatment}} \times 100$$

RESULTS

A total number of 2964 ectoparasites were found corresponding to 5 species in 54 out of 94 examined dogs (57.54%). The collected ectoparasites were identified as 27.43% *Ctenocephalides canis ,1.32% Ctenocephalides felis .26.79% Trichodectus canis , 44.47% Rhipecephilus sanguineous and Sarcoptes scabiei var canis* which infect dogs at 21/94 (22.34%), 5/94 (5.32%), 23/94(24.87%), 20/94(21.28%)and 3/94(3.19%) respectively.

R. sanguineous was the most abundant infesting species in this study followed by *C. canis* while *Sarcoptes scabiei var canis* was the lowest abundant species Table (1) . The table showed also that the mean parasitic intensity of ectoparasites was 45.38 and that of *R. sanguineous* was the highest (57.3 tick/ animal) followed by *Tr. canis* (39.7 lice / anima), *C. canis* (38.71 flea/ animal) ,while the lowest intensity was recorded in *C. felis* (7.8 flea/ animal) . Table (2) showed that, the ectoparasites infestation rate in puppies was 55.26 % and in young dogs 62.5% while in adult dogs from was the lowest (54.56%).

Young dogs were infested by 4 parasitic sp. *(C. canis , Tri. Canis, R. sanguineous and S. scabiei var canis*) and puppies were infested by 3 sp. (*C. canis , C. felis and Tri. Canis*) while adult dogs were infested by two sp. (*R. sanguineous and C. canis).*

Superinfection by two ectoparasites was detected in 18 (33.33%) out of 54 infested dogs . *Tr. canis* was detected with *C. canis* in 11 puppies and with *C. felis* in 2 puppies . In young dogs, it was also detected in with *C . canis* in 3 animals and with *R. sanguineous* in 2 animals . Single infection was detected in 36 (66.67%) animals mainly in adult and old dogs.

Results in table (3) indicated that the highest ectoparasitic infestation in dogs was recorded in spring (43.06%) and summer (33.33%) while the lowest percentages were recorded in autumn(13.89%) and winter (9.72%). *C. felis* was detected in spring (80%) and summer(20%) while *S. scabiei var canis* detected only in summer (100%) .

Table (4) showed that female dogs harbored higher infestation by ectoparasites(74.19%) compared to (49.21%) in males

Control of ovine pediculosis by using deltamethrin and.ivermectin

Table (5) showed that, both dipping of puppies in deltamethrin 0.025% or subcutaneous injection of ivermectin resulted in 100% reduction in the mean number of all fleas during the period extended from 48th hrs to 35th day post treatment . Deltamethrin also resulted in100% reduction in the mean number of *Tr. canis*. during the period extended from 48th hrs to 28th day post dipping and some lice were found dead, dry and attached to the skin of the treated puppies. The efficacies of deltamethrin at 35th day post dipping against *Tr. Canis* was 71.07 % as some lice re-infect puppies.

Subcutaneous injection of ivermectin against lice in puppies resulted in 5.3% reduction in the mean number of lice for 48th hrs post injection and 0.00 % after .

Fig(1) Trichodectus canis female

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Fig (2): Ctenocephalides felis female

Fig (3): Ctenocephalides felis male

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Fig (4) :Cetenocephalides canis female

Table (1): **Incidence and intensity of ectoparasites infesting dogs in Kalubyia Governorate (n=94)**

parasite	Number of Infected dogs	Infestation rate	No. of Collected Parasite	%	Parasite intensity/ ;animal
C. canis	21	22.34	813	27.43	38.71
C. felis	5	5.32	39	1.32	7.8
Tr. cains	20	21.28	794	26.79	39.7
R. sanguineous	23	24.87	1318	44.47	57.30
S. scabiei var canis	3	3.19	-	-	-
Total	54	57.45	2964	-	45.89

