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Seroepidemiology of Bovine Herpesvirus-1 Infection in Cattle Herds from North-East Region of Romania

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Abstract: Infectious Bovine Rhinotracheitis (IBR) is a highly contagious disease caused by the bovine herpesvirus-1. The main biological characteristic of herpesviruses is the establishment of latency in sensory ganglia, the animal remaining infected all their life. The objective of the present study was to describe the seroepidemiology aspects of BHV-1 infection on cattle farms from North-East of Romania. During 2011 to 2012, 1538 blood samples were collected from 87 cattle herds. In these herds serum samples were collected from cows, heifers and calves. A commercial gB ELISA test was used to analyze samples for antibodies against BHV1. The overall prevalence of IBR was found to be 36.02% (554 positive samples out of 1538) in cattle herds from North East of Romania.

Key words: Bovine herpesvirus 1, seroepidemiology, cattle herd, life, ELISA

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR), caused by Bovine Herpesvirus 1 (BHV-1) is a disease of domestic and wild cattle. BHV-1 is a member of the genus *Varicellovirus* in the subfamily Alphaherpesvirinae which belongs to the Herpesviridae family. Three subtypes of BHV1 are recognized worldwide: BHV1.1, BHV1.2a and BHV1.2b (Metzler *et al*., 1985). Viruses belonging to subtypes 1.1 and 1.2a are more virulent and severe than those belonging to subtype 1.2b. The virus may cause latent infection while stress can induce reactivation (Ackermann *et al*., 1990) and intermittent excretion of the virus into the environment (Muylkens *et al*., 2007). Latent infections make it difficult to control transmission to unexposed animals, since they cannot be diagnosed by clinical examination or quarantine (Guarino *et al*., 2008).

In adult cows infection is associated with a severe and prolonged decrease in milk yield, reduced fertility and abortions (Miller, 1991). Many infections run with a subclinical course (Van Oirschot *et al*., 1993). Contaminated materials including semen can transmit the virus (Chandranaik *et al*., 2010).

An infection normally induced an antibody response and a cell-mediated immune response within 7-10 days (Kramps *et al*., 2004). Infected animals remain seropositive all their lives. Maternal antibodies are transferred via colostrum to the young calf which is consequently protected against BHV-1-induced disease (Mechor *et al*., 1987). Maternal antibodies have a biological half-life of about 3 weeks but may be detected occasionally in animals up to 9 months old and rarely in animals over this age.

Herd prevalence of BHV1 virus infection varies from country to country and herds to herds. Infection with BHV1 is recognized as an important health problem in Romanian dairy herds. IBR was first diagnosed in Romania in the mid-1960s. The presence of the BHV-1 infection was described in bull semen by Ionescu (2000).

Several countries in the European Union such as Denmark, Sweden and Finland have eradicated BHV1 by prohibiting vaccination, removing seropositive animals and by additional preventive measures. Besides, some countries, like Austria and Netherlands have an European Union approved national compulsory eradication program (Van Schaik *et al*., 2001). Seroprevalence of BHV-1 in cattle has been reported worldwide in dairy and beef herds and some risk factors for the presence of BHV-1 antibodies in cattle include large herd size, older age, dairy herds with presence of beef cattle, high density of herds within an area, purchased cattle and herds located close to BHV-1 positive herds (Van Schaik *et al*., 1998; Boelaert *et al*., 2005). The objective of the study was to evaluate the prevalence of BHV1 antibodies in cattle population in North-East region of Romania.

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Table 1: Description of the size herds used for field study

No. of herds ≤10 cows (N = 54 herds) (Household system)		No. of herds ≤50 cows (N = 22 herds)		No. of herds >100 cows (N = 11 herds)	
Animals	Total count	Animals	Total count	Animals	Total count
Calves <6 months	60	Calves <6 months	125	Calves <6 months	75
Heifers 6-12 months	106	Heifers 6-12 months	324	Heifers 6-12 months	115
Cows >24 months	98	Cows >24 months	335	Cows >24 months	300
Totals	264	Totals	784	Totals	490

Grand total = 1538

MATERIALS AND METHODS

Type of study and experimental animals: The data used in this study came from 87 dairy herds in North-East region of Romania. The data are presented in Table 1. Farms were visited between 2011 and 2012 and were located in 6 counties. During this period in the studied farms were not made vaccinations against BHV-1 infection. A number of 1538 samples were collected from animals apparently healthy.

Blood samples: Blood samples were collected from the jugular or tail vein into 9 mL sterile vacuum tubes containing a clotting activator using disposable needles (0.9×38 mm). Serum was separated by centrifugation of blood at 3000 rpm for 10 min at room temperature; the aliquots were transferred into 1.5 mL sterile microtube and were kept at -20°C until analysis.

Detection of antibodies against BHV-1: The serum samples were analyzed for BHV-1 antibodies using a commercial HerdChek* IBR gB ELISA test kit (IDEXX Laboratories, Inc., The Netherlands). The sensitivity and specificity of the assay were 100 and 98.9%, respectively.

The experiment was carried out according to the kit protocol. OD of samples and controls were measured at 450 nm by using ELISA reader (Tecan Sunrise, Switzerland) and recorded using a computer. According to test instructions serum sample was considered to be negative if the blocking percentage was <45%, suspect between 45 and 55% and positive when over 55% (IDEXX Laboratories, Inc., The Netherlands).

Statistical analysis: Descriptive statistics was used to calculate the specific seroprevalence (by size herd, age). The Vassar Stats® Software was used to calculate the confidence intervals (set at 95%) of the specific prevalence.

