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Prevalence of bovine herpesvirus-1 in the Belgian cattle population

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Abstract

The national bovine herpesvirus 1 (BHV-1) seroprevalence (apparent prevalence) in the Belgian cattle population was determined by a serological survey that was conducted from December 1997 to March 1998. In a random sample of herds (N=556), all cattle (N=28 478) were tested for the presence of antibodies to glycoprotein B of BHV-1. No differentiation could be made between vaccinated and infected animals, because the exclusive use of marker vaccines was imposed by law only in 1997 by the Belgian Veterinary Authorities. Twenty-one percent of the farmers vaccinated continuously against BHV-1.

In the unvaccinated group, the overall herd, individual-animal and median within-herd seroprevalences were estimated to be 67% (95% confidence interval (CI)=62–72), 35.9% (95% CI=35.0–36.8) and 33% (quartiles=14–62), respectively.

Assuming a test sensitivity and specificity of 99 and 99.7%, respectively, the true herd, individual-animal and median within-herd prevalence for the unvaccinated group of herds were estimated to be 65, 36 and 34%, respectively. The true herd prevalence for dairy, mixed and beef herds were respectively, 84, 89 and 53%; the true individual-animal prevalence for those types of

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herds were, respectively, 35, 43 and 31%; whereas, the true median within-herd prevalences were 36, 29 and 38%.

Keywords: Cattle-microbiological diseases; Bovine herpesvirus-1; Infectious bovine rhinotracheitis; Prevalence; Belgium

1. Introduction

Infectious bovine rhinotracheitis is caused by the bovine herpesvirus type I (BHV-1). It is an enzootic disease on the B list of the Office International des Epizooties (OIE). Programs to eradicate BHV-1 have been implemented in several European countries to facilitate the free trade of cattle, semen and embryos within the European community. Therefore, Belgium will have an incentive to control and eradicate BHV-1.

A preparatory step towards the design of an eradication program consisted in investigating the BHV-1 prevalence. The group of animals which is of epidemiological importance in terms of the transmission and maintenance of infection — and therefore of disease control and eradication — is the herd (Thrusfield, 1995). This is particularly true for BHV-1, because the control and eradication measures implicate the herd — not the animal (OIE, 1996). Therefore, in this survey, the sampling units were defined as the cattle herds.

To date, a few surveys have estimated BHV-1 prevalence at the regional or national level. However, the findings of these surveys are affected by the nature of the study design (sample or census survey), the study population (subclinical or clinical, vaccinated or unvaccinated), the type of prevalence parameters studied (herd prevalence, individual-animal prevalence or within-herd prevalence), the diagnostic-test used, and the age of the tested animals. Comparison of these survey results is therefore difficult. Moreover, few studies adjust the seroprevalence for factors such as test sensitivity and specificity to calculate the true prevalence (creating further difficulty in comparison across studies).

In Europe, the BHV-1 seroprevalence in herds with dairy cows (both dairy and mixed herds), was surveyed several times in the Netherlands. Van Wuyckhuise et al. (1993) investigated a non-random sample of unvaccinated herds by blood testing in March 1992, and found 93% seropositive herds and 36% seropositive animals, whereas the within-herd seroprevalence varied from 0 to 100% based on a within-herd sample survey. Without considering the vaccination status of herds, the Dutch herds with dairy cattle were investigated by a random-sample survey in the winter of 1992–1993 by Van Wuyckhuise et al. (1993), and by a census survey in November 1994 by Van Wuyckhuise et al. (1998); they found the BHV-1 bulk-milk herd apparent prevalence to be 75 and 84%, respectively. In a more-recent sample survey that also did not take into account the vaccination status of herds, Hartman et al. (1997) found the herd apparent prevalence (based on individual blood samples) to be 92%, whereas 89% of the herds were positive by bulk-milk sampling.

In Belgium, regional BHV-1 surveys that did not take into account the vaccination status of herds, estimated the herd seroprevalence in mixed and beef herds to be 62% in 1986 (Van Malderen et al., 1987), whereas the individual-animal and the within-herd

seroprevalence in herds of these types were estimated to be 64 and 51%, respectively, in 1995 (Lemaire et al., 1997). The national prevalence of BHV-1 in Belgian dairy, mixed and beef herds is unknown.

