Bayesian estimation of herd-level prevalence and risk factors associated with BoHV-1 infection in cattle herds in the State of Paraíba, Brazil

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ABSTRACT

A cross-sectional study was carried out to estimate the animal- and herd-level prevalence of bovine herpesvirus 1 (BoHV-1) infection in cattle in the State of Paraíba, and to identify risk factors associated with herd-level infection. The state was divided into three sampling strata, and for each stratum, the prevalence of herds infected with BoHV-1 was estimated through a two-stage sampling survey carried out from September 2012 to January 2013. In total, 2443 animals were sampled from 478 herds. A virus-neutralization test was used for BoHV-1 antibody detection. A Bayesian latent-class model was used to describe the data, taking into account imperfect diagnostic test characteristics and the non-independence of test results from animals within the same herd, and using a dynamic within-model risk factor selection method based on indicator variable selection. The adjusted herd-level prevalence was estimated to be 84% (95% CI: 80–88%) for the State of Paraíba, and the animal-level prevalence was estimated to be 73% (95% CI: 66–84%). Only five of the available risk factors were used by the model, with the three most influential being disposal of aborted foetuses (3.78, 95% CI: 1.11–13.85), sharing resources with other farms (3.0, 95% CI: 1.1–8,6), and a herd size of > 23 animals (2.5, 95% CI: 1.1–6.0). Our findings suggest that the animal- and herd-level seroprevalence of BoHV-1 infection in the State of Paraíba is high. While some risk factors such as herd size and sharing resources were identified as risk factors for BoHV-1 infection, these risk factors are initially likely to be of only minor relevance in a control programme due to the extremely high prevalence of infected farms. However, the results are relevant to the risk of reintroduction of disease on farms that have previously eradicated the disease.

1. Introduction

Bovine herpesvirus 1 (BoHV-1), an alphaherpesvirus of the Herpesviridae family, is an important pathogen of cattle. It is associated with respiratory disease and vulvovaginitis/balano-posthitis also known as infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/balanoposthitis, (IPV/IPB), respectively, as well as reproductive failures such as embryonic death and abortion, and reduction in milk production (Graham, 2013; Elhassan et al., 2015). BoHV-1 can result in severe economic losses directly through the clinical impact such as abortion, infertility, mastitis, conjunctivitis and encephalitis, and indirectly through restrictions on international trade in animals and animal products of the cattle industry (Biswas et al., 2013; Raaperi et al., 2014).

As with all herpesviruses, BoHV-1 is able to establish latent infections that can remain inactive in the sensory neurons. Viral reactivation can occur after natural or corticosteroid-induced stress (Jones et al., 2011; Kook et al., 2015). Once infected, the animal becomes a latent carrier, ensuring the persistence and transmission of the virus within the cattle population (Jones, 2003). Upon reactivation, the virus initiates a new replication cycle, producing infectious particles with or without clinical signs and lesions in the replication sites. Reactivation from latency is responsible for the maintenance of the virus in the herd (Jones et al., 2011; Kook et al., 2015), but animals with latent infection typically do not show clinical signs. However, the acute infection produces antibodies that can be detected by serological tests, which are

