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Prevalence of serum antibodies to bovine herpesvirus-1 in cattle in Galicia (NW Spain)

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Abstract

Infectious bovine rhinotracheitis (IBR) is a highly infectious disease of cattle caused by bovine herpesvirus 1 (BHV-1) that causes considerable economic losses to the dairy and beef industry. This report describes the results of two surveys in which prevalence of serum antibodies against BHV-1 in Galicia (NW Spain) was determined. The first was performed on the entire cattle population of the region (year 2000) and the second on the herds included in a voluntary livestock health programme, which comprises IBR, conducted by a group of farms designated ADSG (Health Defence Groups) in this same region (year 2004). Anti-BHV-1 antibodies were determined by anti-gB ELISA in serum samples from all animals aged over one year in the area's dairy and beef herds. Mean individual animal and herd prevalence recorded in 2000 were 38.4% (ranging 37.3%-39.5%; 43.2% dairy, 26.8% beef) and 50.4% (ranging 46.4%-54.4%; 58.3% dairy, 48.1% beef), respectively. Mean true prevalence by herd and animal for the ADSG farms were 47.2% (44.9%-49.5%; 51.5% dairy, 45.2% beef, 42.3% mixed) and 35.7% (35.3%-36.1%; 37.8% dairy, 33.1% beef, 25.5% mixed), respectively. However, the use of non-maker vaccines make difficult to develop a control and eradication program based on serological analysis.

Additional key words: BHV-1, IBR, seroprevalence.

Resumen

Prevalencia de anticuerpos séricos frente al herpesvirus bovino tipo 1 en el ganado vacuno de Galicia (NO España)

La rinotraqueitis infecciosa bovina (IBR) es una enfermedad infecciosa del ganado producida por el herpesvirus bovino tipo 1 (BHV-1) que causa considerables pérdidas económicas en la industria láctea y cárnica. Este artículo describe los resultados de dos estudios de prevalencia en los que se determina la presencia de anticuerpos frente al BHV-1 en Galicia (NO de España). El primero fue realizado sobre toda la población bovina de la región (año 2000) y el segundo sobre los rebaños pertenecientes a las asociaciones de defensa sanitaria ganaderas (ADSG) en la misma región (año 2004), que realizan un programa de vigilancia que incluye la IBR. La presencia de anticuerpos se determinó con un ELISA anti-gB en las muestras de suero de todos los animales mayores de 1 año de los rebaños estudiados. Las prevalencias medias por animal y por rebaño halladas en 2000 fueron 38,4% (intervalo 37,3%-39,5%; 43,2% en leche y 26,8% en carne) y 50,4% (intervalo 46,4%-54,4%; 58,3% en leche y 48,1% en carne) respectivamente. La prevalencia real media de rebaño y animal para las granjas de las ADSG fue de 47,2% (44,9%-49,5%; 51,5% en leche, 45,2% en carne y 42,3% en mixtas) y 35,7% (35,3%-36,1%; 37,8% en leche, 33,1% en carne y 25,5% en mixtas) respectivamente. Sin embargo, el uso de vacunas no marcadoras dificulta el desarrollo de programas de control y erradicación basados en el análisis serológico.

Palabras clave adicionales: BHV-1, IBR, seroprevalencia.

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Abbreviations used: ADSG (Health Defence Groups), AP (apparent prevalence), BHV-1 (bovine herpesvirus-1), BVDV (bovine viral diarrhoea virus), CI (confidence intervals), HAP (apparent herd prevalence), HSe (herd sensitivity), HSp (herd specificity), HTP (true herd prevalence), IBR (infectious bovine rhinotracheitis), Pe (expected prevalence), sAb (specific antibodies), Se (sensitivity), Sp (specificity), TP (true prevalence).