RESULTS AND DISCUSSION

Of the 1538 animals sampled, 554 were positive to the presence of antibodies against BHV-1. The overall percentage of seropositivity was 36.02% among the animals examined (n = 1538). The total number of antibody-positive herds detected during the studied period was 37, representing 42.52% of studied farms. Seroprevalence in the household system was 29.6% for small herds was 54.5% and in the large herds was 81.8%. With respect to the age of cattle, the lowest seroprevalences obtained in the group of 6-12 months heifers from household system was 7.5% (95% CI, 3.5-14.7%) and from farms with up to 50 animals was 7.4% (95% CI, 4.9-10.9%). The highest seroprevalence was obtained in the group of adults (older than 24 months) coming from large farms, representing 84.7% (95% CI, 79.9-88.4%). The lowest rates were recorded in small farms with up to 50 animals: 24.8% (95% CI, 17.7-33.4%) for calves up to 5 months 7.4% (95% CI-4.9-10.9%) for 6-12 months heifers and 16.7% (95% CI, 12.9-21.2%) for adult cows. The detailed results are presented in Table 2 and 3.

BHV1 antibodies can be found in bovines in all continents and in many wild species. Prevalence varies greatly depending on herd size and management. It was found that the gB-specific ELISAs were most sensitive for the detection of antibodies in serum whereas for assaying milk samples the indirect ELISAs were the tests of choice (Kramps *et al*., 2004).

The results obtained on BHV-1 antibody prevalence in this study (36.02%) were compatible with prevalence rates (35.02 and 38.75%) found by Duman *et al.* (2007) in beef herds in Turkey.

The results regarding the seropositivity for BHV1 are similar of those reported by Woodbine *et al.* (2009) and Rypula *et al.* (2012). Infection rates detected in small farms where fewer animals were grown (up to 10 animals) were lower than those in organized in large farms where many animals were grown intensively. The possible explanation for this might be determined by reduced possibilities of contamination among animals depending on the number of animals in small farms.

The same results were obtained by Raaperi *et al.* (2010) in Estonian dairy cattle. The herd prevalence increased significantly with herd size, being 3.4% in the smallest category (<20 cows) and 85.7% in herds of size over 400. Moreover, Raaperi *et al.* (2012) observed that moderate and high (>50%) seroprevalences of BHV-1

Table 2: Seroprevalence of BHV-1 by age in cattle in Eastern Romania

No. of herds ≤10 cows (N = 54 herds) (Household system)	Calves <6 months				Heifers 6-12 months				Cows >24 months			
	Tested	Positive	Seroprevalence (%)	Confidence interval (95%)	Tested	Positive	Seroprevalence (%)	Confidence interval (95%)	Tested	Positive	Seroprevalence (%)	Confidence interval (95%)
≤10 cows (N = 54)	60	22	36.7	24.9-50.1	106	8	7.5	3.5-14.7	98	27	27.6	19.2-37.6
≤50 cows (N = 22)	125	31	24.8	17.7-33.4	324	24	7.4	4.9-10.9	335	56	16.7	12.9-21.2
>100 cows (N= 11)	75	48	64.0	52.0-74.5	115	84	73.0	63.8-80.7	300	254	84.7	79.9-88.4
Total	260	101	38.8	32.9-45.0	545	116	21.3	17.9-25.0	733	337	46.0	42.3-49.6

Table 3: Seroprevalence of BHV-1 by herd size in cattle in Eastern Romania

No. of herds ≤10 cows (N = 54 herds) (Household system)	Status of the herd			
	Seropositive	Seronegative	Seroprevalence (%)	Confidence interval (95%)
≤10 cows (N = 54)	16	38	29.6	18.3-43.8
≤50 cows (N = 22)	12	10	54.5	32.6-74.9
>100 cows (N = 11)	9	1	81.8	47.7-96.8

among cows were both related to a high frequency of respiratory disease in calves. Penny *et al*. (2002) indicated that respiratory disease is more often seen in calves of primiparous cows. That can be explained by the lack of previous exposure to BHV-1 by their dams. Therefore, a number of calves may contract the disease caused by BHV-1 as a result of weak maternal immunity.

According to OIE, in the same period with the study (2011 to 2012), BHV-1 infection outbreaks were reported in several European countries with developed cattle breeding systems. In 2011, were reported 8 outbreaks in Belgium (1775 suspected cattle), 33 outbreaks in Estonia, 26 outbreaks in Germany and 83 outbreaks in Poland. In 2012, the highest number of IBR outbreaks were reported by Spain (1152 outbreaks), Poland (97 outbreaks), Germany (25 outbreaks) and Russia (22 outbreaks).

Kahrs (1981) suggested the higher seroprevalence in rural farming conditions could be due to natural breeding practices that were followed with virus contaminated semen. Movement of seropositive cattle and trade with BHV-1-positive semen used in artificial insemination are to be considered as the most important ways to reintroduce the virus into IBR-free herd.

Chandranaik *et al*. (2010) opined that the presence of antibodies against BHV-1 in cattle itself cannot confirm an active infection in cattle. Transportation of cattle with latent infection can reactivate the virus and the existence of seronegative latent carriers is a threat for cattle husbandry (Nandi *et al*., 2009). Therefore, in order to find out the PI in cows (seronegative and seropositive cows) vaccination plans in the herds are necessitated (Fulton *et al*., 2004).

Because seronegative cattle play a role in international trade a number of European countries have eradicated BHV1 with very high costs involved. Marker and conventional vaccines can prevent disease but not infection followed by the state of latency (Straub, 2001).

CONCLUSION

The results of this study highlight that farmers should take into account the immune status of cattle related to BHV-1 before introducing it to the herd. Importing countries should consider only vaccinated animals for import. It should be required that the animals are seronegative prior to vaccination.

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