To investigate BHV-1 prevalences in the Belgian cattle population, a survey was conducted from December 1997 to March 1998 in all the provinces of Belgium. The primary goal of this survey is to provide an unbiased estimate of the true national BHV-1 herd-level prevalence in dairy, mixed and beef herds, by random selection of herds to form samples. A second objective is to estimate the true national BHV-1 individual-animal and within-herd prevalences in dairy, mixed and beef herds.

2. Material and methods

2.1. Survey design

The survey was organized using the coordinates for the cattle herds registered in SANITEL-Cattle, the central computerized database for the identification and registration of the Belgian cattle population (Ministry of Small Enterprises, Traders and Agriculture, Belgium). By law, all Belgian cattle keepers have to be registered in SANITEL-Cattle and have the duty to report all the necessary data that are needed for making up their herd and cattle-movement inventories. This information is updated daily in SANITEL-Cattle by the Regional Veterinary Investigation Centers. In SANITEL-Cattle, a herd is defined as a stock of bovids kept in a geographical entity — containing one or several buildings with adjacent premises — that makes up a clear and distinct unit on the basis of epidemiological bounds set by the veterinary inspection. Therefore, in this survey, the sampling units were defined as the cattle herds.

The survey was conducted on herds of all types from December 1997 to March 1998. A stratified random sample design was followed. The total number of herds to be sampled was set at one percent of the total number of Belgian cattle herds. The sample was stratified by province. The number of herds to be sampled in each province was determined by proportional allocation (Thrusfield, 1995). Herds were randomly selected from SANITEL-Cattle using a software random-number generator function of Visual Basic 3.0 (Microsoft Corp., 1993). In the selected herds, all animals were blood sampled. A herd was defined to be BHV-1-seropositive if at least one BHV-1-seropositive bovid was present.

The number of herds on the computer-generated sampling lists exceeded the calculated number, to allow for replacement of herds which did not have cattle on the sampling date. As soon as a herd was confirmed as not having cattle, it was replaced by the next herd on the reserve list. In 185 instances, herds were replaced.

2.2. Collection of samples and herd and management characteristics

The blood samples were taken by the veterinary practitioners and sent to the Veterinary and Agrochemical Research Center. The age of the cattle was known from the SANITEL-Cattle herd inventories. The veterinary practitioners also interviewed the farmers to

obtain information concerning the herd and management characteristics: herd type (dairy herd, mixed herd or beef herd), herd size (number of cattle on the premises), and whether the farmer vaccinated continuously, intermittently, or not, against BHV-1 (before 1996, during 1996–1997 and in 1998), or whether he did not know the BHV-1 vaccination status.

2.3. Serological testing and interpretation

The serum samples were tested for antibodies against BHV-1 with a commercially available blocking ELISA (HerdChek®, Idexx, France), specific for BHV-1 glycoprotein B (gB) (Kramps et al., 1994). All samples were tested according to the manufacturer's instructions. Doubtful test results were classified negative in the data analysis.

2.4. Statistical methods used to calculate the true prevalence for unvaccinated herds

The seropositive herds will have one or more infected animals, or have veal with maternal antibodies, or have vaccinated animals. Because of the latter, the vaccination status of tested herds was investigated, and the BHV-1 true prevalences for unvaccinated herds were calculated as follows. First, assumptions found in the literature concerning the intrinsic properties of the gB-blocking ELISA were made: a diagnostic-test sensitivity (SENS) of 99% (Kramps et al., 1994), and a diagnostic-test specificity (SPEC) of 99.7% (de Wit et al., 1998). Second, the true within-herd prevalence (TPWH) for each of the BHV-1-seropositive herds was estimated according to the standard equation of Marchevsky (1974). Third, for each of the BHV-1-seropositive herds, the infected herd detectability (IHD) was calculated based on the following probabilities formula:

$$\mathrm{IHD} = 1 - [(1 - \mathrm{SENS})^{m \times \mathrm{TPWH}} (\mathrm{SPEC})^{m(1-\mathrm{TPWH})}]$$

where m is the sample or herd size, because all cattle present were sampled.

