ABSTRACT

Viral respiratory infections of cattle represent a syndrome characterized by acute, subacute or chronic polifactorial inflammation of respiratory tract. These diseases currently known in increasing incidence in bovine farms, being with enteropathies the main diseases diagnosed in cattle and particularly in young bulls. At the same time are the most important causes of economic losses recorded in cattle.

Economic losses are made by mortality, early slaughter, decreased weight gain, treatment costs, prevention and control. These losses are added that clinical recovery is not equivalent to animal and economic recovery, the animals passed through the disease remains underdeveloped and prone to relapse, is at the same time, sources of infection to permanent shelters.

Surveillance and control of viral respiratory diseases limit or prevent their spread and eradication efforts must be national veterinary priorities. The two morbid entities studied in this thesis, infectious bovine rhinotracheitis and bovine viral diarrhea, are recognized as diseases that produce significant losses of livestock system.

Stringency of control measures implemented depend on epidemiological characteristics of each entity throughout the morbid side, so they must be viewed individually by means of transmission, degree of contamination and action of each pathogen responsible for producing each disease separately.

Thesis entitled "***Researches on respiratory virosis of cattle***" is spread over 240 pages and is made in accordance with current legal provisions, the two main parts: the first part entitled "*Bibliographical study*" includes 59 pages, 6 tables and 55 figures, Part II "*Personal contribution*" occupies 133 pages, 41 tables and 92 figures.

The first part consists of six chapters which are summarized, information from literature on the subject of the sentence which was subsequently used to interpret and compare data obtained in the second part.

The first chapter entitled "*Bibliographic data on history, distribution, importance and etiology of infectious bovine rhinotracheitis - infectious pustular vulvovaginitis (IBR-IPV)* " presents data on the first descriptions of disease and etiological agent, the literature on the prevalence and importance of this disease.

Buchner and Trommsdorf describe in Germany during the 19th century "Blaschenausschlag (vesicular exanthema coition), a disease of cattle likely caused by bovine herpesvirus type I (BoHV-1). Viral etiology was described in 1928 by Reisinger and Reimann, who showed that venereal disease, is transmitted by a filterable agent. By the early 1950s manifestation of BoHV-1 infection was known as "infectious pustular vulvovaginitis (IPV) in

cows and" infectious pustular balanoposthitis (BPI) in bulls, is attributed only to diseases of the genital tract. Meanwhile respiratory disease occurs in cattle in North America. The disease was more severe due to BoHV-1 infection, called "infectious bovine rhinotracheitis (IBR). Infectious bovine rhinotracheitis has spread rapidly in Europe, because of imports of dairy cows from North America in order to improve milk production in Europe.

The second chapter entitled "*Aspects of the epidemiology, pathogenesis, clinical and pathological changes in infectious bovine rhinotracheitis - infectious pustular vulvovaginitis (IBR-IPV)* " is divided into five chapters, summarizing data from the literature referring to epidemiology, pathogenesis, immunology, clinical and pathological changes. Sources of infection are presented, revealing how pathogenic mechanism of contamination and infectious bovine rhinotracheitis virus infection. Are also presented symptoms and lesions produced in various forms of the disease.

In the third chapter titled "*Diagnosis, prevention and control of infectious bovine rhinotracheitis virus infection - infectious pustular vulvovaginitis (IBR-IPV)*" are presented the main diagnostic methods, referring to the etiology and differential diagnosis, and measures to limit spread of infection.

In chapter IV, entitled "*Bibliographic data on history, distribution, importance and etiology of bovine viral diarrhea-mucosal disease (BVD-MD)*" presents data on the first descriptions of disease and of etiological agent, the literature on the prevalence and importance of this disease.

The first description of this complex was made by Mac Callum et al., 1946, as the New York viral diarrhea, manifested as an acute illness, fever, rinderpest-like lesions in the mouth and serious enteritis. It was later established that the disease is caused by a specific virus, different from that of rinderpest. A similar disease was described in cows and calves by Pritchard et al. (1956), in Indiana as the Indiana virus diarrhea or gastroenteritis erosion. At the same time in different U.S. states were reported enzootic disease affecting the digestive and respiratory mucosa, generically called "Mucosal disease".

Mucosal disease term was introduced by Ramsey et al. (1953), referring to Iawa disease of cattle characterized by lesions of the mouth and severe diarrhea. Hoag et al. in (1956) described the mucosal disease of Virginia.