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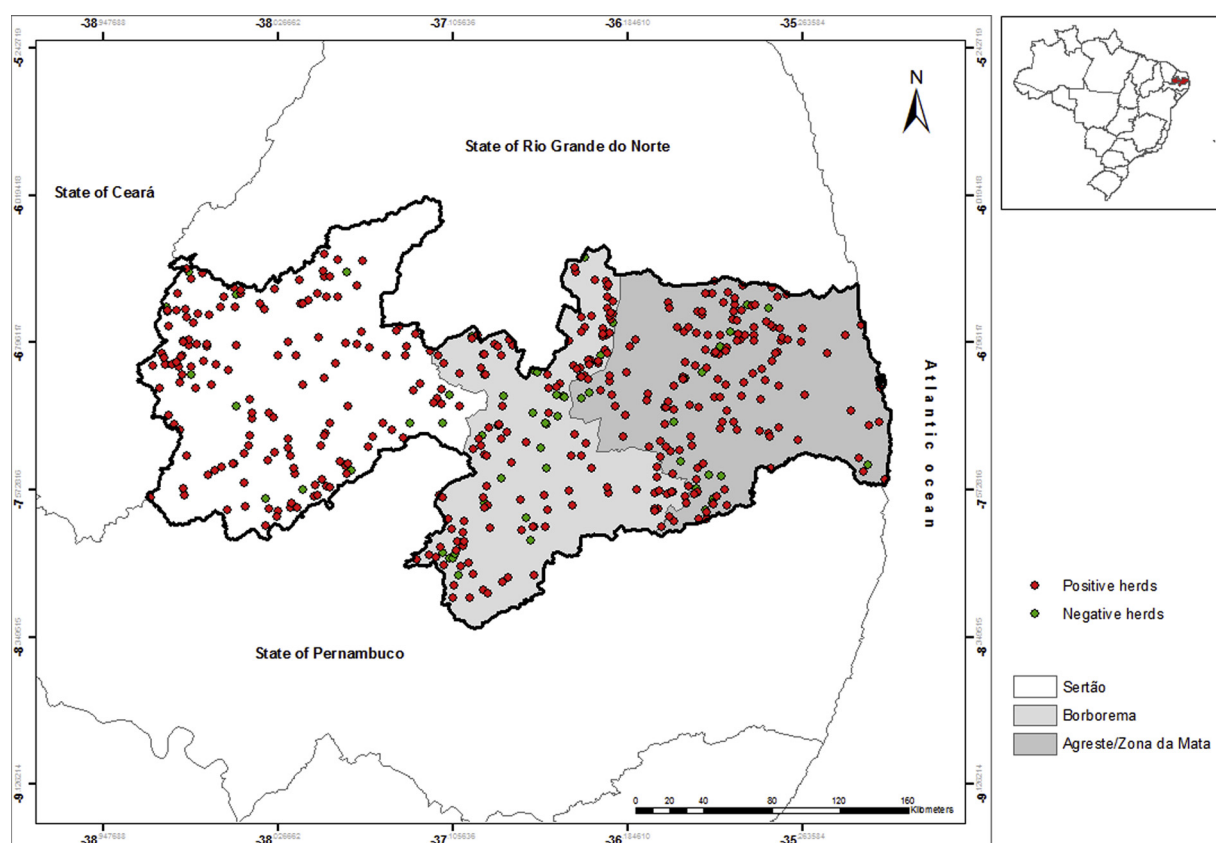*E-mail address:* sergio@vps.fmvz.usp.br (S.S. de Azevedo).

Fig. 1. Division of the State of Paraíba into three sampling groups. Detail shows the Paraíba State within Brazil.

useful for the identification and quantification of infected animals (Jones, 2003; Biswas et al., 2013).

The tests most commonly used and recommended by the World Organization for Animal Health (OIE) for the detection of antibodies are the virus neutralization test (VNT) and the enzyme-linked immunosorbent assay (ELISA) (OIE, 2010). VNT is considered the reference standard of BoHV-1 infection, with a reported sensitivity of 93% to 96%, and a relatively high specificity of 96% (Kramps et al., 2004; Cárdenas et al., 2006). In Brazil, most epidemiological surveys of BoHV-1 use VNT as the standard serological test, with animal-level prevalence estimates ranging from 46.6% to 84.5% (Rocha et al., 2001; Vieira et al., 2003; Barbosa et al., 2005; Thompson et al., 2006; Affonso et al., 2010; Bezerra et al., 2012) suggesting that the virus is widespread in the bovine population of the country. However, the majority of these serological surveys were carried out in restricted geographical regions, or were based on a limited number of samples. A serological survey conducted in the State of Paraíba during 2006 showed that 46.6% of the tested cattle had neutralizing antibodies against BoHV-1 (Thompson et al., 2006), however no risk factors could be identified from this study.

One of the difficulties involved with analysing these types of data is that the diagnostic test is imperfect, meaning that an animal may falsely test as positive even on a farm that does not have BoHV-1 infection. Furthermore, the probability of each animal testing positive within a herd is related to the common infection status of the farm, so the true status of the animals is not independent. In order to deal with both of these issues, it is possible to use a Bayesian latent class model to analyse the data according to this hierarchical structure. Bayesian methods have steadily been increasing in popularity in several fields, and have previously been used to estimate both prevalence and risk factors (Liapi et al., 2011; Koop et al., 2013; Sanogo et al., 2013; Angelidou et al., 2014; Paul et al., 2014; Wolf et al., 2014), as well as in the evaluation of diagnostic tests (Toft et al., 2007) and similar problems involving

unobserved latent class structures (Denwood et al., 2011). These methods allow the conditional probability of animals testing positive to depend on the unobserved farm positive status, and for the imperfect sensitivity and specificity of the diagnostic test to be taken into account in order to obtain unbiased risk factor and prevalence estimates.