This formula is the equivalent of the HSENS formula developed by Martin et al. (1992), adapted to sampling of all animals present in the herds. The overall and herd-type-specific IHD were calculated as the median IHD of the BHV-1-seropositive herds and of the seropositive dairy, mixed and beef herds, respectively. Fourth, the herd-level specificity (HSPEC) was calculated according to Martin et al. (1992):

$$\mathrm{HSPEC} = (\mathrm{SPEC})^{m}$$

where m is the median sample or herd size because — as for the IHD — all cattle present were sampled.

Fifth, based on the calculated IHD and HSPEC, the true herd-level prevalence was estimated according to the standard equation of Marchevsky (1974).

The true individual-animal prevalence (TAP) was calculated as

$$\mathrm{TAP} = \frac{\sum_{i=1}^{n} d_i}{N}$$

where d_i (the number of infected animals) was estimated for each seropositive herd by multiplying the sample or herd size by the TPWH, and N the total number of animals held in the unvaccinated herds.

The overall and herd-type-specific TPWH were estimated based on the survey results from the BHV-1-seropositive herds, assuming non-reactor herds were non-infected. This calculation consisted of calculating the median of the estimations of the TPWH for each of the BHV-1-seropositive herds.

Lastly, the IHD, HSPEC, and herd true prevalence were estimated according to a range of test sensitivities and specificities of 70–99% and 96–99.7%, respectively.

2.5. Data analysis

The prevalences were analyzed per herd type to allow comparison with other published BHV-1 prevalence figures. Data originating from herds without herd-type specification were excluded from the analysis. The median herd size of different herd types were compared using a Wilcoxon rank-sum test. The proportion of farmers of different herd types that vaccinated continuously against BHV-1 were compared using a logistic regression model using PROC GENMOD (Statistical Analysis Systems Institute, 1996) with herd type as independent variable and vaccination status as the response variable. All tests were two-tailed and a p-value <0.05 was considered as significant.

3. Results

3.1. General features of the target and study population

The sample consisted of 594 randomly selected herds (Table 1). There were 38 non-responding herds (6% of the 594 herds) from which no samples were received and for which no replacement occurred either. The reasons for non-response were: (1) the farmer had ceased his activities (24 herds, 4.0%); and (2) no cattle were blood sampled in due time, due to lack of coordination between different project partners (14 herds, 2.3%). The study population consisted of 106 (20%) dairy herds, 113 (21%) mixed herds and 309 (59%) beef herds. At the animal-level, the total numbers of cattle held in dairy, mixed and beef herds were 8360 (31%), 10 206 (37%) and 8892 (32%), respectively. The median and the range of the herd size were: 81, 2–238 for dairy herds; 73, 4–252 for mixed herds; and 11, 1–326 for beef herds.

Table 1
Sample composition and national seroprevalence of bovine herpesvirus-1 (BHV-1) in Belgium, 1998

	No. of herds		No. of cattle	
	N	Total (%)	*N*	Total (%)
Total[a]	58811	–	3242600	–
To be sampled	594	100	33264	100
Actually sampled	556	94	28478	86
Actually sampled unvaccinated herds	309	52	11284	34
BHV-1 seroprevalence for unvaccinated herds (95% CI[b])	207	67 (62–72)	4060	35.9 (35.0–36.8)

[a] SANITEL-Cattle, 1997. Ministry of Small Enterprises, Traders and Agriculture, Belgium.
[b] Confidence intervals.

Table 2
Vaccination status against bovine herpesvirus-1 (BHV-1) and herd size of cattle herds, per herd type, Belgium, 1998

Type of herd	Variable being cited (number)	Vaccinated		Unvaccinated[b]	Unknown vaccination status
		Continuously[a]	Intermittently		
All, combined	Herds	102[c]	64	309[d]	59[d]
	Cattle	9313	4686	11284	2394
Dairy	Herds	23	11	59	12
	Cattle	2073	1156	4112	968
Mixed	Herds	29	22	54	8
	Cattle	3624	2305	3724	553
Beef	Herds	47	31	194	37
	Cattle	3446	1225	3378	843

[a] Herds that were vaccinated continuously.
[b] Herds that were never vaccinated.
[c] Three herds of unknown herd type.
[d] Two herds of unknown herd type.

