

## ABSTRACT

### Key words:

**Cows, antibiotics, enteropathies, pneumopathies**

The PhD Thesis entitled “THE MONITORING OF THE ANTIBIOTICS USAGE IN TAURINE INFLAMMATORY ENTERO-PNEUMOAPATHIES” comprises 250 pages and is structured in two main parts, according to the current provisions. The first part entitled – Bibliography Study – of the studied subject represents 67 pages (26,8%) of the entire work, and the second part entitled – Own Research- comprises 183 pages (73,2%) of the entire work.

The obtained results are supported by 42 tables and 56 pictures and images.

The first part is dedicated to the bibliography study and contains data taken from the specialised literature, presented in a synthetic manner, concerning the studied subject. This part is structured on 4 chapters.

In the first chapter, entitled “*Enteritis Etio-pathogenesis*”, the focus is on the basic notions related to the most important causes, the factors involved and the enteritis occurrence at bovines.

The second chapter “*Pneumopathies Etio-pathogenesis*” is concerned with the numerous causes triggering the occurrence and the evolution of the pneumopathies at bovines bred in intensive system. This chapter also outlines the importance of some determining factors and the pathogenesis of different pneumopathies.

The third chapter named “*General data about antibiotics*” specifies the antibiotics terminology, classification and action mode. This chapter- vital for the thesis construction – aimed to thoroughly classify the  $\beta$ -lactam antibiotics, the aminoglycosides, the gentamicin, the tobramycin and the amikacin, as well as other aminoglycoside antibiotics.

This chapter also outlines the importance of large spectrum “classical” antibiotics and, as a corollary, the complexity of accidents caused by the usage of anti-infectious medication.

The fourth chapter of the bibliography study relates to the importance and complexity of the pharmacovigilance, with the mention that, following these studies, the pharmacovigilance regulation authorities shall take measures which may include the modification of the Product Characteristics Summary (RCP) and the medicine prospect, the usage restriction on certain population groups, establishing a stricter surveillance of the respective medicine, its withdrawal from the market. This chapter also highlights – in a quite obvious manner- the legislation

concerning the pharmacovigilance, with the mention that the final measures must be adopted in accordance with the community procedure.

The second part of the thesis entitled **"Own Research"** is structured in 6 chapters.

Each chapter of this part has several sub-chapters, presenting the material and the method, the results obtained following the research and the partial conclusions drawn following the completion of each study.

The main objectives of the thesis were:

- Studying the extensive and intensive action of the germs which cause enteritis and pneumopathies of parasite and infectious nature (bacterial, viral) on bovine within a milk cows commercial unit and within a commercial unit with fattening young bovine.
- Establishing the enteritis and pneumopathies occurrence on bovine depending on their etiology;
- Monitoring the clinical and para-clinical aspects in enteritis and pneumopathies of parasite and infectious nature (bacterial, viral) on bovine;
- Aspects regarding the differential diagnosis of enteritis and pneumopathies on young and adult bovine.
- Monitoring of antibiotics usage on inflammatory enteritis and pneumopathies of taurine.
- Identification and determination of the secondary reactions previously unknown;
- Monitoring the frequency of the known adverse reactions;
- Identification of animal sub-groups presenting a particular risk for adverse reactions occurrence (risk related to the given doses, age, gender or associated disorders);
- The Pharmacovigilance of the antibiotics therapy on the livestock in the period 2007 – 2011,

In order to accomplish the proposed study, the research was performed in Neamt county on bovine farms with intensive breeding system .

In chapter seven, after setting the data regarding the studied livestock sample, the material and methods used in the thesis are largely explained.

In the first stage, the livestock, the exploitation and micro-climate conditions.

Throughout the study period, several exams were performed (coproparasite, bacterial, viral and mycology exams) and subsequently, the animals were treated in accordance with the symptomatology similar to that of the animals examined in lab conditions.