Initially, under the term of mucosal disease complex in the broad sense of the term, were included, the Iawa and Virginia mucosal disease, two viral diarrhea (New York and Indiana) and infectious bovine rhinotracheitis.

Subsequent serological and viral investigations have established that New York and Indiana viral diarrhea and mucosal disease of Iawa and Virginia are produced by identical virus,

while infectious rhinotracheitis is caused by a distinct virus. In 1960, Gillespie et al., proved the identity of etiologic agents, showing that in fact it is just two manifestations, somewhat different, of the same disease called mucosal disease and bovine viral diarrhea. For this reason, most authors now use the name of bovine viral diarrhea - mucosal disease. In Romania, the disease has been identified serologically and virology by Coman and col. in 1968.

Fifth chapter entitled "*Aspects of the epidemiology, pathogenesis, clinical and pathological changes in bovine viral diarrhea-mucosal disease (BVD-MD)* " is structured in four chapters, summarizing data from the literature with regard to epidemiological characters, pathogenesis, clinical and pathological changes.

In chapter VI, entitled "*Diagnosis, prevention and control of bovine viral diarrhea virus infections, mucosal disease (BVD-MD)*" are presented the main diagnostic methods, referring to the etiology and differential diagnosis, also the measures to limit the spread of infection.

In chapter VII are described the aims and objectives of the doctoral thesis. Throughout the period of study, the research took into account five key goals:

- Epidemiological investigations on the prevalence of infectious bovine rhinotracheitis - pustular vulvovaginitis (IBR-IPV) and bovine viral diarrhea - mucosal disease (BVD-MD) in Romania during 2006-2008.
- Serological research on the prevalence of infectious bovine rhinotracheitis - pustular vulvovaginitis (IBR-IPV) and bovine viral diarrhea - mucosal disease (BVD-MD) in four counties of Moldova region (Iasi, Vaslui, Botoşani and Suceava) in 2006-2009.
- Research on the detection of infectious bovine rhinotracheitis virus in eastern Romania.
- Research on the detection of bovine viral diarrhea virus in the eastern Romania
- Developing recommendations on the strategy to control infectious bovine rhinotracheitis - pustular vulvovaginitis (IBR-IPV) and bovine viral diarrhea-mucosal disease (BVD-MD).

Chapter VIII presents the results of investigations on the presence and prevalence of infectious bovine rhinotracheitis - pustular vulvovaginitei (IBR-IPV) using serological tests.

For these tests have been used different ELISA kits for detection of specific antibodies anti - BoHV-1. The first part of the chapter presents the results of serological tests carried out in Romania during 2006-2008. 12,601 samples were tested and a number of 959 reacted seropositive, representing a prevalence of 7.61%. In the year 2006 were serologically tested 6523 serum samples of which 147 (2.25%) were positive and 6376 (97.75%) were negative for IBR-IPV. In 2007, 5196 serums were tested serologically and 739 samples (14.22%) were identified positive and 4457 (85.78%) negative. In 2008 from 882 serum samples tested 73 (8.27%) were IBR-IPV positive and 809 (91.73%) were seronegative.

The second part of the chapter presents the results of serological tests carried out in four

counties (Iasi, Vaslui, Botosani, Suceava) in Moldova, undertaken in 2006-2009. During this period 1800 samples were tested, 200 in 2006, 400 in 2007, 535 in 2008 and 665 in 2009, and a total of 747 samples were found seropositive for infectious bovine rhinotracheitis - pustular vulvovaginitis..

In 2006 from 200 samples tested, were identified 77 positive sera (38.5%) and 123 (61.5%) negative samples. In 2007 the seroprevalence was 37.5% (150/400). In 2008, 535 sera were tested, 242 (45.23%) samples were identified as positives respectively, the remaining 293 (54.77%) negative. The seropositivity in 2009 was 41.8%, representing 278 positive serums and 387 (58.2%) samples were negative. During the investigation made in farms during 2006-2009, in the four counties were identified 577 seropositive samples representing 44.28% of 1303 samples analyzed. The household system serological test detect 170 positive samples (34.20%) and 327 (65.79%) negative samples out of 497 tested serums.

Chapter IX presents the results of research on detection of infectious bovine rhinotracheitis virus - pustular vulvovaginitis (IBR-IPV). The research used the direct fluorescent antibody reaction and molecular biology techniques (PCR) for detection of BoHV-1 in animals from different breeding systems.