3.2. Vaccination against BHV-1

The vaccination status of the 534 herds that were obtained in response to a questionnaire, are shown in Table 2. The median herd size of herds vaccinated continuously was: 88 overall; 91 for dairy herds; 114 for mixed herds; and 48 for beef herds. In contrast, the median herd size of unvaccinated herds was: 19 overall; 60 for dairy herds; 59 for mixed herds; and 7 for beef herds. Herds that were vaccinated continuously had thus a larger herd size compared to unvaccinated herds, overall and specific for all herd types.

The proportion of farmers that vaccinated continuously against BHV-1 was 21% (102/(102+309+64)). Compared to the proportion of beef herds that were vaccinated continuously (17%), more mixed herds were vaccinated (28%). This proportion did not differ with that for dairy herds (24%).

Table 3
National seroprevalence of bovine herpesvirus-1 (BHV-1), per herd type, in Belgium, 1998

Type of herd	Herd seroprevalence		Individual-animal seroprevalence		Median of within-herd seroprevalence (quartiles)
	No. tested	%Positive (95% CI[a])	No. tested	%Positive (95% CI)	
Dairy	59	86 (77.7–95.2)	4112	35 (33.5–36.4)	35 (10–60)
Mixed	54	91 (83.0–98.5)	3724	42 (40.5–43.7)	29 (13–64)
Beef	194	54 (47.1–61.1)	3378	31 (29.6–32.7)	38 (20–67)

[a] Confidence intervals.

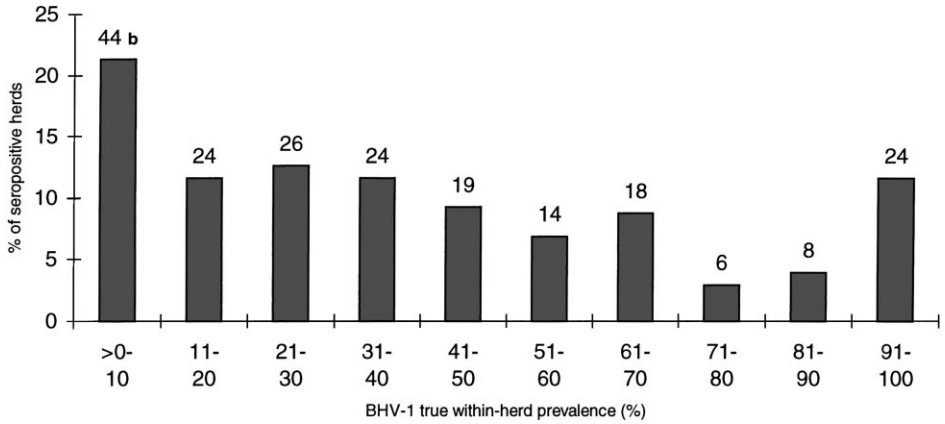

Fig. 1. Frequency distribution of the BHV-1 true within-herd prevalence in non-vaccinated herds in Belgium, 1998 (assuming a test sensitivity and specificity of 99 and 99.7%, respectively); b indicates the number of positive herds.

3.3. BHV-1 seroprevalence in unvaccinated herds

The BHV-1 overall herd and individual-animal seroprevalences for unvaccinated herds were, respectively, 67 and 35.9% (Table 1). The overall median (quartiles) and average within-herd seroprevalence were, respectively, 33% (14–62) and 41%. The herd-type-specific seroprevalences are summarized in Table 3.