The objective of this study was to estimate the animal- and herd-level prevalence of BoHV-1 infection in cattle in the State of Paraíba, and to identify the risk factors associated with occurrence of the pathogen within the herd. This will facilitate the future development of preventive and control measures and their application in an effective and efficient manner.

2. Materials and methods

2.1. Target population and characterization of the study area

The target population consisted of all cattle in the State of Paraíba, located in the Northeastern region of Brazil. The state is characterized by warm weather throughout the year. The state is geographically subdivided into the following four mesoregions (subdivisions of Brazilian states), based mostly on vegetation type and rainfall: (i) Zona da Mata, (ii) Agreste, (iii) Borborema, and (iv) Sertão. The Zona da Mata and Agreste have relatively higher rainfall regimes, predominating the humid tropical climate, with autumn-winter rains and dry season during the summer. On the coast, the mean annual rainfall is ≈ 1700 mm and temperatures average 24 °C. Formed by the Atlantic forest, the coastal vegetation consists of forests with the presence of evergreen tall trees, mangroves and savannas (Cabrera and Willink, 1973). Both Borborema and Sertão (the semiarid region) are typically within the Caatinga biome, which encompasses an area of 900,000 km² (11% of Brazilian territory) and is the only major biome that occurs exclusively in Brazil. Caatinga is xeric shrubland and thorn forest, which consists primarily of small, thorny trees that shed their leaves

seasonally. Cacti, thick-stemmed plants, thorny brush and arid-adapted grasses make up the ground layer; however, during the dry periods there is no ground foliage or undergrowth. The weather is characterized by a hot and semiarid climate, with temperatures averaging 27 °C, and the mean annual rainfall is typically ≈ 500 mm. There are typically two seasons: a rainy season from February to May, and a long drought period from June to January. However, occurrences of droughts sometimes lasting for longer than one year is also a characteristic of the region (Andrade-Lima, 1981).

2.2. Division of the State of Paraíba into stratified sampling groups

To compare the regional differences of epidemiological parameters of BoHV-1 infection, such as the different production systems, management practices, operating purposes and herd size, the State of Paraíba was divided into three sampling areas: sampling area 1 (mesoregion of Sertão), sampling area 2 (mesoregion of Borborema), and sampling area 3 (mesoregions of Zona da Mata and Agreste) (Fig. 1). The biome, the social and economic characteristics of the mesoregions and the operational capacity of the Agricultural and Livestock Defense Service of the State of Paraíba (SEDAP), based on the areas of action of its regional units in order to ensure that the agency could conduct the fieldwork, were considered for the stratification scheme proposed.

2.3. Study design and sampling

A two-stage sampling survey was carried out for each sampling area. First, a pre-established number of herds with reproductive activity (primary sampling units) were randomly selected; in the second stage, a pre-established number of cows aged ≥ 24 months were randomly selected (secondary sampling units).

In farms with more than one herd, the cattle herd of greater economic importance was chosen as the target of the study; the animals in the selected cattle herd were subject to the same type of management system as the other herds, i.e. had the same risk factors as the other herds. The selection of the primary sampling units was based on simple random sampling from the records of farms of the SEDAP. If a selected herd could not be visited, the herd was replaced by another in the vicinity with the same production characteristics. The number of selected herds per sampling area was determined by using the formula for simple random sampling to estimate a prevalence (Thrusfield, 2007). The assumptions used for the calculation were as follows: 95% confidence level, 50% estimated prevalence, and 8% error. Further, the operational and financial capacity of the SEDAP was taken into consideration when determining the sample size of the sampling area. The pre-established number was 150 herds for each sampling area.

For the secondary units, the minimum number of animals to be examined within each herd was estimated in order to allow its classification as an infected herd. For this purpose, the concept of herd sensitivity and specificity was used (Dohoo et al., 2003). For the calculations, the following values were adopted: sensitivity and specificity of the virus neutralization test of 94.4% and 96% (Kramps et al., 2004; Cárdenas et al., 2006), respectively, and 50% for the intra-herd estimated prevalence. Herdacc version 3 (University of Guelph) software was used during this process, and the sample size was selected so that the herd sensitivity and specificity values would be ≥ 90%. Therefore, 10 animals were sampled in herds with up to 99 cows aged over 24 months; 15 animals were sampled in herds with 100 or more cows aged over 24 months; and all animals were sampled in those with up to 10 cows aged over 24 months. The selection of the cows within the herds was systematic (Thrusfield, 2007), and only non-vaccinated females were sampled.

