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Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population

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Abstract

The national bovine paratuberculosis (PTB) 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 ($N = 556$, 9.5%), all adult cattle of 24 months of age or older ($N = 13,317$, 0.4%) were tested for the presence of antibodies using a commercially available absorbed ELISA test kit. The PTB median within-herd seroprevalence (proportion of detected animals within the seropositive herds) and the PTB individual-animal seroprevalence (proportion of detected animals) were, respectively, 2.9% (quartiles = 1.6–5.6) and 0.87% (95% confidence interval (CI) = 0.71–1.03). The PTB herd seroprevalence (proportion of detected herds) was 18% (95% CI = 14–21).

Assuming a test sensitivity and specificity of 45 and 99% [Sweeney et al., 1995. *J. Vet. Diagn. Invest.* 7 (4), 488; Sockett et al., 1992. *J. Clin. Microbiol.* 30 (5), 1134], respectively, the median true within-herd prevalence and the true individual-animal were estimated to be 7 and 2%, respectively. The true herd prevalence of *Mycobacterium paratuberculosis* infection was first estimated according to currently accepted methodology. This calculation revealed that the specificity of the used test has a dramatic effect on the estimation; assuming a test sensitivity of 45% and a true within-herd prevalence of 7%, the true herd prevalence estimation decreased from 36 to 0.8% if the test specificity decreased from 99.9 to 99%, respectively. This sensitivity analysis showed that the practical limits of the accuracy of the used screening test jeopardize the estimation of the true herd prevalence within reasonable confidence limits, because the within-herd PTB true prevalence was low.

For this reason we augmented the herd specificity for herds with larger adult herd size (>5). This was done by increasing the cut-off number of positive cattle required (≥ 2) to classify a herd truly positive and including herds with one positive test result if there was historical

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evidence of PTB (previous diagnosis and/or clinical signs). This approach resulted in an estimated true herd prevalence of *M. paratuberculosis* infection of 6%. The true herd prevalence for dairy, mixed and beef herds was, respectively, 10, 11 and 3%. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cattle-microbiological diseases; Paratuberculosis; Johne's disease; Prevalence; Belgium

1. Introduction

Paratuberculosis (PTB), or Johne's disease, is a chronic infectious disease of ruminants caused by infection with *Mycobacterium avium* subsp. *paratuberculosis*. It is an enzootic disease on the B List of the 'Office International des Epizooties' (OIE), and is characterized by chronic, granulomatous degenerative enteritis that causes intermittent but persistent diarrhea, progressive weight loss, and eventually, death. The disease is untreatable and slowly progressive. Paratuberculosis is probably the most widespread infectious disease of domestic animals and causes important economic losses in ruminants, particularly cattle, worldwide (Buergelt and Duncan, 1978; Chiodini et al., 1984; Chiodini and Van Kruiningen, 1986; Benedictus et al., 1987). Expanded efforts to control this disease, including regulatory programs in some countries, may lead to future market restrictions.

In Belgium PTB is not a notifiable disease, and hence no official control or eradication program is executed. Vaccination has been recommended in heavily, clinically infected herds. However, vaccination precludes the serodiagnosis of PTB-infected cattle, and is administered under the authority of the Veterinary Inspection since it interferes with the diagnosis of bovine tuberculosis.

When dealing with infectious diseases, 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 PTB because the control and eradication measures implicate the herd, not the animal (OIE, 1998). Therefore, in this survey, the sampling units were defined as the cattle herds.

To date, few methodological serological surveys have been organized to estimate the PTB prevalence at the regional or national levels. Moreover, these surveys are affected by the nature of the study design (sample or census surveys), the study population (subclinical or clinical), the type of prevalence parameters studied (herd, individual animal or within-herd prevalence), the diagnostic test used, and the age of the tested animals. Comparison of these survey results is virtually impossible. Moreover, only very 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.

Estimations of the herd seroprevalence in the USA range from 50% in Wisconsin (Collins et al., 1994) to 74% in Missouri (Thorne and Hardin, 1997) for dairy herds. In Louisiana beef herds, Turnquist et al. (1991) found a herd seroprevalence of 30%, whereas Thorne and Hardin (1997) estimated it to be 40% in Missouri. At the individual-animal level, serological surveys results range from 7.3% in Wisconsin (Collins et al.,

1994) to 17.1% in Florida (Braun et al., 1990) for dairy cattle, and from 1.2% in Finland (Hintikka and Seuna, 1998) to 25.2% in Texan beef cattle (Alexander et al., 1993). In Belgium, a regional survey in southern Belgium found 12% of the cattle seropositive to PTB (Vannuffel et al., 1994).