3.4. BHV-1 true prevalence in unvaccinated herds

The median TPWH was 34%. The overall frequency distribution of the BHV-1 within-herd prevalence is shown in Fig. 1; of the positive herds, 57% had a TPWH of $\leq$40% — but 12% of the positive herds had a TPWH of >90%. The overall IHD was 100%. Based

Table 4
Infected herd detectability, herd specificity, and true prevalences at the herd-level, animal-level and within-herd, of bovine herpesvirus-1 (BHV-1), per herd type, Belgium, 1998[a]

Type of herd	Infected herd detectability (%)	Herd specificity (%)	True herd prevalence (%)	True individual-animal prevalence		Within-herd prevalence	
				N	Npos (%)	Median (quartiles)	Average
Dairy	100	84	84	4112	1448 (35%)	36 (10–60)	39
Mixed	100	84	89	3724	1584 (43%)	29 (13–64)	39
Beef	100	98	53	3378	1059 (31%)	38 (20–68)	44

[a] A true overall within-herd prevalence of 34%; a true within-herd prevalence of 36% for dairy herds, 29% for mixed herds, and 38% for beef herds; a median of 19 animals per herd, a median sample size of 60 for dairy herds, 59 for mixed herds, and 7 for beef herds; a test sensitivity and specificity of 99 and of 99.7%, respectively; and non-reactor herds non-infected are the assumptions made.

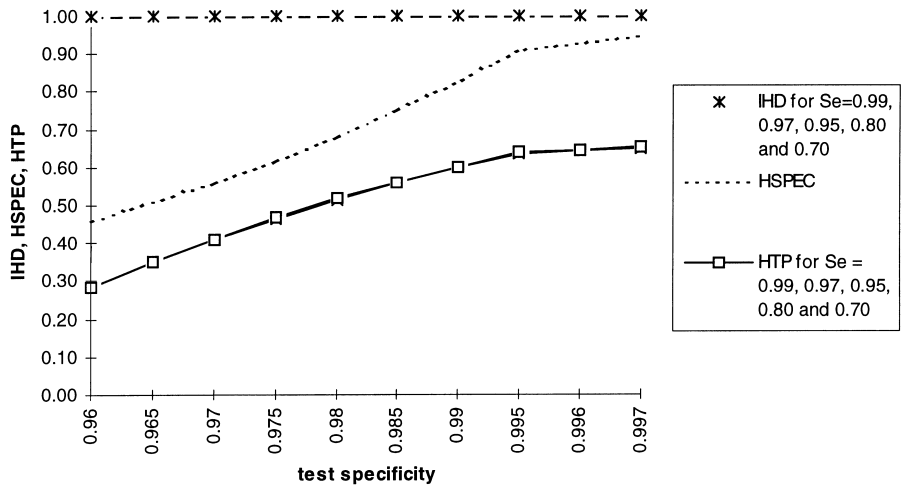

Fig. 2. BHV-1 infected herd detectability (IHD), herd specificity (HSPEC) and herd true prevalence (HTP) in non-vaccinated herds, calculated for a range of test sensitivities (Se) and specificities, Belgium 1998 (assuming a true overall within-herd prevalence of 34% and a median herd size of 19 animals).

on a median sample size of 19, the overall HSPEC was 94%. Based on the aforementioned parameters, the true overall herd prevalence was 65%. The true overall individual-animal prevalence was 36% (4095/11 284). The herd-type-specific prevalence parameters are summarized in Table 4.

In Fig. 2, the IHD, HSPEC, and herd true prevalence are estimated according to a range of test sensitivities and specificities of 70–99% and 96–99.7%, respectively; a median sample or herd size of 19 animals, and a BHV-1 overall within-herd prevalence of 34%. Assuming a SENS of 99% and a SPEC of 99.7%, the herd true prevalence estimation was 65%, whereas with the same SENS and a SPEC of 96%, the herd true prevalence estimation was 28%.

4. Discussion

The present sample survey aimed to provide an unbiased estimate of the true national BHV-1 herd prevalence, and so the herds were randomly selected. Because the percentage of non-responding herds was low (6%), this random sample of herds can be considered to be representative for the Belgian cattle population (Thrusfield, 1995). Because all cattle in the selected herds were tested, the reactor herds provided data without sampling bias for estimation of the apparent and true within-herd prevalence, compared to studies with a within-herd sample-based design.

4.1. Vaccination against BHV-1

The vaccination percentage for dairy herds (24%) was in line with those of Dutch dairy herds (about 20% before the start of a BHV-1 eradication program based on compulsory

vaccination) (Vonk Noordegraaf et al., 1998). The herd-type-specific vaccination percentage was highest for mixed herds. Since vaccination against BHV-1 is usually practised with the aim of clinical protection, this might indicate that herd type is a possible risk factor for BHV-1 infection.

