Seroprevalence and risk factors associated with bovine herpesvirus type 1 (BHV1) infection in non-vaccinated cattle herds in Andalusia (South of Spain)

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Abstract

An epidemiological and serological survey of bovine herpesvirus 1 (BHV1) infection was conducted in Andalusia from January to April of 2000. A total of 4,035 blood samples were collected from 164 herds. A questionnaire, which included variables potentially associated with infection, was filled out for each herd. Serum samples were obtained to identify specific BHV1 antibodies and were tested using a blocking ELISA test. The observed crude odds ratio (OR) (estimate of the chance of a particular event occurring in an exposed group in relation to its rate of occurrence in a nonexposed group) for vaccination is 9.8 (95 % confidence interval: 8.3-11.7). The vaccinated group comprised large dairy farms. This study can only be considered as representative of unvaccinated, small to medium size dairy farms and beef farms in Andalusia. True seroprevalence of the BHV1 virus in nonvaccinated bovine populations in Andalusia reached 45.7% of individuals and 70.4% of herds. Risk factors for BHV1 infection in bovine Andalusian nonvaccinated herds are nonexistence of specific cattle infrastructure (OR: 3.07), beef crossbreeding (OR: 7.90), affiliation with Livestock Health Defence Associations (OR: 2.57), a history of reproductive disorders (OR: 8.39), external replacement (OR: 2.74), proximity to an urban area (OR: 6.11) and herd size (41.98). To control for confounding effects, a binomial logistic regression model was developed. From this regression, BHV1 infections are concentrated in large herds, with external replacement, located close to urban areas. This is the first published report on BHV1 prevalence in the South of Spain.

Additional key words: dispersion, infectious bovine rhinotracheitis, logistic regression, multivariate model.

Resumen

Seroprevalencia y factores de riesgo asociados a la infección por el Herpesvirus Bovino tipo 1 (BHV1) en rebaños bovinos no vacunados de Andalucía

Desde enero a abril de 2000 se ha realizado un estudio epidemiológico y serológico sobre la infección por el herpesvirus bovino tipo 1 (HVB1) en Andalucía, donde se tomaron un total de 4035 muestras de sangre procedentes de 164 rebaños, donde además se cumplimentó un cuestionario que incluye variables potencialmente asociadas a la infección. Mediante un kit ELISA de bloqueo se evaluó la presencia de anticuerpos frente al HVB1. El valor de odds ratio (OR) (probabilidad que tiene un suceso de ocurrir en un grupo expuesto a un factor en relación a la probabilidad en un grupo no expuesto) cruda para la vacunación frente al HVB1 es de 9,8 (IC 95%: 8,3-11,7). Al haberse vacunado las granjas grandes, que fueron eliminadas, el estudio es representativo sólo de granjas de pequeño y mediano tamaño. La prevalencia real en animales no vacunados es del 45,7%, mientras que la dispersión es del 70,4%. Los factores de riesgo detectados son: inexistencia de infraestructuras específicas (OR: 3,07), animales de razas no puras (OR: 7,90), pertenencia a asociaciones de defensa sanitaria (OR: 2,57), historial de trastornos reproductivos (OR: 8,39), reemplazo externo (OR: 2,74), proximidad a zonas urbanas (OR: 6,11) y tamaño del rebaño (41,98). Para limitar el impacto de variables confusoras, se generó un modelo mediante regresión logística, que señala que las infecciones se concentran en grandes rebaños con reemplazo externo en las proximidades de áreas urbanas. Este es el primer trabajo acerca de la prevalencia del HVB1 en el sur de España.

Palabras clave adicionales: dispersión, modelo multivariante, regresión logística, rinotraqueítis infecciosa bovina.

Introduction

Bovine herpesvirus-1 (BHV1) is an Alphaherpesvirus belonging to the family *Herpesviridae*. It is responsible for infectious bovine rhinotracheitis (IBR) and infectious bovine vulvovagenitis. Following the clinical disease, the virus becomes latent in the host.

Clinical outbreaks and seroprevalence surveys confirm a worldwide distribution of BHV1 infection. Some countries such as Norway, Sweden, Switzerland, Denmark and Austria have a free status due to eradication programs against this infection. On the other hand, herd prevalence is higher than 50% in other European countries (Tekes *et al*., 1999; Boelaert *et al*., 2000).