Table (2):Incidence of ectoparasites in dogs by age

age	No . of dogs	No. of positive dogs	Detection rate	Parasite sp.	Single infection	Superparasitism
Puppies (less than 6 months)	38	21	55.26	*1- C. canis 16l21* *2-C. felis 5/21* *3- Tri. Canis* 13/ 21	5 3 -	13 3&1 (11) 3&2 (2)
Young dogs (6 m.- 1 year)	24	15	62.5	1- C. canis 3/15 2- Tri. Canis 5/15 3- R. sanguineous 9/15 4- S. scabiei var canis 3/15	- - 7 3	5 2&1 (3 animals) 2&3 (2 animals)
Adult dogs (From 1- 4 years)	22	12	54.56	R. sanguineous 11 C. canis 1	11 1	-
Old dogs (More than 4 y)	10	6	60	R. sanguineous 3 C. canis 1 *Tr. Canis 2*	3 1 2	- - -
Total	94	54	57.45		36	18

Table (3): Seasonal incidence of ectoparasites in dogs

Parasite	Spring	Inf.%	Summer	Inf.%	Autumn	Inf.%	Winter	Inf.%
C. canis	9	42.86	7	33.33	3	14.29	2	9.52
C. felis	4	80	1	20	-	-	-	-
Tr. cains	8	34.78	8	34.78	4	17.39	3	13.04
R. sanguineous	10	50	5	25	3	15	2	10
S. scabiei var canis	-	-	3	100	-	-	-	-
total	31	43.06	24	33.33	10	13.89	7	9.72

Inf.% = Infestation rate

Table (4):Incidence of ectoparasites in dogs by sex

sex	No . of dogs	No. of positive dogs	Detection rate
Males	63	31	49.21
Females	31	23	74.19
Total	94	54	57.45

Table (5) : Effecacy of Deltamethrin and Ivermectin against ectoparasites in puppies

Group	parasite	Insecticide	**Mean number of alive parasites**						**Effecacy %**	
			Before treat.	**48hrs**	**7th**	**14th**	**28th**	**35th**	**48 hrs**	**35th day**
Group A	*C. canis* *Tr. Anis*	Deltamethrin 0.025 %	38.25 39.75	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 11.5	**100** **100**	**100** 71.07
Group B	*C. canis* *Tr.canis*	Ivermectin	39.25. 37.5	**0.00** **35**	**0.00** **35.75**	**0.00** 35.25	**0.00** **41.75**	**0.00** **44.25**	**100** 5.33	**100** **0.00**
Group C	*C. canis* *Tr.cani*	-	40.67 39.66	**39.33**	**34.67** 40	**29** 43	**18.67** **43.67**	**17.33** 44.67	**0.00** 0.00	**0.00** 0.00

DISCUSSION

In the present study 2964 ectoparasites belonged to 4 species were collected from dogs in rural areas of Kalubyia Governorate and identified as 7.43% *Ctenocephalides canis* ,1.32% *Ctenocephalides felis* .26.79% *Trichodectus canis , 44.47% Rhipecephilus sanguineous* beside *S.scabiei var canis*. Similar results were recorded **Aldemir (2007)** in Turkey, **Chee et al., (2008**) in the republic of Korea and **Farkas et al., (2009)** in Hungary. Higher number of ectoparasites 5193 were collected from dogs in Argetina by **Gonzalez et al 2003.**

The present study revealed that , the overall infestation rate by ectoparasites in different dog was 76,6% . There are no previous studies dealt with most ectoparasites in dogs in Egypt. However, Lower incidences were recorded by **Aldemir (200**7), and higher incidences were recorded by **Liberato (1998), , Bostamante (1998)** and **Chee et al., (2008).** Such differences may be attributed to breeds of dogs, environmental condition as well as management system of breeding.

was the most abundant infesting species among dog parasites found in this study and were evidence in 24.87% of infested dogs with high density 57.30. This result was in agreement with data of **Gonzalez et al., (2003)** in Argentina , and disagreed with **Aldemir (2007)** in Turkey who recorded that *C. canis* was the most frequent sp. infecting dogs. **Silveira et al., (2009)** in Brazil also recorded infestation rate of *R. sanguineous* in dog 7.8 %. Such difference might be due to changes in the nature of stray dogs in rural areas in Kalubyia Governorate and the examined dogs from the pet care Dept. of small animal veterinary clinic in Brazil .