The field activities included blood collection, provision of an epidemiological questionnaire (see below), and sending the samples to the laboratory. The veterinarians and agricultural and livestock technicians from the SEDAP were involved in the fieldwork. Blood samples (10-mL

volume) were collected from September 2012 to January 2013, from cows aged ≥ 24 months by jugular vein puncture with a disposable needle and a 15-mL capacity vacuum tube (without anticoagulant). An 11-digit code was used for identification of the tubes, of which the first nine digits referred to the herd code and the final two digits to the number sequence of the sampled cow. Serum samples were frozen and transferred and stored in plastic microtubes.

2.4. Data collection

A structured questionnaire including closed-ended questions was designed to obtain information concerning (a) the identification and location of the herd; (b) management practices; (c) structure and composition of the herd; and (d) presence of other domestic and wildlife species in the farm. Questionnaires were administered to the owner or person in charge of the herd either by the primary author or by a veterinarian from the SEDAP at the same time of the visit to blood collection. The description of the questions included in the questionnaire can be found in the supplementary material.

Data obtained with the epidemiological questionnaires were screened for inclusion as risk factors within the model. Due to extremely strong correlation between some of the related variables in the raw dataset, a number of questions and categories from the original questionnaire were excluded or combined. The final candidate variables and respective categories were as follows: management system (extensive vs confined), milking (no vs yes), predominant breed (purebred/crossbreed), herd size categorised (greater than 23 vs 23 or fewer), sheep or goats present (yes vs no), horses present (yes vs no), pigs present (yes vs no), wildlife present (yes/no), disposal of aborted foetuses (bury/burn vs no/feed), animal purchasing (yes vs no), share resources (yes vs no), flooded pastures (yes vs no), maternity pens (yes vs no), and veterinary assistance (yes vs no).

2.5. Virus-neutralization

For the serological diagnosis of the BoHV-1 infection the virus-neutralization test (VNT) was used (OIE, 2010). The cytopathic viral strain BoHV-1 was used and supplied by the Virology Institute of the University of Veterinary Medicine of Hannover, Germany. In the screening, a sample was deemed positive when it presented a titer ≥ 2. The neutralizing antibodies titers were considered the reciprocal of the higher serum dilutions capable of inhibiting the viral replication and the consequent production of cytopathic effect (CE) of BoHV-1. The infectious titer used was $10^{6.25}$ DICT$_{50}$/50 µL, determined by the Reed and Müench method (1938).

2.6. Modelling methods

A latent-class model was constructed within a Bayesian framework to describe the observed cow-level data as a series of Bernoulli trials conditional on the unobserved farm infection status, within-herd prevalence of disease (for infected farms), and diagnostic test sensitivity and specificity. The unobserved farm infection status was modelled as a second Bernoulli trial, with probability given by a standard logistic regression model to estimate the farm-level risk factors for infection. The diagnostic test sensitivity and specificity were given informative Beta (33.5, 2.7) and Beta (78, 4) priors respectively, which were derived from the literature (Kramps et al., 2004) combined with expert opinion. These correspond to median (95% CI) estimates of 93% (82–99%) and 90% (90–99%) for sensitivity and specificity, respectively. The animal-level prevalence was assumed to take the same value for all infected farms in order to aid model identifiability, and was given a minimally informative Beta (1, 1) prior. The overall intercept for the logistic regression was given a minimally informative Normal (0, 1000) prior, but weakly informative Normal (0, 2) priors were used for the risk factor effects to restrict the effect sizes to within biologically

plausible ranges.

Inference from the model was made using Markov chain Monte Carlo (MCMC) sampling, implemented using JAGS (Plummer, 2003) called from within R (R Core Team, 2016) using the runjags package (Denwood, 2016). The model was run initially using a burnin period of 5000 iterations and sampling period of 50,000 iterations with two chains. Convergence was assessed visually and a sufficient sample size was ensured by examining the effective sample size of the auto-correlated Markov chains (Denwood, 2016; Toft et al., 2007).