Several authors have identified a variety of risk factors associated with infection by the BHV1 (van Schaik *et al*., 1998; Boelaert *et al*., 2005). The main risk factors are age, vaccination, herd size, production type (dairy, beef) and animal import.

In Spain, a few IBR control programmes have started and it is only a matter of time before the beginning of an official national programme. The first step in an eradication program would be vaccination with gE-deleted vaccines to reduce the disease prevalence. Once this objective is reached all positive animals should be removed. This study focuses on detecting IBR seroprevalence distribution and identification of the major risk factors associated with seroprevalence in the Andalusia region of Spain as a first step before development of an eradication program.

Material and methods

In 1999, the cattle population of Andalusia was about 500,000 in 10,000 herds (official data). A previous survey, in 1998, by the authors, of southwest Andalusia was used to calculate sample size. In that survey the individual seroprevalence to IBR in 968 unvaccinated animals was 53.01% (95%: CI: 44.28-61.92), while herd prevalence was about 70% (95% CI: 62-78%).

Sample size was calculated for herd prevalence. The free software Survey Toolbox® (Cameron and Baldock, 1998a,b) was used. Herd and animal selection was randomly taken from the entire bovine population of Andalucía. Using a confidence level of 95%, 70% of herd prevalence and 7% assumed error 164 herds were selected. Twenty five blood samples were collected from every herd. This number can probe the herd status (positive or negative) with an intra-herd prevalence of 12% or higher. Intra herd prevalence found for BHV1 is around 30-35% (Boelaert *et al*., 2000). Sterile, vacuum, 7 ml tubes (Vacutainer®) were used for blood extraction. Tubes were identified, anti-crash packed and sent to the laboratory in isotherm containers. Blood samples were centrifuged (15 min, at 2,500 rpm at 4ºC) and transferred to 2.5 mL Eppendorf polystyrene tubes. Samples were then lyophilised and stored at 4ºC until serological testing. Sampling started in January 2000 and concluded in April 2000.

A questionnaire was completed for each farm by means of a personal interview with the owner. It included variables about production characteristics (farming system (extensive/intensive); breeding on the same farm; beef crossbreeding; dairy, beef or mixed production; open/close breeding facilities; herd size); contact with other animal species (coexistence of sheep, goats or pigs; use of common pastures; external replacement; participation in markets and exhibitions), animal health data (respiratory or reproductive disorders (disease occurrence), membership of a herd defence association (ADSG: farmers' associations set up to develop common animal health programmes at a local level) and area data (province, urban area, farm density).

In Andalucía, use of marker vaccines is exceptional, so antibodies produced by natural infection or by vaccination with conventional vaccines is indistinguishable. Vaccination with IBR was used in 54 out of 164 herds. The use of IBR vaccines in different types of herds showed that 8.5% of beef herds, 44.1% of dairy herds and 64.7% of mixed herds were vaccinated.

Vaccination blurred BHV1 infection status and is a major confounding factor. To eliminate vaccination confounding effect, all results refer to the unvaccinated cattle population (2,393 animals from 110 herds). With this size sample, expected error at the herd level is 8.56%. The number of animals from the herd (median around 21 animals) remains significant.

For BHV1 antibody detection, a blocking ELISA test (INGEZIM IBR compact®, INGENASA S.A., Madrid, Spain) was used. This blocking-ELISA is based on the use of a monoclonal antibody to gB of BHV1 and it has

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Abbreviations used: ADSG (asociaciones de defensa sanitaria ganaderas; livestock health defence associations), BHV1 (Bovine herpesvirus-1), CI (confidence interval), IBR (infectious bovine rhinotracheitis), OR (odds ratio).

a sensitivity and specificity of 99% (as claimed by INGENASA). According to the manufacturer, all samples with a blocking percentage higher than 30% should be considered as positive.

True prevalence was calculated from apparent prevalence using the sensibility and specificity values. A Pearson χ^2 test of contingency tables was carried out to identify significant associations between seroprevalence (dependent variable) and variables in the questionnaire (independent variables). To assess strength of the associations, odds ratio (OR) with a CI at 95% confidence level and non-parametric tests Phi (dichotomous variables) and V of Cramer (non-dichotomous) at 95% of confidence level were performed. All variables with associated *p*-values lower than 0.05 were considered significant and included in the binomial logistic regression model.