4.2. BHV-1 seroprevalence

In Belgium, only live non-marker vaccines were commercialized before 1997. Since it is not possible to discriminate between antibody response following vaccination and the antibody response following infection, vaccination with these non-marker vaccines has in fact only increased the prevalence of gB-seropositive cattle. In this survey, seropositive unvaccinated herds could thus have purchased animals vaccinated with conventional vaccines or non-infected veal with maternal antibodies — and not necessarily one or more infected animals. This could have resulted in an overestimation of the true overall prevalences. However, the unvaccinated herds had a smaller herd size compared to vaccinated herds. Consequently, the seroprevalences for the unvaccinated herds might also underestimate the true overall prevalences, because larger herd size is a possible risk factor for BHV-1 infection (Van Wuyckhuise et al., 1998).

The overall herd seroprevalence for unvaccinated mixed and beef herds, 62% (49+105/54+194) equals the regional seroprevalence figure obtained by Van Malderen et al. (1987). The BHV-1 herd seroprevalence of Belgian herds with dairy cows, taking into consideration dairy and mixed herds, was 88.5% (51+49/59+54) with a 95% confidence interval (CI) of (82.6–94.8), and is comparable to the herd seroprevalences of analogue Dutch herds in 1992 of 93% reported by Van Wuyckhuise et al. (1993) and in 1997 of 92% reported by Hartman et al. (1997). The former Dutch study concerned only unvaccinated herds. The individual-animal seroprevalence for herds with dairy cows, 38.3% (1436+1567/4112+3724) with a 95% CI of (37.2–39.4), was comparable to the Dutch analogous figure of 36% in unvaccinated herds in 1992 (Van Wuyckhuise et al., 1993). The individual-animal seroprevalence for mixed and beef herds, 36.9% (1567+1051/3724+3378) with a 95% CI of (35.7–38.0), was lower than the regional figure of 64% obtained by Lemaire et al. (1997). Those authors found a regional average within-herd seroprevalence figure for mixed and beef herds of 51%, whereas in the present study the average (quartiles) within-herd seroprevalence was 42% (16–67).

4.3. BHV-1 true prevalence of unvaccinated herds

The testing procedure could also have been a source of information bias, because the aforementioned apparent prevalences assume a perfect test sensitivity and specificity of 100%. No published data exist for sensitivity and specificity of the ELISA kit used. Kramps et al. (1994) estimated the diagnostic sensitivity of the gB-blocking ELISA to be 99%. In their study, no information was reported on the reference test (gold standard) or the vaccination status of the positive reference sera. The estimated diagnostic specificity of the gB-blocking ELISA in a BHV-1 free and unvaccinated animal population ranged from 96% (Kramps et al., 1994) to 99.7% (de Wit et al., 1998). For the true-prevalence calculations, the most-recent published test specificity figure was assumed. The gB

ELISA is very sensitive, inducing a negligible problem of false-negative animals. The assumed specificity is very high — implying only a minor problem with false-positive animals. The problem of false-positive test results was further diminished by the protocol of the data analysis, where doubtful OD-test values were classified as negative. The estimations of the TPWH (34%) and of the IHD (100%) assumed non-reactor herds to be non-infected. Corrected for testing procedures, the true prevalence figures were comparable to the apparent prevalences.

As these intrinsic test characteristics could have been too optimistic, the herd true prevalence was verified for a set of test characteristics. For these calculations, a fixed true within-herd prevalence of 34% was assumed, because this parameter did not vary substantially according to the different test characteristics mentioned. The calculations show that the herd true prevalence is the same for a varying test sensitivity ranging from 70 to 99%. The IHD was 99.99–100% for all parameter combination under consideration. However, the lack of test specificity has a dramatic effect on the estimation of the herd true prevalence; the true herd-prevalence estimation decreased from 65 to 28% if the test specificity decreased from 99.7% (de Wit et al., 1998) to 96% (Kramps et al., 1994), respectively, for a varying test sensitivity ranging from 70 to 99%. These calculations showed that the practical limits of the accuracy of the used screening test, a gB ELISA, jeopardize the estimation of the herd true prevalence within reasonable confidence intervals.