The paraclinical research was performed in the Sanitary-Veterinary and Food Safety Laboratory of Neamț ( picture10) and confirmed by the Animal Diagnostic and Health of Bucharest.

Chapter 8, entitled “Results and debates” includes 7 studies:

The first study is called: **”Epidemiology study regarding the occurrence of digestive and breathing disorders on age categories in the period 2007 – 2011”** .

Thus, after the determination of digestive and breathing disorders of the studied livestock, it was noted that both digestive and breathing disorders have an important position (6,34%, respectively 3,68%) as compared to the rest of the disorders observed at the studied livestock, which awards a greater relevance to the study, also outlining that, regardless of the increase or decrease of the animal number, the sickening rate preserved its values around 6%, .

It was noted that the digestive disorders may have a seasonal character, being usual in particular in the cold or wet seasons of the year, namely in October, November, December or at the beginning of the year, in the months January, February and March. The enteropathies are influenced by a series of maternal, bioclimatic or nutritional factors which may act both upon gestant females and upon young bovine with age from 0 – 6 months, thus generating the increase of the sickness frequency of young taurine.

The study of the causes which determined the occurrence of breathing disorders pointed that mainly the bioclimatic factors acted, namely: the low temperature in some winter periods, the high humidity of the bedding or the cold water washing of the floor as well as the failure to close of doors and windows, which led to the increase of air currents speed.

It was also noted that a great part of the young taurine involved in the development of breathing disorders were between 0 – 6 months old.

The second study called **”Study regarding the germs involved in the bovine digestive and breathing disorders in the period 2007 – 2011”** found that, following the lab analyses, in the etiology of enteral disorders, both bacterial and viral and parasite germs are involved, in different combinations. As to the importance of the involvement, it was noted that the most frequent germ is bacterial, namely *Escherichia coli*, found at 31% cases, followed by

*Pseudomonas aeruginosa* diagnosed at 23% of the cases. On the third place, in the frequency order, the presence of the bacteria *Salmonella enteritidis* was noted with the same rate as the parasite *Eimeria bovis* respectively, 12%, followed by Coronavirus in 9% of the cases, *Salmonella typhimurium* in 7% of the cases and on the last position it was *Rotavirus* in 6% of the studied cases.

As to the weight of pneumopathogen agents causing the breathing disorders, the following were noted: in the evolution of breathing and eating disorders, the involvement of different bacterial, viral and parasite agents was also noted, which most of the time, evolve together, in combinations of 2 bacteria, a virus and a bacteria, virus – parasite, bacteria – parasite, or combinations between a bacteria – a virus and a parasite. It was noted that in the case of the combination between the bacterial agent *Pseudomonas aeruginos* and *Streptococcus spp*, the sick animals were greatly adult cows and less heifers or calves, and the sickness evolution was similar to a purulent bronchopneumonia associated to cough, dyspnea, mucous-purulent jet, fever syndrom. In the case of *Actinomyces pyogenes* involvement in combination with different viral or parasite germs, the involved category was represented by young calves.

The etiological involvement of other germs was also noted, the most frequent being *Pasteurella haemolytica* sometimes associated to *Haemophilus somnus*, *Pneumocystis carinii*, *Mycoplasma bovis*, or even *Fusobacterium necrophorum*. On calves aged 0 – 6 months, a fibrinopurulent pneumonia was frequently identified, namely at 20 cases. All cases presented the germ *Streptococcus zooepidemicus* with the appropriate symptomatology (fever syndrom, dyspnea, cough, purulent secretions, high loss of weight).