Regarding *Siphonptera* occurrence , *C. canis* was most predominant flea in dogs *than C. felis* as the respective infestation rates were 22.34% and 5.32% . These results agreed with results of **Gonzalez et al., (2003)** in Argentina **and Aldemir (2004)** in Turkey. In contrast with **Amin (1966)** in Egypt , **Painter and Echeri (1985)** in USA **Gracia et al., (2008)** in Spain who recorded that *C. felis* is the predominant sp. This discrepancy is not easy to explain , but changes in the environmental and climatic factors as well as social and development of cultural aspects related to urban and rural ways of life might be play a role . it may also attributed to site of collection as **Amin (1966**) carried out his study on *Siphonaptera* in Nile valley and delta while our study was carried out in the rural areas in Kalubyia Governorate. **Gracia 2008** mentioned that , the mean annual temperature is positively related to *C.felis* abundance and negatively related to C. canis abundance

Tr. canis was recorded only in dogs less than 1 year and its prevalence was 24.87 % and intensity 39.70. Our results was higher than results of **Chee et al., (2008)** in Republic of Korea who recorded *Tr. Canis* for the first time in 1% in the examined dogs and suspected a higher infestation rate if more dogs and animal shelters were involved in the investigation. The difference between the two results may be due to epidemiological factors such as weather , seasonal variation, geographical location, innate resistance and particularly the age of the animals examined.

Our results showed that female dogs harbored more ectoparasites than males. This observation was in agreement with studies of **Alcaino et al., (2002)** , **Edward and Kristensen (1969)**, **Gonzalez et al., (2003) Kristensen et al., (1978) and Aldemir (2007)** who have also reported a greater susceptibility of females to ectoparasites . This may due to hormonal changes in females during reproductive period that could favored reinfestation by ectoparasites . In **contrast Nayak et al (1997), Rodriguez – Vivas et al ., (2003)** suggested that both sexes are equally susceptible **. Chee et al ., (2008)** found that , the prevalence of ectoparasites was more frequent in males than females. Also , **Silveira et al.,, (2009)** reported that the incidence of *R. sanguineous* were significantly higher in females than in males Such differences may attributed to some environmental factors as well as differences in breeds of dogs.

In the present study, dogs less than 1 year are more susceptible to ectoparasites as the detection rate in puppies less than 6 months was 89.47% and in young dogs 83.33% compared to 60% in dogs ranged from 1-3 years . This result agreed with **Nayak et al., (1997), Weisbroth et al., 1974) Kwochka (1987) and Chee et al., (2008).** This might be due to their constant exposure to carrier mothers **(Scott 1979)**. It was also recorded that young dogs were infested by most ectoparasites. Such higher infestation resulted from bad management under Egyptian condition and / or lack of immunity under the effect of weaning. The higher infection old dogs compared to dogs ranged from 1-3 years Might be due to decline of immunity and exhaustion of the immune system as a result of poor feeding and superparasitism.

Concerning seasonal abundance , the overall ectoparasitic infestation rate significantly increased in warm seasons (spring and summer) and decline in autumn and winter. Similar r results were recorded in Egypt by **Amin (1966**) . Also **Alcaino et al (2002)** established that ectoparasites were predominant in spring in Chile but their population began to decline at the beginning of summer and completely disappeared in autumn. This might be due to sudden increase in the temperature in spring after winter which accompanied with high relative humidity that is important factors in acceleration of life cycles of ectoparasites.

Concerning control of fleas and lice in puppies , our results showed that, both dipping of puppies in deltamethrin 0.025% or subcutaneous injection of ivermectin resulted in 100%