Selection of risk factor variables was not possible using the deviance information criterion, because of the presence of the mixture component within the model (Denwood et al., 2011). Instead, the risk factors were selected directly within the JAGS model formulation using the method of Kuo and Mallick (1998), which involves a set of variable selection indicators with prior probabilities of 0.25. This allows risk factors with little support for effect sizes substantially different from zero to be dynamically 'turned off' by the model, leaving the model inference to be driven predominantly by risk factors that are supported by the data. For a general overview of this method of variable selection within Bayesian models, see O'Hara and Sillanpää (2009). The full JAGS code incorporating both the parameter estimation and variable selection elements within the same model is given in Appendix A, and the R code necessary to run this model is available from the authors on request.

An adjusted between-herd prevalence estimate for the State of Paraíba was calculated based on the estimated probabilities that each of the sampled farms was infected as obtained from the model described above. For each iteration, an estimate of each region-level prevalence was drawn from a Beta conjugate prior based on the infection states of each farm within the same region. These region-level prevalence estimates were then re-weighted by the total number of farms in that region to obtain an estimated state-level prevalence. This was repeated for 10,000 iterations in order to obtain mean and 95% confidence interval estimates.

3. Results

3.1. General features of the target and study population

The census data and the sample studied in each sampling area are shown in Table 1. Herd size ranged from 1 to 335 (median = 10; 3rd quartile = 23). In total 2443 animals (990 Sertão, 729 Borborema and 724 Agreste/Zona da Mata) from 478 herds (159 Sertão, 160 Borborema and 159 Agreste/Zona da Mata) were tested. The apparent seroprevalence of animals with antibodies against BoHV-1 in the state was 64.8% (n = 2443), with region specific test prevalence estimates as shown in Table 1. The overall apparent herd-level prevalence was 87.4%, with apparent animal-level prevalence estimates ranging from 16.1 to 100% (mean = 73.2%) depending on the herd. Table 2 presents the descriptive statistics with area-specific prevalence estimates of BoHV-1 test-positivity for each risk factor collected on the questionnaire.

3.2. General model results

The model converged and achieved the required effective sample size within the initial run of 50,000 iterations. The posterior estimates from the model indicate that 84% (95% CI: 78–88%) of the sampled farms were truly infected, and the within-herd prevalence was estimated to be 73% (95% CI: 66–84%). The region-level prevalence was estimated to be very similar in the Sertão (84%; 77–90%) and Agreste/Zona da Mata (88%; 81–93%) regions, but slightly lower in the relatively over-sampled Borborema region (78%; 70–85%). The adjusted between-herd prevalence estimate for the State of Paraíba (accounting for the sampling fraction within each geographical area) was estimated to be 84% (95% CI: 80–88%). The posterior 95% confidence interval for the sensitivity was 83–99%, which is very close to the prior 95% confidence interval of 82–99%. The posterior 95% confidence interval for the specificity was 78–90%, compared to 90–99% for the prior.

3.3. Risk factor estimates

Of the 14 potential risk factors, only 5 were associated with a substantial ($>10\%$) probability of being activated by the model (Table 3). The remaining variables were associated with a 90–95% probability of being deactivated by the model, due to a lack of support for an effect size greater than zero. The estimated odds ratios (based on only those iterations at which the relevant variable was switched on by the model) are also shown in Table 3. The three most influential risk factors were disposal of aborted foetuses by burying or burning with an odds ratio of 3.8 (95% CI 1.1–13.9), sharing of resources with an odds ratio of 3.0 (95% CI 1.1–8.6) and a herd size of greater than 23 animals with an odds ratio of 2.5 (95% CI 1.1–6.0). The estimated odds ratios for all other risk factors were associated with 95% confidence intervals that included one.

4. Discussion

This is the first study carried out in Brazil using planned sampling and Bayesian latent class analysis methods to estimate both the animal- and herd-level prevalence and risk factors of BoHV-1 infection. The Bayesian approach allows for the incorporation of prior information on the sensitivity and specificity to account for the imperfect diagnostic test characteristics of the VNT, which is still one of the most sensitive tests for the detection of antibodies in serum (OIE, 2010), obtaining exact inference without the need for large-sample approximations and using a two-stage cluster-sampling design (Branscum et al., 2004). The inclusion of sensitivity and specificity within the model is only possible using relatively strong priors for these parameters, without which the model would not be identifiable. The prior and posterior for sensitivity were practically identical, but the posterior estimate for specificity was markedly reduced compared to the prior. This may indicate that the field specificity of the test is less than that previously reported by Kramps et al. (2004), but we also note that this model formulation was not primarily intended for diagnostic test evaluation, so caution is merited when interpreting this estimate.