5. Conclusion

The results of this survey show that the Belgian cattle population is endemically infected with BHV-1. Eradication of BHV-1 will be only economically feasible by first lowering the prevalence, possibly followed by test and removal procedures. In anticipation of such BHV-1 eradication program, the Belgian Veterinary Authorities imposed by law the exclusive use of marker vaccines since 1997. Vonk Noordegraaf et al. (1998) simulated the epidemiological and economic consequences of various control strategies of BHV-1 infection in the Dutch pure dairy herds, and found that compulsory vaccination would be necessary to reach a BHV-1-free status. The proportion of 24% of Belgian dairy farmers vaccinating continuously against BHV-1, would be too low. However, the model developed by Vonk Noordegraaf et al. (1998) should be run on the basis of parameters characterizing the Belgian dairy cattle population.

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References

de Wit, J.J., Hage, J.J., Brinkhof, J., Westenbrink, F., 1998. A comparative study of serological tests for use in the bovine herpesvirus-1 eradication programme in The Netherlands. Vet. Microbiol. 61, 153–163.

Hartman, A., Van Wuijckhuise, L., Frankena, K., Franken, P., Wever, P., de Wit, J., Kramps, J., 1997. Within-herd BHV-1 prevalence prediction from an ELISA on bulk milk. Vet. Rec. 140, 484–485.

Kramps, J.A., Magdalena, J., Quak, J., Weerdmeester, K., Kaashoek, M.J., Maris-Veldhuis, M.A., Rijsewijk, F.A.M., Keil, G., Van Oirschot, J.T., 1994. A simple, specific, and highly sensitive blocking enzyme-linked immunosorbent assay for detection of antibodies to bovine herpesvirus-1. J. Clin. Microbiol. 32, 2175–2181.

Lemaire, M., de Wergifosse, B., Nols, L., Limbourg, B., Deblicquy, P., Onclin, M., Czaplicky, G., Bughin, J., Antoine, H., Wellemans, G., Pastoret, P.-P., Thiry, E., 1997. A study of the seroprevalence of bovine herpesvirus-1 infection in the Walloon region of Belgium. Epidémiologie et Santé animale 32, 11.C.21.

Marchevsky, N., 1974. Errors in prevalence estimates in population studies: a practical method for calculating real prevalence. Zoonosis 16, 98–109.

Martin, S.W., Shoukri, M., Thorburn, M.A., 1992. Evaluating the health status of herds based on tests applied to individuals. Prev. Vet. Med. 14, 33–43.

OIE, 1996. International Animal Health Code. OIE, Paris, France.

Thrusfield, M., 1995. Veterinary Epidemiology, 2nd Edition, Blackwell Science, Cambridge, UK.

Van Malderen, G., Vanopdenbosch, E., Wellemans, G., 1987. Bovien herpes virus 1 en 4: een sero-epidemiologisch onderzoek van de Belgische rundveestapel. Vl. Diergeneesk. Tijdschr. 56 (4), 364–371.

Van Wuyckhuise, L., Bosch, J., Franken, P., Hage, J., Verhoeff, J., Zimmer, G., 1993. The prevalence of infectious bovine rhinotracheitis (IBR) in the Netherlands. In: Proceedings of the Dutch Society for Veterinary Epidemiology and Economics (VEEC), pp. 7–15.

Van Wuyckhuise, L., Bosch, J., Franken, P., Frankena, K., Elbers, A.R.W., 1998. Epidemiological characteristics of bovine herpesvirus-1 infections determined by bulk milk testing of all Dutch dairy herds. Vet. Rec. 142, 181–184.

Vonk Noordegraaf, A., Buijtels, J.A.A.M., Dijkhuizen, A.A., Franken, P., Stegeman, J.A., Verhoeff, J., 1998. An epidemiological and economic simulation model to evaluate the spread and control of infectious bovine rhinotracheitis in the Netherlands. Prev. Vet. Med. 36, 219–238.