reduction in the mean number of all fleas during the period extended from 48th hrs to 35th day post treatment . Deltamethrin also resulted in100% reduction in the mean number of *Tr. canis*. during the period extended from 48th hrs to 28th day post dipping and its efficacy at 35th day post dipping against *Tr. canis* was 71.07 %.. Subcutaneous injection of ivermectin against lice in puppies resulted in 5.3% reduction in the mean number of lice for 48th hrs post injection and 0.00 % after . Our result agreed with the previous results of **Ramadan (2004)** and **Ramadan and Abdel, Mageid (2008)** who mentioned that, spraying goats and sheep by low dilution of deltamethrin reduced the number of lice and all sprayed goats were free from lice until 28th day post spraying. The lower effect of subcutaneous injection of ivermectin against *Tr. canis* may be attributed to the feeding habit of this lice as it is a chewing lice , feed on debris, tissues and skin scales and not suck blood **Kettle (1985).** The prolonged efficacy of deltamethrin against ovine lice may attributed to its loucidal and ovicidal effect. In this respect **Wall and Shearer (1997)** noted that the microencapsulation of the pyrethroids prolonged their activity to weeks with greater stability as the microcapsules adhere to the insect ectoskeleton and the pyrethroid is absorbed through the chitin to produce its toxic effect.

It was concluded that, stray dogs specially puppies were infected by *C. canis, C. felis, Tr. canis , R. sanguineous and S. scabiei var canis* with higher infestation during spring . Fleas and lice were successfully treated by using deltamethrin dipping once in low concentration 0.025% which was more effective than ivermectin injection. The zoonotic nature of the detected fleas, lice and ticks of dogs in this study could be regarded as public health alert. To prevent the possibility of continuous transmission of the ectoparasites from dogs, practicing veterinarians should be advice who contact with stray dogs to pay attention and to be aware of ectoparasites of zoonotic importance and also to choose the more effective and save insecticide in its control.

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ESTIMATION OF VEGETAL EXTRACTS EFFICIENCY IN DOMESTIC BIRDS ECTOPARASITOSES TREATMENT AND PROPHYLAXIS

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Abstract: *Parasitoses in domestic birds provoke big losses. At the same time, some parasitic diseases (zoonoses) represent a major social problem because they can extend and infect people. Antiparasitic preparations of a chemical origin are toxic for the animal body and their elimination takes a long period of time. In some cases their use may provoke the phenomenon of chemical resistance to parasites. In order to avoid these facts it is highly recommended to give priority to pharmaceutical products of a vegetal origin.*
Keywords: invasion extensity, meat quality indices, antiparasitic treatment.

INTRODUCTION

One of the main social problems of the Republic of Moldova is that of supplying people with dietetic alimentary products (fowl) and eggs. Bird breeding is an economically advantageous industrial system as it guarantees a maximum production increase with minimal expenses due to their density at a unit of surface. Despite the economical advantages, the industrial increase doesn't exclude the risks conditioned by the appearance and extensity of some parasitoses whose evolution brings about big losses. At the same time some parasitic diseases (zoonoses) represent a major social problem because they can extend and infect people.(1, 7, 9) Nowadays, parasitoses watch, prevention and fighting represents one of the most important options of parasitological science and practice. Against ectoparasites it is usually applied a local treatment with acaricide and insecticide substances of a chemical origin, under different forms which, due to their nature, act as a "foreign body" for the organism. These imported preparations are of a chemical origin and consequently are toxic for the animal body. Their elimination takes a long period of time, fact which imposes restrictions and precautions regarding the use of the products and sub-products obtained from birds treated with them. Repeated treatments are stressing for the birds, reduce productivity or provoke the phenomenon of chemical resistance to parasites (5). In order to avoid these facts it is highly recommended to give priority to pharmaceutical products of a vegetal origin.

The study of vegetal extracts has registered a qualitative rise lately, due to some favorable results concerning the increase of animal viability and productivity. It is known the fact that some phytocides from plants like: sweet basil, lavender, white wormwood, peppermint, pine, cedar, fir tree etc have an acaricide effect and were used to treat different cutaneous affections (scab) at rabbits and porcine provoked by *Psoroptes* and *Sarcoptes acarians.* (2, 4)

MATERIALS AND METHODS

The parasitological investigations were carried out in the Parasitological and Helminthology Laboratory of the Institute of Zoology, ASM during 2007-2009, on Adler Silver chickens race of 4 months. The Malophagans were collected from living birds according to a new procedure which is more informative. (6)