A *"step-forward"* binomial logistic regression model was run, at herd level, with variables associated in the bivariate analysis (input value 0.05, output value 0.10, 95% confidence). Wald's statistic was used for variable selection. Previously, colinearity was checked and variables removed. Mode "indicator" was chosen, so the reference value in the other category was always 1. Coefficients were estimated and OR values corrected.

Results

Individual and herd seroprevalence

Individual seroprevalence in the 2,393 unvaccinated animals reached 45.8% (true prevalence 45.71%). A herd was considered "positive" if at least one individual serum from an unvaccinated herd had specific antibodies to BHV1. Herd prevalence was 70% (true prevalence 70.41%).

Bivariate analysis

Table 1 shows variables that were significantly associated with BHV1 infection in the bivariate analysis. Farming system (intensive/extensive), breeding (yes/no), beef crossbreeding (yes/no), production type (dairy, beef, mixed), standard breed (local/non local), type of facilities (open field/other) were evaluated looking for associations and only "open field production" and "beef crossbreeding" were associated with seroprevalence in the unvaccinated herds. Detailed results for these variables are shown below.

Infrastructure: Seroprevalence was 58.9% of herds with specific facilities (indoor systems), while the percentage was 81.5% in open field herds. There was a significant association between infection and the open field system (χ^2: 6.628; *p*: 0.018) and OR: 3.07 (CI 95%: 1.29 - 7.29).

Beef cross-breeding: There was a significant association between cross-breeding (between local races and Limousine or Charolais) for beef production and infection (χ^2: 11.201; *p*<0.001). The OR value was around 8 (OR: 7.9; CI 95%: 2.2-28.1).

Contact with other herds or species: The following variables were analysed: existence of sheep, goats or pigs, origin of replacement stock, origin of fattening calves and participating in markets or common pastures. Only external replacement revealed a significant association (OR: 2.74; CI 95%: 1.2-6.4).

Table 1. Herd prevalence of BHV1 infection (%). Bivariate analysis

		Prevalence	χ^2 (p-value)	Phi Coefficient	OR[1]	95% CI[2] of OR
Open field		81.5	6.628 (0.018)	0.246	3.07	1.29-7.29
Crossbreeding		91.9	11.201 (0.001)	0.340	7.91	2.22-28.13
External replacement		79.9	4.707 (0.030)	0.227	2.74	1.18-6.38
ADSG[3]		75.0	4.231 (0.040)	0.196	2.57	1.03-6.42
Reproductive disorders		94.1	4.294 (0.044)	0.196	8.34	1.06-66.19
Urban area		84.1	15.672 (0.001)	0.398	6.11	2.51-14.87
Herd size *(quartiles)*	1-19	14.8	57.386 (<0.001)	0.722	41.98[4]	12.02-146.60
	20-49	71.4				
	50-99	94.4				
	≥100	96.3				

[1] OR: odds ratio. [2] CI: confidence interval. [3] ADSG: Livestock Health Defence Association. [4] When 1st quartile is tested in contrast to others.

Coexistence of sheep, goats or pigs: the differences between with and without another species were not significant.

Common pastures: There was no statistical significance between groups.

Participation in markets and exhibitions: No significant differences were detected.

Membership of Livestock Health Defence Association (ADSG): ADSG are farmer' associations set up to develop common animal health programmes at the local level. Seropositivity was found in 75.0% of ADSG herds *vs.* 53.8% in herds where farmers did not belong to an ADSG. The difference was significant (OR: 2.57; CI 95%: 1.1-6.4)

A history of respiratory and reproductive disorders: 90.9% and 94.1% of unvaccinated herds that had recently suffered respiratory or reproductive disorders were seropositive, compared with 67.7% and 65.6% for the group with no history of such disorders. There was only a significant association with reproductive disorders (OR: 8.39; CI 95%: 1.1-66.2)

Province: There was a wide range of variation among provinces (sub-region) in the number of positive herds: 83.3% in Almería; 76.9% in Cádiz; 78.6% in Córdoba; 69.2% in Granada; 50.0% in Huelva; 71.4% in Jaén; 61.1% in Málaga; 72.7% in Seville. Although differences were statistically significant (χ^2: 3.972; $p<0.001$) the sampling was not designed to estimating province prevalence, so these values are only included for descriptive purposes.