The third study was entitled ”**Study for the etiology determination, clinical expression, diagnosis and therapy of breathing disorders at young bovine** “ . It was noted that, depending on the animal age, several types of clinical manifestations may be identified: bronchopneumonia ab-ingestis at new-born calves (in particular, those fed using a feeding bottle), enzootic bronchopneumonia of calves in stables and transport disease or “transport fever” of animals bred for fattening (occurring several days or weeks after transport). As to the etiology, it was noted that there are 2 types of factors involved in the occurrence and evolution of bronchopneumonias of young calves, namely factors caused by the animal and factors caused by the environment. Among the factors caused by animals, the most important are: maturity of the breathing function, lack of breathing function, body immunity condition, energetic (protein and calories)

malnutrition, vitamin lack, or those lacking the trace (minor) elements and pathological disorders. Among the factors caused by environment, the most important are: sudden diet changes due to pasturing or withdrawal to the shelter, the nutrition modifications, switching to the fattening intake, environment changes, sudden temperature changes or inappropriate ventilation of the shelter, high temperature variations, inappropriate air pressure, dust and toxic gas presence, hierarchy changes, necessary veterinary manipulations and manoeuvres, inappropriate lot breakdown and last, but not least, keeping the sick animals within the collective, over-population which leads to the occurrence of a cumulative stress. The most important factor in the occurrence of breathing disorders is represented by the involved pathogen agents. Also isolated different parasites associated with bacterial agents, mycoplasma or chlamydia.

The symptomatology found was quite diverse. The main symptoms found were: the mucous jet (from serous to purulent) associated with other clinical signs (respectively, cough, fever, tachypnea, lack of appetite to anorexia, and dyspnea, either inspiring or expiring. The physical signs noted were the increase of the breathing frequency and the occurrence of overlapped noises (cracking and wheezing), in particular on ventral side and in front of the auscultation point.

The existence of 4 clinical phases was noted in the complex of breathing disorders of the ruminants: Phase 1 – with sub-clinical evolution where there is a control exerted by the body concerning the multiplication of pathogen agents, and the animal frequently cures spontaneously; Phase 2 – with compensated clinical evolution and more or less important inflammatory reaction and the animals cure after a couple of days of therapy using a single antibiotic; Phase 3 - with decompensated clinical evolution and quite severe inflammatory reaction, severe symptomatology, pathological wounds requiring a complex treatment both antibiotics and anti-inflammatory, as fast and as persistent and Phase 4 – characterised by an irreversible clinical evolution with lung wounds incompatible with survival and productive performance, with dramatic symptomatology where treatment delusive and uneconomical.

The diagnosis in the considered pneumopathies initially required a simple differential diagnostic as compared to other breathing disorders than those involved in the respective study and a differential diagnostic as compared to other more complex disorders, such as emphysema, interstitial pneumonia, lung oedema, pleurisy, laryngitis- tracheitis, vermin bronchitis, or, more

rarely, a neoplastic disease of the lungs or trans-diaphragm hernia. Among the bacteria, the most frequently isolated were *Pasteurella haemolytica* and *Multocida*, as well as *Haemophilus somnus*. In the evolution of a chronic bronchopneumonia or a chronic lung purulence, *Arcanobacterium pyogenes* and coliform bacteria were isolated, observing frequent associations of a bacteria and a mycoplasma or a virus and one or several bacteria.

As to the prognosis related to most cases, it depended on the disease evolution stage. In this respect, it was proceeded to the determination of the plasma lactate of the young bovine with breathing disorders to establish the disease gravity and thus to reduce the economic loss. A serious prognostic was established for all the diagnosed cases in stage 4 of the disease or, following the plasma lactate determination (lactacidemia), its value was above 4 mmol/L.

The treatment considered 4 main objectives: reducing the pathogen action, namely the removal of pathogen agents, limiting the inflammatory reaction, correction of mechanical disorders and general support treatment. In order to reduce the pathogen action, a series of antibiotics following the antibiogram performance, respectively: Ampicillin LA, Cefquinome, Ceftiofur, Cefalexin, Amoxycillin LA, Enrofloxacin (Baytril), Florfenicol, Lincomycin-Spectinomycin, Oxytetracyclin LA, Oxytetracyclin HCl, Spiramycin, Sulfadimidin-Baquiloprim, Tilimicosin, Tylosin.