The quantitative and qualitative collection of ectoparasites from living birds, in different stages of development, reaches around 100% unlike the classical method where the parasites were collected from dead birds, fact that made the parasites leave the dead hosts before being collected. (3)

The collected material was examined later on with a MBS-9 (ob. x 4) magnifying glass and with a MBI-3 (ob. x 10) microscope. In the antiparasitic therapy it was used, in different concentrations, a new preparation of a vegetal origin– *Ectostop-P,* obtained through synthesis by the collaborators of the Laboratory of Parasitology and Helmithology of the Institute of Zoology of ASM in collaboration with the Center of Advanced Biologic Technologies from the Institute of Plant Genetics and Physiology of ASM, Republic of Moldova.

To clarify the indices of meat chemical composition (pH, quantity of proteins, lipids and humidity), the chickens poliparasited with malophagans were slaughtered after the antiparasitic treatment with *Ectostop-P.* According to the classical methods, there were collected by 100-150g samples of meat from each bird and later on they were analyzed in the laboratory of the Republican Center of Veterinary Diagnosis. (8)

RESULTS AND DISCUSSIONS

The main objective of the researches carried out in the period April 2007- December 2009, was to establish *in vivo* the effect of *Ectostop-P* vegetal extract in different concentrations upon malophagans in domestic birds. During the experiment there were used ***Arthemisia absinthium L.*** (wormwood) extract as compared to witnesses (distilled water). To reach this goal, there were formed 5 groups, by 10 samples per group, of Adler Silvery race chickens of 4 months old, spontaneously infected with malophagans of: *Lipeurus caponis*, *Cucgroupogaster heterographus, Goniocotes gallinae*, *Goniocotes maculatus, Menopon gallinae*, *Goniodes dissimilis*, *Eomenacanthus stramineus*, *Menacanthus cornutus*, *Menacanthus pallidulus.*

Group I- experimental, group II- infected and treated with *Ectostop-P* solution of 3,0%, group III- infected and treated with solution of 4,0%, group IV- infected and treated with solution of 5,0%, group V- infected and treated with solution of 6,0 %. The application of *Ectostop –P* was carried out by spraying each bird with 50 ml per/ body. Each group of chickens was isolated in separate places. The efficiency of the preparation administered in different concentrations was determined 2, 12, 24 and 72 hours later. The obtained results are given in figure 1.

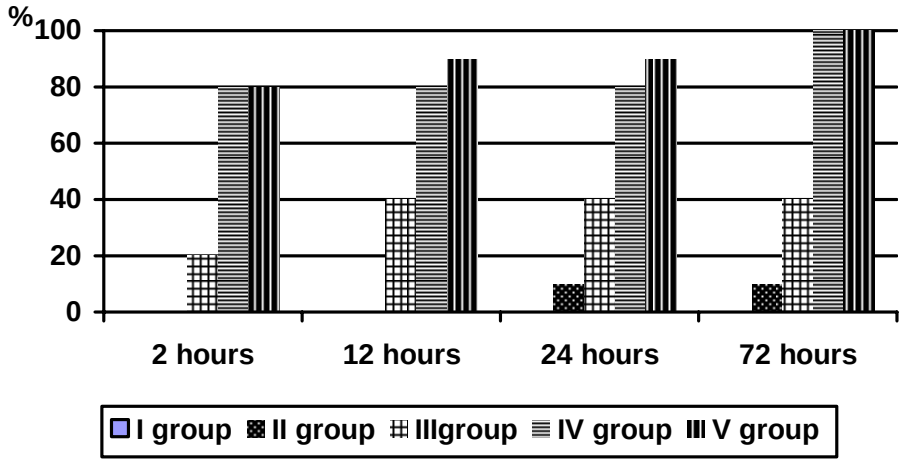

Fig.1. Efficiency of *Ectostop-P* preparation in different doses and periods of time.

The researches were carried out in the aim to establish the insecticide effect of *Ectostop-P* vegetal extracts pointed out different aspects. In groups IV and V, where it was used the *Ectostop-P* preparation in concentrations of 5,0 % and 6,0 %, it was established a high efficiency against different species of malophagans like: *Lipeurus caponis*, *Cucgroupogaster heterographus, Goniocotes gallinae, Goniodes dissimilis*, *Goniocotes maculatus Menopon gallinae, Eomenacanthus stramineus*, *Menacanthus cornutus*, *Menacanthus pallidulus.*
After the application of the parasitic treatment the hens clinical state improved, the birds calmed
and appetite increased.