The herd-level prevalence found in our study was high in the State of Paraíba (84%) and this is in accordance with that previously found in

Table 1

Census data of the cattle population and the results obtained in samples of bovine serum analyzed by virus neutralization test for BoHV-1 in the State of Paraíba.

Sampling stratum	No. of herds with reproductive activity				No. of cows $\geq$ 24 months of age			
	Total	Sampled	no. of positives	% pos.	Total	Sampled	no. of positives	% pos.
Sertão	24,356	159	147	92.5	288,764	990	634	64.0
Borborema	11,603	160	131	81.9	83,428	729	432	59.2
Agreste/Zona da Mata	18,398	159	140	88.0	192,320	724	518	71.5
State of Paraíba	54,357	478	418	87.4	564,512	2443	1584	64.8

Table 2

The frequency distributions of BoHV-1 infection, and distribution of responses to all survey questions from 478 cattle herds in Paraíba State.

Independent Variable	Category	No. of herds	BoHV-1 (-)	BoHV-1 (+)	%
Region	Sertão	159	14	145	91
	Borborema	160	30	130	81
	Agreste/Mata	159	20	139	87
Breeding type	Meat	59	11	48	81
	Milk	139	20	119	86
	Mixed	280	33	247	88
Management system	Confined	29	10	19	66
	Semi-confined	270	38	232	86
	Extensive	179	16	163	91
No. of milking	Not milking	124	23	101	81
	1 times per day	305	37	268	88
	2 or 3 times per day	49	4	45	92
No. of lactating cows	0 – 4	382	61	321	84
	> 4	96	3	93	97
Daily milk production	0 – 15	368	60	308	84
	> 15	110	4	106	96
Use of artificial insemination	No	475	63	412	87
	Yes	3	1	2	67
Predominant breed	Zebu	25	4	21	84
	European dairy breed	43	7	36	84
	Crossbred	405	53	352	87
	Other breeds	5	0	5	100
No. of cows aged ≥ 24 months	0 – 9	368	58	310	84
	> 9	110	6	104	95
No. of calves ≤ 12 months	0 – 6	370	60	310	84
	> 6	108	4	104	96
Herd size	1 – 23	364	60	304	84
	> 23	114	4	110	96
Presence of goats and sheeps	No	296	37	259	88
	Yes	182	27	155	85
Presence of horses	No	219	37	182	83
	Yes	259	27	232	90
Presence of swine	No	324	47	277	85
	Yes	154	17	137	89
Presence of wildlife	No	302	38	264	87
	Yes	176	26	150	85
Presence of cervids	No	471	64	407	86
	Yes	7	0	7	100
History of abortion	No	447	60	387	87
	Yes	31	4	27	87
Disposal of aborted foetuses	Leave in the field	379	57	322	85
	Used to feed pig or dog	7	1	6	86
	Buried or burned	92	6	86	93
Animal purchasing	No	317	50	267	84
	Yes	161	14	147	91
Pasture rental	No	366	56	310	85
	Yes	112	8	104	93
Sharing of pastures	No	398	53	345	87
	Yes	80	11	69	86
Flooded pastures	No	304	44	260	86
	Yes	174	20	154	89
Maternity pens	No	356	50	306	86
	Yes	122	14	108	89
Veterinary assistance	No	404	57	347	86
	Yes	74	7	67	91
Share sources of water	No	404	57	347	86
	Yes	74	7	67	91

Table 3

Risk factor estimates from the Bayesian latent-class logistic regression model, for the rationalised risk factors based on the questionnaire survey responses from 478 cattle herds. Odds ratios are shown as mean and 95% confidence interval estimates from the model posterior, using only those iterations where the risk factor was activated by the variable selection mechanism within the model. The activation probability represents the proportion of iterations for which the risk factor was used by the model.