Introduction

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease that affects cows worldwide, although the different countries show very different prevalence. Through eradication programmes, some European Union (EU) countries or regions have managed to reach the status of «IBR-free», and others are presently adopting control or eradication programmes. A third group of countries, including Spain, have no official specific IBR programme (Kramps *et al.*, 2004; Borchers, 2006; Müller, 2006). Given that IBR appears in the World Organization for Animal Health list of bovine diseases and that European countries vary greatly in their IBR status, restrictions are now being imposed to the free circulation of animals and their products among the countries of the EU.

Nowadays, there are two approaches in IBR control and eradication in Europe (EFSA, 2006). The first line, used in countries with an initial low prevalence (Denmark, Austria, Finland, Sweden, Switzerland and Norway) is a direct test-slaughter policy. The second line, implemented in countries with higher prevalence (Germany) began with use of a marker vaccine to first reduce gB seroprevalence and stop spreading of bovine herpes-virus-1 (BHV-1).

There are not data available that reflect the IBR status of Galicia, Spain's largest cattle population region. Therefore, in 2000 a seroprevalence study to know the infection status in this region was considered necessary.

After that, in Galicia in 2004, a voluntary sanitary program was initiated in herds through Health Defense Groups (ADSG in its Spanish Acronym). These herds are committed to carry out a sanitary program that includes the prohibition of conventional vaccines use and its substitution for gE deleted (marker) vaccines, as well as other sanitary measures and control programs [cattle trade control, disinfection, decontamination and bovine viral diarrhoea virus (BVDV), Johne's disease and neosporosis control].

The objective was to know the ADSG epidemiology BHV-1 situation with the aim of establishing the bases for the beginning of a control and eradication program.

Material and methods

Area description and herd selection

The northwest region of Spain, Galicia, is the country's largest bovine population region accounting for 16.8% of Spain's cattle, and milk and beef production quotas that are on the rise, according to data obtained in 2000 (25.7% milk and 6.0% beef production) and 2004 (35.0% milk and 12.0% beef production). Spain has no official IBR control programme and any control measures in Galicia are voluntary campaigns undertaken through the ADSGs, organisations set up in 2004. They grouped initially 4.6% of the herds and 13.0% of Galician bovine census. An annual increase in the number of the ADSG took place until 2008 when they included 28.9% of the total herds from Galicia and 36.0% of the census. Control program against BHV-1 in ADSG involves serological follow up of the farms, control of all purchased animals and the ban of non-marker vaccines.

In 2000, an initial survey of the IBR situation from Galicia's entire cattle industry (36,481 dairy and 38,241 beef cattle farms) was performed. The sample size was calculated based on a confidence level of 95%, a 5% error and an expected prevalence (Pe) of 50.0%. Given no previous epidemiological data for IBR in Galicia, it was used the figure of 50.0% as the Pe despite the need for a high sample size (Thrusfield, 1995). No mixed farms were included in this first survey since these are not representative of the whole population.

Serum samples were obtained from all animals older than 1 year in 375 dairy herds (6,038 animals) and 385 beef herds (2,690 animals). The mean herd size was 11.5 animals (16.1 for the dairy and 6.9 for the beef herds).

These herds were selected by stratified (dairy/beef) random sampling according to the contribution of each district to the total census for the region.

In the second survey performed in 2004 from farms of the same region in all herds belonging to any ADSG, serum samples were obtained from all animals over the age of 1 year in 1,147 dairy herds (38,120 animals), 1,464 beef herds (21,219 animals) and 141 mixed dairy/beef herds (2,504 animals). The mean herd size was 21.8 cows (33.2 dairy, 14.5 beef, 17.8 mixed). In this case, mixed farms were included, since the aim was trying to know the serologic profile in all the farms from ADSG.

To the 1,147 dairy herds analyzed in 2004, the vaccination history of 584 was known, data provided by the veterinarian in charge of the sanitary program for each ADSG.