Thus, after having administered the *Ectostop-P* preparation of 5%, according to the proposed procedure, ectoparasites extensity decreased significantly (to 100%). *Ectostop-P* preparation is a natural extract, biologically active, obtained from vegetal raw material, it isn't toxic and dangerous because the dose and the way of administration have an inoffensive impact on them. The procedure is not expensive and doesn't require much work; it has no restrictions in using products and subproducts from the birds treated with it. To treat hen ectoparasites the method of spraying in two stages is used, with an interval of 14 days, in a dose of 50 ml per bird, according to the biologic cycle peculiarities of different ectoparasites groups. In a prophylactic purpose it is recommended to administer the preparation in a single stage, fact which ensures their recovery and prevents infection

According to the concepts World Organization of Health, it is required that the products of an animal origin were ecologically pure and inoffensive for people and environment.
One of the intended objectives was to establish the impact of mixt-invasion with malophagans upon meat quality in the chickens involved in the experiment. That's why, at the end of the experiment the chickens were slaughtered (14 days later). The analyses proved that the chemical indices studied at the hens (from groups IV and V) poliparasited with malophagans and treated with *Ectostop-P* preparations of 5,0% and 6,0% of a vegetal origin, the content of proteins (20,5%±0,20) and pH-ul (5,3±0,12) increased, but the index of humidity decreased (65,4%±0,21), reaching the level of the non infected group (experimental).

In group II (infected but not treated), the level of proteins diminished, constituting 16,22%± 0,03, the index of humidity increased constituting 74,52%±0.18, the pH reached the limit of 7,2±0,15 and the report of fat/humidity in meat is in the favor of humidity 2,54%±0,15/74,52±0,18.

Thus, the meat of the hens from the treated groups has a higher content of proteins, a low pH and a low humidity, fact which permits to keep the fowl a longer period of time, being consequently of a higher quality as compared to the non-treated infected group. Besides, there are no more restrictions for using products and sub products from the birds treated with *Ectostop-P.*

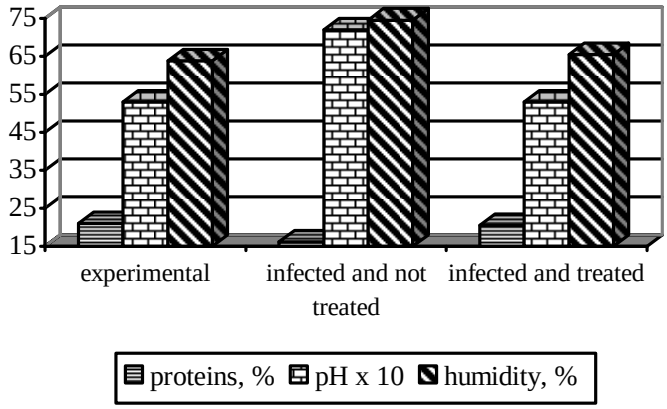

Fig. 2. The chemical composition of the skeletal muscular tissue (humid mass) in hens poliparasited with malophagous after antiparasitic treatment.

CONCLUSIONS

1. It was established that *Ectostop-P5,00%* has a high therapeutic efficiency (to 100%) against various species of hen malophagans: *Lipeurus caponis*, *Cucgroupogaster heterographus, Goniocotes gallinae, Goniodes dissimilis, Menopon gallinae*, *Eomenacanthus stramineus*, *Menacanthus cornutus*, *Menacanthus pallidulus.*
2. To treat hen parasites it is used the spraying method in two stages within an interval of 14 days, in a dose of 50 ml per bird.
3. The meat of the hens treated with *Ectostop-P 5,00%* preparation of a vegetal origin has a higher content of proteins, a low pH and a low degree of humidity, fact which permits to increase the keeping term and excludes the restrictions of use.

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