Risk Factor	Estimated odds ratio			Activation Probability
	Mean	L95% CI	U95% CI	
Disposal of aborted foetuses by bury or burn	3.8	1.1	13.9	0.58
Sharing Resources	3.0	1.1	8.6	0.56
Herd Size greater than 23	2.5	1.1	6.0	0.45
No Horses Present	0.5	0.3	1.1	0.23
Purebred Predominant	0.6	0.3	1.5	0.11
Sheep Goats Present	0.7	0.3	1.4	0.10
Extensive Management System	1.4	0.7	3.1	0.08
Animal Purchasing	1.2	0.6	2.9	0.07
Presence of Veterinary Assistance	1.1	0.5	3.1	0.07
Presence of Maternity Pens	1.0	0.4	2.2	0.07
Pigs Present	1.0	0.5	2.4	0.06
Wildlife Present	1.0	0.5	2.0	0.06
No Milking	0.9	0.4	2.1	0.06
Presence of Flooded Pastures	0.9	0.4	1.8	0.06

sampling strata (Sertão: 84%; Agreste/Zona da Mata: 88%, Borborema: 78%), indicating that the infection is widely spread in Paraíba. We note that detection of latent carriers is critically dependent on the use of highly sensitive serological tests that are able to detect low levels of BoHV-l-specific antibodies. VNT has imperfect sensitivity and animals with low levels of BoHV-l- specific antibody are not detectable by this test. On the other hand, such low levels may be due to the presence of maternal antibodies or as a result of vaccination by a killed vaccine, and of course, should be excluded in these seropositive animals (Kramps et al., 1996; Takiuchi et al., 2003). However, in this study we sampled non-vaccinated cows over 24 months (since none of the owners confirmed that they vaccinated the herds against BoHV-1) confirming that these antibodies found originate from the exposure of the animals to the virus, suggesting active spread of infection within the cattle herds.

The animal-level prevalence estimate of around 74% (within infected herds) is relatively high compared to the estimated animal-level sero-prevalence of BoHV-1 infection of 34% in Belgium (Boelaert et al., 2000), 37.8% in Estonia (Raaperi et al., 2010), 43% (7–80%) in Costa Rica (Raizman et al., 2011) and 57.1% (2.1–99.2%) in south west England (Woodbine et al., 2009). However, none of these estimates were infection prevalences and therefore not really comparable. We attempted to fit a distribution of animal-level prevalence between herds, but ultimately the assumption that animal-level prevalence is consistent across herds was required in order for the model to be identifiable. It is possible that this assumption may have affected the posterior estimates for sensitivity and specificity if a subset of truly low-prevalence herds existed in the data (these would either be described by the model as negative herds with false positive tests or high-prevalence herds with false negative tests). However, estimation of sensitivity and specificity was not the objective of our study. An identifiable model may have been attainable given additional data or stronger prior information regarding the distribution of animal-level prevalence across herds. Previous survey studies in Brazil did not estimate the herd-level prevalence of BoHV-1 infection, however, one study determined that the proportion of seropositive animals per herd ranged from 20 to 100% in the state of Maranhão (Bezerra et al., 2012). The authors also suggested that the source of infection was likely from their own herds because of the observed variation between farms with recent acquisition of animals compared to those which did not buy in animals. The

Paraíba (100%) (Thompson et al., 2006), in the State of Maranhão (100%) (Bezerra et al., 2012), and in Goiás (98.5%) (Barbosa et al., 2005). A lower seroprevalence was reported in Paraná (71.3%) (Dias et al., 2013). A high herd-level true prevalence was found in all of the

animal-level prevalence is relevant because it directly correlates with economic losses due to the BoHV-1 infection (Raizman et al., 2011), and once introduced in a herd, it quickly spreads within-herd. Despite this, and the high herd-level prevalence of BoHV-1 infection found in this study, most farmers in Paraíba are not aware of the impact of the infection and the economic losses that it can cause, possibly because most farms are family or subsistence farms (Clementino et al., 2015), and in general IBR is an unknown disease for most of them.

The risk factor assessment allowed identification of the herd and management characteristics that are more likely to be associated with infection, and would therefore potentially play a role in the dissemination or maintenance of the infection in the national herd. In this study, we found that farms that practice proper disposal of placental tissue and aborted foetuses were associated with an elevated odds of having BoHV-1 infection (OR 3.8; 95% CI: 1.1–13.9) compared to those who do not. At first glance this result seems to be inconsistent with expectation, and with the findings of previous studies (Quevedo et al., 2011; Dias et al., 2013). However, it must be remembered that our study was observational, so causation cannot necessarily be inferred from the associations prevented. In fact this result may be due to reverse causality: those farms with the highest number of abortions caused partly by BoHV-1 infection may be more concerned with carcass disposal than farms with fewer abortions as a direct result of the high prevalence of BoHV-1 infection on the farm. In this respect, 'disposal of aborted foetuses' may be considered as a proxy for other risk factors, (e.g. 'veterinary care', 'biosecurity'), and it should not be considered as a cause for the occurrence of the disease, but rather as a consequence of the infection. Perhaps this can be explained by the high prevalence in the state, preventing the discrimination of any condition as a risk factor, since the risk was already imminent (Barbosa et al., 2005).