Diagnostic technique

BHV-1 antibodies were determined using a commercial blocking ELISA test kit (Idexx HerdChek IBRgB) that detects specific antibodies (sAb) against gB

Table 1. Apparent (AP) and true (BHV-1) seroprevalence (TP) at the individual cow and herd level

Cattle	Year	Herd-level (%)		Animal-level (%)	
		AP	TP (CI)[a]	AP	TP (CI)[a]
Dairy	2000	64.5	58.3 (52.6-64.0)	42.4	43.2 (41.9-44.5)
	2004	65.3	51.5 (47.7-55.3)	36.9	37.8 (37.3-38.3)
Beef	2000	47.3	48.1 (42.2-54.0)	26.5	26.8 (25.0-28.6)
	2004	52.7	45.2 (42.2-48.2)	32.4	33.1 (32.4-33.8)
Mixed	2004	51.8	42.3 (32.4-52.2)	25.3	25.5 (23.7-27.3)
Total	2000	55.8	50.4 (46.4-54.4)	37.5	38.4 (37.3-39.5)
	2004	57.9	47.2 (44.9-49.5)	34.9	35.7 (35.3-36.1)

[a] CI: confidence intervals.

glycoprotein. This test detects serum antibodies both from vaccine and real infection. The sensitivity (Se) of the method is 95.4% and its specificity (Sp) is 99.0% (Kramps *et al.,* 2004). The controls and cutoff values indicated by the manufacturer were used to score each serum sample as negative (< 45% inhibition) or positive (≥ 45% inhibition).

Statistical analysis

χ^2-tests (packages SPSS 11.5 and Grahpad Instat) were used to compare prevalences obtained by category (dairy/beef), herd size, age of the animals and seroprevalence level according to intervals proposed by Pritchard (2001): prevalence 0%, 0-5%, 5-20%, 20-60% and > 60%). Results were considered significant when $p \leq 0.05$. True prevalences (TP) at the individual-animal level were calculated as a function of Se, Sp and the apparent prevalence (AP): TP = (AP + Sp – 1)/ (Se + Sp – 1) (Noordhuizen *et al*., 1997). Likewise, true prevalence at the herd level (HTP) was calculated as a function of herd sensitivity (HSe), herd specificity (HSp) and apparent herdlevel prevalence (HAP): HTP = (HAP + HSp – 1)/ (HSe + HSp 1). HSe and HSp values were calculated as per Martin *et al.* (1992). Confidence intervals (CI) were established using the equation described by Greiner and Gardner (2000). Thus TP was used only as statistical term, based on Se and Sp of the serological test.

Herds were declared positive if one or more animals tested positive.

Results

Apparent and true prevalences are presented in Table 1. The true prevalence were significantly higher for both dairy herds and individual cows in dairy farms compared to the beef cattle in the studies conducted in 2001 and 2004 (Table 1).

The vaccination status from 584 dairy herds in 2004 was known: 122 (20.9%) used vaccines while 462 (79.1%) did not vaccinate. It could be observed that 96.7% of the vaccinated herds were in groups of higher prevalence (> 20%) whereas 80.7% of the non vaccinated were in groups of prevalence ≤ 20% (Table 2).

When the herds were stratified by size, prevalence for both dairy and beef herds in both surveys increased significantly as the herd size increased ($p < 0.0001$ for both categories and studies) (Table 3).

As the age of the animals increased, a significant increase in the percentage of seropositive animals for BHV-1 ($p < 0.0001$) was recorded. In addition, during the study conducted in 2004, significantly lower seropositivity rates were obtained for the animals 12-24 months ($p < 0.0001$) and 25-36 months ($p < 0.0001$) of age than observed in 2000, yet this did not occur in the group of animals aged > 36 months (Table 4).

Separate analysis of the farms that have seropositive animals from 12 to 24 months of age indicate that seroprevalence in this group is lower in 2004 (22.5%) than in 2000 (26.6%) (Table 5).