The metaphylaxis consisted of a mass treatment on all the animals with minor problems or if the symptomatology concerned a small number of animals. It was considered epidemic if more than 10% of the animals were affected on a day, if more than 25% of the animals were affected on a period of 3 - 5 days or if the food consumption suddenly decreased; as a prevention measure, a mixture of Tetracyclin + Tilmycosin + Spiramicin was given; if following this metaphylactic treatment there was another sick animal, than the antibiotics was changed.

The fourth study was entitled **”Study concerning the therapy means in eating and breathing disorders”**. The major objectives set to solve the eating disorders were: stopping water and electrolyte losses, re-hydration and re-salinization, fighting acidosis and nitrate retention, supporting the main functions, fighting infections, digestive therapy and artificial & diet feeding. In order to stop losses, it was turned to administering mucilages, anti-diarrhea teas or digestive „dressings” associated to adsorbents and astringents for most animals. As digestive „dressing” the following were used: calcium carbonate, magnesia, zinc oxide, bismuth salts, and as adsorbent – bismuth salts and medical coal were used. The **re-hydration and re-salinization**

of the animals involved were performed both hypodermic and in particular intravenous, using **isotonic, alkaline and energising solutions** (glucose 5%, dextrose 5%, soda 1,3-1,4 %, Ringer solution or Ringer lactate solution).

The **prevention and fighting of the bacteria complications** were done following the antibiogram, using antibiotics and chemotherapy active on negative and positive Gram germs function of the symptomatology and antibiotics sensitivity of the pathogen agent concerned, on all sick animals. The **antibiotics** used were those able to provide a good concentration in the systemic circulation, in particular - penicilins, aminosides, cephalosporins - and those which were appropriate for the pulmonary tissue, namely the tetracyclins and macrolides. The more frequently used antibiotics in order to fight the digestive disorders were: **Enroxil 10%, Penstrep, Colistin and Sulfadiarom, Betamox LA, Amoxilrom 10% and Gentamicin.**

In order to heal the bovine bronchopulmonary disorders, the following were prescribed: anti-infectious medicines, anti-inflammatory medicines, corticoides, broncho-dilatators, anti—cough and pectoral medicines. In terms of **anti-inflammatory medicines**, both **specific** (such as Ketotifen) and efficient anti-inflammatory **corticoids**, with secondary effects reducing immunity, the prednisone and the hemisuccinate hydrocortisone being more frequently used.

These were also used for the purpose of reducing fever, but the Algocalmin (in phials) was more successful. The antibiotics more frequently used were those which had an adequate action spectrum and a good diffusion. When using **sulfamides**, these were selected so as to have a larger spectrum and a satisfying diffusion, respectively the chemicals based on sulfamides doubled by Trimetoprim. As to the antibiotics usage frequency on the animals diagnosed with breathing disorders, it was noted that the product **Depocillin** was most frequently used, followed by **Enroxil 10%, Amoxilrom, Synulox, Floron, Linco Spectin, Resflor Gold, Penstrep** and last -**Oxitetracilin**.

The fifth study called "**Study regarding the pharmacovigilance following the treatment performance for breathing and eating disorders**" outlined the fact that, for the antibiotics therapy applied to the animals diagnosed with enteropathies and pneumopathies, we tried to achieve as many objectives of the pharmacovigilance activity. Thus, first of all, the secondary effects and the adverse reactions observing that the total of 584 cases undergoing antibiotic therapy, only 1.71% of the animals showed side were monitored and the findings were that of the animals presented secondary effects and 1,02% presented adverse reactions.

**The secondary effects** mentioned were: oedema at the inoculation spot, intense itching at the inoculation spot, abatement, lack of appetite, mucosa congestion, epiphora, under-fever condition, persistent thirst, muscle trembling, ataxia, frequent urination and even slight anaphylactic reaction, and the adverse reactions noted consisted of lethargy, muscle trembling, convulsions, violent anaphylactic reactions, collapse, dyspnea, pulmonary oedema, diarrhea, lack of urination, ataxia and death. As to the identification of animal sub-groups presenting a particular risk for adverse reactions occurrence, one must mention that several aspects were considered: the administered dose, age, gender and related disorders, finding individual reactions, not group reactions, while the administered dose was the one mentioned in the prospect of each given (medicine).