Urban area: Rural farms were 46.3% positive whereas farms located near urban areas were 84.1% (χ^2: 15.672; $p<0.001$). This variable appears to be important (OR: 6.1; CI 95%: 2.5-14.8).

Farm density: There were no significant differences.

Herd size: Among unvaccinated herds, positive farms had a mean herd size of 87.4 animals in contrast with 24.6 animals on negative farms ($p<0.001$). Further, there was a strong association (χ^2: 57.39; $p<0.001$) when herd size was categorised as *"small"* (herds up to

19 animals) were 14.8% positive; *"medium-size"* (20-49 head) were 71.4%; *"large"* (50-99 head) 96.3%; and *"very large"* ($\geq$ 100 head) were 96.3%.

Predictive model

Coefficients of the final model and the OR values through the steps are given in Table 2. The best-fit as obtained with three steps and could correctly classify 94.5% of farms according to the results. External replacement (OR 116.77), proximity to an urban area (OR 7.58) and herd size (14.57) were included in the model as major factors associated with BHV1 infection in Andalusia.

Discussion

Vaccination was the main confounding factor in BHV1 seroprevalence because only conventional (non-deleted) vaccines are used in Andalusia. The OR of herd vaccination was 1.43. This could be incorrectly considered as a low value because prevalence in the vaccinated group (100%) limits the OR maximum value. When the survey was designed, it was supposed that most of farms were using gE-deleted vaccines, and it would be possible to differentiate antibodies arising from vaccines, so it was decided to include vaccinated animals. During the study, it was noted that only cheaper, conventional vaccines were used, because vaccination was detected mainly on farms with a history of clinical disorders (respiratory or reproductive) to reduce economic losses without considering potential eradication. This is why vaccinated animals were initially included in the survey.

Of the seven variables, which were significantly associated to infection in the bivariate analysis, only three continued in the multivariate model. Relationships between membership of an ADSG, open fields and large

Table 2. Binomial logistic regression model for herd seropositivity in the last step (step 3). Coefficients and odds ratio (OR) estimates of variables in equation

Variable	Wald	df [1]	p	OR	CI 95.0% for OR[2]	
External replacement	20.6	1	0.000	116.78	14.94	912.33
Urban	4.7	1	0.030	7.58	1.21	47.24
Large herd	8.28	1	0.004	14.57	2.35	90.39
Constant	15.44	1	0.000	0.01		

[1] df: degrees of freedom. [2] CI confidence interval: lower limit (left column) and higher limit (right column).

herds were detected. Consequently, in the final model, the two first variables were removed.

Many authors have described the role of closed systems to prevent external infections (van Schaik *et al.*, 1998; van Schaik *et al.*, 2002; Vonk Noordegraaf *et al.*, 2004; Boelaert *et al.*, 2005), so incorporation of the variable "External replacement" as a risk factor seems to be logical and agrees with these authors.

The mean density of beef cattle farms in Andalusia is low compared with other regions or countries (<0.1 herds km^2). Only farms near urban areas are relatively close to each other and can permit airborne virus transmission (Belleti and Cordioli, 1995; Mars *et al.*, 2000). Therefore, there is only a slight association between BHV1 infection and farm density in this study. From another perspective, an urban area is an indirect parameter that means density because it reflects farm nuclei where density is relatively high but not high enough to change the density/km^2 of the municipality. In this study, there was a strong association between urban area proximity and BHV1 infection.

Finally, most researchers agree with the result than bigger herd size is an important risk factor for infection with BHV1 (Mazzucchelli, 1995; McGowan and Murray, 1998; Nardelli *et al.*, 1999; Solis-Calderon *et al.*, 2003; Boelaert *et al.*, 2005).

Summarizing, conventional (non-deleted) BHV1 vaccines are commonly used in Andalusia. Therefore, vaccination was identified as the principal confounding factor in estimation of BHV1 seroprevalence. After vaccination was controlled for individual and herd seropositivity to BHV1 infection in Andalusia reached 45.71% and 70.41%, respectively.

A binomial logistic regression model was used to improve estimations of risk factors. Three variables (external replacement, herd size and proximity to an urban area) were conserved in the best fit model.

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