Published estimations of the true prevalence are only available for dairy herds. The true herd prevalence is estimated to range from 1.3% in England (Cetinkaya et al., 1998) to 34% in Wisconsin (Collins, 1994). The true individual-animal prevalence in dairy cattle is estimated to range from 4.8% in Wisconsin (Collins, 1994) to 6.1% in Ontario (McNab et al., 1991). Hardly any data exist on the true within-herd prevalence of PTB. Estimations range from 5% (Obasanjo et al., 1997) based on whole herd examination by fecal culture, to 20% based on sample surveys by absorbed ELISA (Collins et al., 1994).

To investigate PTB prevalences in the Belgian adult cattle population, a pilot survey was conducted from December 1997 to March 1998 in all the provinces of Belgium. The goal of this survey was first to provide an unbiased estimate of the national herd-level seroprevalence of *M. paratuberculosis* infected dairy, mixed and beef herds, by random selection of herds to sample, and second to calculate the true national PTB herd prevalence.

2. Material and methods

2.1. Survey design

The survey was organized using the co-ordinates 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, Belgium). SANITEL-cattle constitutes a permanent basis for efficient organized disease control. 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 cattle 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 these 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 1% 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 generator function of Visual Basic 3.0 (Microsoft Corp., 1993). In the selected herds, all of the adult herd, i.e. all cattle over 24 months of age were blood sampled. A herd was defined to be PTB-seropositive if at least one PTB-seropositive adult bovine 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. 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 by the SANITEL-cattle herd inventories. By means of a questionnaire, the veterinary practitioners also interviewed the farmer concerning the following herd and management characteristics: herd type (dairy herd, mixed herd or beef herd), herd size (number of cattle on the premises), whether the farmer vaccinated yearlong against PTB, and whether there was historical evidence of PTB (previous diagnosis and/or clinical signs).

2.3. Serological testing

The serum samples were tested for antibodies to *M. paratuberculosis*, using a commercially available Absorbed ELISA (HerdChek[®], IDEXX, France). All samples were tested using one batch of test kits, according to the manufacturer's instructions. Sera with corrected optical density (OD)-values <0.2 and ≥ 0.3 were considered negative and positive, respectively. Intermediate OD-values were considered doubtful and classified as negative in the data analysis.

2.4. Data analysis

The inclusion criteria were as follows: (1) the samples had to be obtained from adult cattle and (2) the samples had to be obtained from herds that never vaccinated against PTB. Data originating from herds with all sampled cattle outside the required age category or that ever vaccinated against PTB were excluded from the analysis. The prevalences were analyzed per herd type to allow comparison with other published PTB prevalence figures. Data originating from herds without herd type specification were excluded from the analysis.

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

The overall and herd type specific true within-herd prevalence (TPWH) were estimated based on the survey results from the PTB seropositive herds, assuming that non-reactor herds were non-infected. This consisted in calculating the median of the estimations of the TPWH for each of the PTB seropositive herds. The TPWH for each of the PTB seropositive herds was estimated according to the standard equation of Marchevsky (1974).

The true individual-animal prevalence (TAP) was calculated according to:

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

where d_i is the number of infected animals that was estimated for each seropositive herd

by multiplying the sample size by the TPWH, and where N is the total number of adult animals held in the unvaccinated herds.

Estimation of the true herd prevalence of *M. paratuberculosis* infection should incorporate factors, such as test sensitivity and specificity, true within-herd prevalence, sample size and the cut-off number of reactors required to call a herd truly positive, that lead to uncertainty in the observed herd prevalence (Martin et al., 1992). First, the following assumptions found in the literature concerning the intrinsic properties of the absorbed ELISA were made: an overall diagnostic test sensitivity (SENS) and specificity (SPEC) of, respectively, 45% and greater than 99% (Collins, 1996). Second, true within-herd prevalence (TPWH) for each of the PTB seropositive herds was estimated as described above. Third, the infected herd detectability (IHD) was calculated based on the following exact probabilities formula (Boelaert et al., 2000):

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

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

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

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

where m is the median sample or adult herd size because, as for the IHD, all adult 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 IHD, the HSPEC, and herd true prevalence were also estimated according to a range of test sensitivities and specificities of, respectively, 25–55 and 99.0–99.9%.