Virological investigations using direct immunofluorescence technique on presence of BoHV-1 from outbreaks in three counties: Iasi, Iasi, Galati has performed in 2008-2010. In this study were examined a number of 41 animals of which 5 aborted calves. Were performed 99 smears using target tissues for bovine herpesvirus type 1: lung, pulmonary lymph nodes, tracheal mucosa, liver (abortion), and nasal discharge. Of all animals tested were found positive 7 animals of which 2 two aborted calves.

In the investigations of molecular biology on a presence of bovine herpesvirus type 1 were used two protocols. In 2008 we used ExtF and ExtE set of primers to amplify a 468 nucleotide sequence of the glycoprotein B, of 20 animals tested were identified two positive responses (10%). In 2009 we used two sets of primers: for gB and gC, identifying an positive animal of 12 tested. The sample was identified as positive only for GC showing the genetic variability of wild-type BoHV-1 circulating in cattle populations.

Chapter X presents the results of research on bovine viral diarrhea prevalence and. For serological tests were used different ELISA kits to detect specific antibodies anti-BVDV.

The first part of the chapter presents the results of serological tests carried out in Romania during 2006-2008. Following investigations on the prevalence of BVDV antibodies in Romania, from 5823 samples tested were identified as positives 226 serums, representing a prevalence of 3.88%. In 2006 from 2998 serological samples tested, 80 (2.66%) were positive and 2918 (97.34%) negative. In 2007, out of 2637 samples tested serologically were identified 127

(4.81%) seropositive and 2510 (95.19%) seronegative. Bovine viral diarrhea seroprevalence in 2008 was 10.1% (19/188).

The second part of the chapter presents the results of serological tests carried out in four counties (Iasi, Vaslui, Botosani, Suceava) in Moldova reagion, undertaken in 2006-2009. The serological investigations out of 978 samples, 117 in 2006, 302 in 2007, 425 in 2008 and 134 in 2009, a total of 391 were found seropositive for bovine viral diarrhea - mucosal disease. In 2006, from 117 samples tested were identified 63 positive sera (53.85%) and 54 (46.15%) negative. In 2007 out of 302 serological samples tested a number of 121 samples were positive which represent 40.07%. In 2008, 425 sera were tested and 129 were found IBR positive (30.35%) and 296 (69.65%) seronegative. Seropositivity in 2009 was 58.21% (78/134).

Analysis of data obtained from serological tests in 2006-2009, in the four counties, on cattle farm, revealed a seropositivity of 44.99% (314/698). In household system serological test results for anti-BVDV antibodies revealed 77 positive samples (27.5%) and 203 negative (72.5%) out of 280 sera tested.

Chapter XI presents the results of research on detection of bovine viral diarrhea virus (BVDV). During investigation were used two methods: direct immunofluorescence and molecular biology techniques (RT-PCR). The first part of the chapter presents the results of viral investigations using direct immunofluorescence method to detect the presence of bovine viral diarrhea virus. 27 animals were tested and 15 (71.43%) positive were identified, all positive cattle were from household system.

From slaughtered animals were collected the following tissues: lung, trachea, tracheo-bronchial lymph nodes, mesenteric lymph nodes, thymus and small intestine sections. Age of the tested animals was between 10 and 16 months. After testing all samples from farms animals was not found any positive response.

The second part of the chapter presents the results of molecular investigations on bovine viral diarrhea virus in three districts of Moldova region, both on farms and in households. Samples were collected from cattle slaughtered, being represented by: mesenteric lymph nodes and portions of small intestine. The animals came from areas where have been cases of disease and were found positive by direct fluorescent antibody method. Out of 19 animals studied, aged between 11 and 17 months, were collected 38 tissue samples, identifying three (15.79%) positive animals that came from households.

It notes that of 13 positive samples were confirmed by direct immunofluorescence only a percentage of 23.07% (3 samples). This is confirmed by the literature showing that gene amplification technique is more specific than direct immunofluorescence, recommending the use of molecular methods for detection of persistently infected animals.

In **Chapter XII**, because of the presence and movement of the two viral respiratory infections in cattle are given some recommendation to limit BoHV-1 and BVDV infection within the herd, in line with European regulations.

In **Chapter XIII** 21 final conclusions are summarized.