Table 2. Prevalences distribution of dairy herds according with their vaccination status in 2004

Prevalence	Vaccinated	Non vaccinated
< 5%	(2/284) 0.7%	(282/284) 99.3%
5-20%	(2/93) 2.2%	(91/93) 97.8%
20-60%	(10/67) 14.9%	(57/67) 85.1%
> 60%	(108/140) 77.1%	(32/140) 22.9%
Total	(122/584) 20.9%	(462/584) 79.1%

Table 3. Individual-animal level BHV-1 seroprevalences (in percentages) stratified by sizes

Herd size (head)[a]	Dairy		Beef		Mixed	Total	
	2000	2004	2000	2004	2004	2000	2004
1-9	45.0	40.7	38.4	32.0	27.5	40.7	32.8
10-17	70.2	53.1	73.3	57.0	67.6	71.5	56.2
18-29	76.1	63.3	70.0	77.5	55.6	74.6	69.9
>29	92.3	76.6	100.0	89.2	73.1	93.1	78.7

[a] Four categories based on the quartiles of herd size.

Table 4. Individual-animal level IBR seroprevalences (in percentages) by age group

Age (months)[a]	Dairy		Beef		Total	
	2000	2004	2000	2004	2000	2004
12-24	29.2	21.1	15.4	19.6	25.8	20.8
25-36	40.7	30.6	16.5	25.3	35.1	29.3
>36	43.4	42.3	27.9	33.6	38.3	38.9

[a] Three age groups were established; seropositives younger than 24 months were interpreted as recent infections.

Percentages of herds belonging to different prevalence status showed that 44.2% and 42.1% of the farms in 2000 and 2004 respectively were BHV-1 free from serological point of view (Table 6).

Discussion

Previous studies performed in Spain are poorly representative of the real situation and are limited to serological screening of small sample sizes or surveys on small populations. Investigations on dairy herds have established IBR prevalence in the range 17.3-54.0% (Espuña *et al.,* 1988; Prieto, 1991; Guitián, 2001).

The IBR status of countries in the european economic area is highly variable. Studies established herd prevalence for Europe of 10-80%. After several decades of eradication programmes, Finland, Norway, Sweden, Austria, Denmark, Switzerland and the Bolzano region of Italy, have been declared IBR-free by the OIE since 2003 (Borchers *et al.,* 2006).

Table 5. Percentage and number of farms with seropositive animals younger than 24 months in both studies and aptitudes

Cattle	2000 (Galicia)	2004 (ADSG)[a]
Dairy	(70/232) 30.2%	(260/1040) 25.0%
Beef	(37/171) 21.6%	(180/916) 19.7%
Total	(107/403) 26.6%	(440/1956) 22.5%

[a] ADSG: Health Defence Groups.

The prevalence obtained in this study for Galicia region is similar or slightly lower to that observed in the European countries that have not tried to control IBR. These prevalences include 47.2% of beef cattle in Portugal (Soares *et al.,* 2006), 61.0% of the unvaccinated dairy herds of Italy (Cavinari, 2006), and 84.0% of the dairy herds, 53.0% of the beef herds, 89.0% of the mixed herds and 35.0%, 31.0% and 43.0% of the dairy cows, beef cows and mixed dairy/beef animals of Belgium (Boelaert *et al.,* 2000). The same can be said of the prevalences recorded at the start of eradication programmes such as the 50.0% for Germany (Teuffer, 2006) or 80% for Hungary (Pálfi and Foldi, 2006).

The ELISA used cannot distinguish between sAb induced by the conventional vaccine and those raised against the field virus such that the seroprevalences obtained are higher than true infection values (Nardelli *et al.,* 1999). Thus, an animal found seropositive for BHV-1 may have been vaccinated or naturally infected at some point, since the virus remains latent throughout its lifetime and may be reactivated by factors causing stress or immunosuppression (calving, corticosteroids, overcrowding, transport, other illnesses, etc.) (Álvarez *et al.,* 2002; Ackermann and Engels, 2006; Muylkens *et al.,* 2007).