Sharing resources was also considered a risk factor, which suggests direct and indirect contact between herds. Herds from properties sharing resources had a 3.0 times (95% CI: 1.1–8.6) higher odds of BoHV-1 infection. The practice of sharing resources, such as pastures and water sources, with other herds has been considered a potential risk factor and is linked to biosecurity deficiency in study farms. Sharing of resources is a practice that must be discouraged in order to avoid not only the dissemination of the BoHV-1 but also other infectious agents.

This study found that a herd size of greater than 23 animals is a risk factor for the BoHV-1 infection in herds (OR: 2.5, 95% CI: 1.1–6.0). In most studies, there is a positive association between large herd size or high density of animals and BoHV-1 seropositivity (Solis-Calderon et al., 2003; Boelaert et al., 2005; Guarino et al., 2008; Dias et al., 2008; Gonzalez-Garcia et al., 2009; Woodbine et al., 2009; Raaperi et al., 2010; Segura-Correa et al., 2010). This finding may be explained by the fact the BoHV-1 causes latent infections, and seropositivity represents the presence of infection in the animal for life, and consequently life-long virus dissemination can also occur (Raaperi et al., 2014). Considering the frequency of seropositive animals per herd, which ranged from 16.1% to 100%, we suggest that the virus is widespread in the herds from the State of Paraíba.

Finally, we have demonstrated a method of selecting risk factor variables that can be used within a Bayesian framework without the necessity to pre-select variables based on univariable analysis or run repeated models with varying numbers of predictors. The results presented in this manuscript were obtained from a model using the relatively simple method of Kuo and Mallick (1998), but the same results were also obtained using Gibbs Variable Selection (Dellaportas et al., 2002). This more complex method involves running the same model twice: once in order to obtain estimates for plausible effect sizes for each of the candidate variables, and then a second time to obtain final results using the output of the first model as pseudo-priors for the effect sizes when the relevant variable is turned off within the model. The purpose of these pseudo-priors is purely to facilitate mixing of the final MCMC chains, which may improve the overall computational performance of the model for some applications. The equivalent procedure for

the Kuo & Mallick method is to adjust the prior probability for the risk activation to optimise convergence and mixing of the model. For our data, we found that a value of 0.25 lead to optimum performance, but this is likely to depend on the data and number of parameters for other applications. We refer interested readers to O'Hara and Sillanpää (2009) for a more detailed description along with a more general discussion of stochastic variable selection methods within a Bayesian framework. Although these methods of model selection generally require a certain degree of experience on the part of the user in order to remove strongly correlated predictors and to provide weakly informative priors to exclude biologically implausible effect sizes, the use of a model selection mechanism within the same framework as the risk estimation is conceptually attractive and gave fewer problems with convergence and model identifiability than might be expected. Although we did not consider interactions for this application, these would be theoretically possible to include within the model in the usual way if the interaction was assumed to only be 'turned on' within the model conditional on both dependent variables also being 'turned on' at that iteration. However, further work is needed to identify an appropriate way in which to specify these variables in order to obtain sufficient mixing of the chains (O'Hara and Sillanpää, 2009). We therefore encourage the more widespread investigation of the applicability of these methods for similar studies, and invite interested researchers to contact us for more details of the methods including the R code necessary to implement the JAGS model code given in Appendix A.

5. Conclusion

In conclusion, our results suggest that: (i) both the animal- and herd-level prevalence of BoHV-1 infection are very high in the State of Paraíba, Northeastern Brazil, (ii) disposal of aborted foetuses, herd size and resource sharing were identified as risk factors for BoHV-1 infection. These identified risk factors are not likely to be of major relevance in the initial stages of a control programme due to the overall high prevalence of BoHV-1, but are potentially relevant in terms of assessing the risk of re-introduction of disease to farms on which it has previously been eradicated. Based on the high herd-level seroprevalence observed (along with the fact that vaccination is not currently practiced in the region), our findings also suggest that future implementation of control strategies for BoHV-1 in the State of Paraíba are merited.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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