It was also noted that 4 of the 6 cases presenting adverse reactions died. It was more relevant to establish the most vulnerable age for the occurrence of secondary effects and adverse reactions. The most often affected by secondary effects or adverse reactions were the calves aged 0 – 6 months the greatest rate: 44%, due to the administration errors, to the failure to correctly assess the weight and due to the body's failure to get used to the administered medicine).

To establish the efficiency of the antibiotics used, it was proceeded to classify them function of the administration frequency to cases. Thus, it may be said that the first position is held by Enroxil 10%, followed by Penstrep and Amoxilrom 10% , used both in enteropathies and in pneumopathies, then Sulfadiarom, Colisin, Betamox LA, Gentamicin, Depocillin, Synulox, Floron inj.sol. 300mg/ml, Linco Spectin, Resflor Gold inj.sol., followed by Oxytetracycline.

The sixth study was entitled **”Study for the testing of the active substance Enrofloxacin given to calves right after birth in order to mitigate the frequency and severity of neonatal diarrhea“** and outlined that the values of the erythrocyte indexes of the studied calves presented a decrease tendency by the end of the neonatal period. The lowest values of the haemoglobin and hematocrit were noted at the 11-day-old calves, which coincides with the exchange of mother milk with collected milk. The Pearson test pointed a positive correlation statistically significant ( $P = 0,001$ ) in the evolution of the haptoglobin, on one hand, and the erythrocyte indexes, on the other hand. The calves age had a statistically significant effect ( $P < 0,05$ ) on thrombocytes; the lowest values were recorded at the 2-day-old calves and the greatest values were noted at the 11, respectively 18-day-old calves. The Pearson test pointed an indirect correlation statistically

significant ( $P = 0,04$ ) between the haptoglobin evolution and the average values of trombocytes. In the neonatal period of the studied calves, the presence of reticulocytes was noted starting with the age of 1 day up to the age of 16-18 days. The average values of the neutrophils decrease progressively, starting with the age of 1 day up to the end of the neonatal period. The values of serum Fe recorded an increase starting with the first day of life until the age of 28-29 days, the values fitting the calves physiological limits. No correlations were established between the evolution of haptoglobin and serum Fe.

The calves age had a statistically significant effect on total proteins and globulins; The Pearson test pointed a positive correlation statistically significant ( $P = 0,004$ ) between the average values of ceruloplasmin and total globulins.

The results of this study indicated that the physiological values of the haematological and biochemical profile of adult bovine may determine diagnosis errors if applied to calves during neonatal period. It was found that prophylactic antibiotic in the first days of life is inconclusive, the losses are greater than the number treated.

The last study is called **”Economic Study regarding the efficiency of antibiotics usage on bovine in the treatment of eating and breathing disorders**. In the case of clinical breathing disorders, the estimated loss was of 4 - 6 litres of milk/day/cow. The economic calculation was performed based on an average deficit of 5 litres of milk/day/cow, for a delivery price per unit of 1,3 ron.

Of young taurine the losses include treatment costs, costs associated to the increased mortality, premature sacrifices, reduced growth progress, reduced fertility and reduced milk production during the first lactation.

In the case of clinical breathing disorders, the estimated losses are of 2 - 6 litres of milk/day/cow.

As a conclusion, it may be said that, economically speaking, the analysis of these disorders highlighted lower costs of the preventive therapy on a high number of cows as compared to the healing therapy on a lower number of cows.

The bibliography references comprise 210 titles from national and international literature. The thesis content also includes the results of my own research published in scientific work volumes presented at international symposia organized by the Faculty of Medicină Veterinary Science.