Table 6. Herd BHV-1 seroprevalence (in percentages) by herd prevalence status

Prevalence group	Dairy		Beef		Mixed	Total	
	2000	2004	2000	2004	2004	2000	2004
0%	35.5	34.7	52.7	47.3	48.2	44.2	42.1
< 5%	3.5	9.5	0.5	3.8	7.1	2.0	6.3
5-20%	16.8	15.3	13.2	17.3	17.7	15.0	16.5
20-60%	17.6	12.4	18.7	11.8	11.3	18.2	12.0
> 60%	26.7	28.1	14.8	19.9	15.6	20.7	23.1

The known vaccination status of the 584 dairy farms included in ADSG study meant that could determine that the seroprevalence increased as the proportion of vaccinated herds increased, supporting the idea that the true prevalence of IBR in Galicia is lower than that detected. Then, the success of a BHV-1 control program depends on sttoping indiscriminate vaccination with non marker vaccines that allows differenciate between infection and vaccine antibodies. In fact, non marker vaccines are nowadays biding in farms belonging to ADSG in Galicia.

To this respect, a study carried out on foetus analyzed in the Laboratory of Animal Health of Galicia during the period from January to October 1999 (132 foetus from dairy farms) conclude that there was a positive direct immunofluorescence result for BHV-1 in 6.8% of the cattle abortions investigated. However, isolation of BHV-1 was not possible and there was always another viral or bacterial agent involved (Mora *et al.*, 2000).

The higher prevalence observed for the dairy farms in the present study could be attributable to the fact that a farm was classified as positive for IBR when a single animal was scored as seropositive for the BVH-1 virus. Given the significantly larger sizes of the dairy herds, it was therefore more likely that an individual animal would be positive for the virus (McDermott *et al.,* 1997; Nardelli *et al.,* 1999). Additionally, the larger dairy herds were subjected to more extensive vaccination programmes, which lead to over-reporting of seroprevalence figures since the ELISA used in both studies fails to discriminate between the vaccine and field virus (Nardelli *et al.,* 1999).

Individual-animal prevalences were also higher for the dairy compared to the beef herds for several reasons. Intensive management and a higher density of dairy cows (compared to the extensive or semi-extensive management of beef or mixed cattle) promote viral spreading and increase the chances that healthy susceptible animals will come into contact with infected animals (Guitián *et al.,* 1998; Muylkens *et al.,* 2007). Further, in larger herds there is usually more movement of animals (purchasing of replacement animals, etc.) increasing the risk of infection (Solis-Calderón *et al.,* 2003), but also being more likely to have been vaccinated. Our findings confirm the fact that herd size is a risk factor for IBR seroprevalence (van Wuijckhuise *et al.,* 1998; Boelaert *et al.,* 2005), but we cannot rule out it is a confounding effect of vaccination.

Findings also indicate an increased proportion of seropositive animals with increasing age. The older the animal, the more likely it is that it will have been vaccinated or exposed to a natural infection source (Nardelli *et al.,* 1999; Solis-Calderón *et al.,* 2003). Antibodies against natural infection start to appear 8-10 days post-infection and persist throughout the animal's life (Álvarez *et al.,* 2000; Muylkens *et al.,* 2007). Vaccine-derived antibodies persist for several years (Van der Poel *et al.,* 1995; Nardelli *et al.,* 1999).

Once obtained a serologic profile of the herds of the ADSG, seronegative farms could be identified (42.1%). On these, periodic controls in bulk tank milk and/or the younger animal rearing group were done to detect new infections.

The lower percentage of farms with seropositive young heifers (12-24 months) in 2004 comparing to 2000 showed that measures established in the ADSG seem to be efficient to get future long term eradication of BHV-1.

Herds whose prevalences were < 5% could in the short term become seronegatives by means of culling seropositive animals and adequate biosecurity measures to avoid new infections. Herds with higher prevalences could besides include vaccination with gE deleted vaccines so that in a determined period (according to prevalence) they diminished the presence of infection antibodies (Solís-Calderón *et al.,* 2003; Franken, 2006).

In conclusion the herds belonging to ADSG constitute a suitable population for the beginning of IBR control program, as much by their epidemiologic situation as by the fact to be grouped in a defined geographic area, that carry out a common sanitary program. To develop a control program based on serological analysis biding of non-maker vaccines would be necessary.

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