Apart from the above method of true herd prevalence calculation, we used another approach to augment the HSPEC for herds with more than five adult cattle since for these herds the HSPEC drops below 95% if the SPEC is 99% (Martin et al., 1992). This approach consisted in increasing the cut-off number of positive cattle required to classify a herd truly positive, as described by Jordan (1996), and adding herds with one positive test result if there was historical evidence of PTB (previous diagnosis and/or clinical signs) on the farm.

3. Results

3.1. General features of the target and study population

In 1997 there were 3,242,600 cattle and 58,811 cattle herds in Belgium. The average herd size was 55. The sample consisted of 594 randomly selected herds (Table 1). There

Table 1
National seroprevalence of paratuberculosis in Belgium, 1998

	Number of herds		Number of cattle of 24 months of age or older	
	N	%	N	%
Total ^a	58811	—	3242600	—
To be sampled	594	100	15635	100
Actually sampled	511	86	14699	94
Actually sampled non-vaccinated herds	464	78	13317	85
Actually sampled non-vaccinated herds, with herd-type specification	458	77	13150	84
PTB seroprevalence (95% CI ^b)	82	18 (14.2, 21.1)	116	0.87 (0.71, 1.03)

^a SANITEL-cattle (1997). Ministry of Small Enterprises, Traders and Agriculture, Belgium.

^b Confidence intervals.

were 83 non-responding herds (14% of the 594 herds) from which no samples were received and for which no replacement occurred either. The major reasons for no response were: (1) the farmer had ceased his activities (53 herds, 8.9%); (2) no adult cattle were present (26 herds, 4.4%) and (3) no cattle were blood sampled in due time, due to lack of coordination between different project partners (4 herds, 0.7%). A total of 14,699 adult cattle from 511 herds (86.0%) were tested for *M. paratuberculosis* during this survey. There were 47 tested herds that did not meet the inclusion criteria for data analysis. The reasons for this were: (1) no complete information was available about the PTB vaccination scheme (29 herds, 4.9%); (2) no questionnaire was sent in (14 herds, 2.4%) and (3) the herd was vaccinated against PTB (4 herds, 0.7%). A total of 13,317 adult cattle from 464 (78.1%) herds that did not vaccinate against PTB, met the inclusion criteria. The median, the average and the range of the herd size were 38, 55, and 1–326. The study population was made up of 98 (21%) dairy herds, 101 (22%) mixed herds and 259 (56%) beef herds. At the animal-level the total numbers of animals held in dairy, mixed and beef herds were 7775 (31%), 9137 (36%) and 8303 (33%), respectively. The median and the range of the herd size of herds were 81, 2–238 for dairy herds; 72, 4–252 for mixed herds; 14, and 1–326 for beef herds.

3.2. PTB seroprevalence in unvaccinated herds

The PTB overall herd and individual-animal seroprevalence (95% CI) for unvaccinated herds were, respectively, 18% (14.2–21.1) and 0.87% (0.71–1.03) (Table 1).

The distribution of the herd test results is depicted in Table 2. A total of 73% of herds testing positive (60/82) had only one single positive test result. The overall median (quartiles) and average within-herd seroprevalence were, respectively, 2.9% (1.6–5.6) and 7.1%. The frequency distribution of the PTB within-herd seroprevalence is shown in Fig. 1. Of the positive herds, 90% had a maximum within-herd seroprevalence of 10%. The herd-type specific seroprevalence parameters are summarized in Table 3.

Table 2
Distribution of test results of non-vaccinated herds with adult cattle seropositive to paratuberculosis in Belgium, 1998

Number of adult cattle tested for <i>M. paratuberculosis</i> per herd ^a			Number of <i>M. paratuberculosis</i> -seropositive adult cattle per herd ^b			
Number of cattle sampled	Number of herds		-	Number of test positive cattle	Number of herds	
	N	%			N	%
≤5	129	27.8	0	382	82.3	
6–25	132	28.4	1	60	12.9	
26–50	106	22.8	2	17	3.7	
51–75	57	12.3	2	2	0.4	
76–100	26	5.6	4	1	0.2	
101–250	14	3.0	5	1	0.2	
			6	0	0.0	
			7	1	0.2	
Total	464	100		464	100	

^a Average: 29; minimum: 1; first quartile: 5; (median): 19; third quartile: 43; maximum: 213.

^b Average: 1.4; minimum: 1; first quartile: 1; (median): 1; third quartile: 2; maximum: 7.

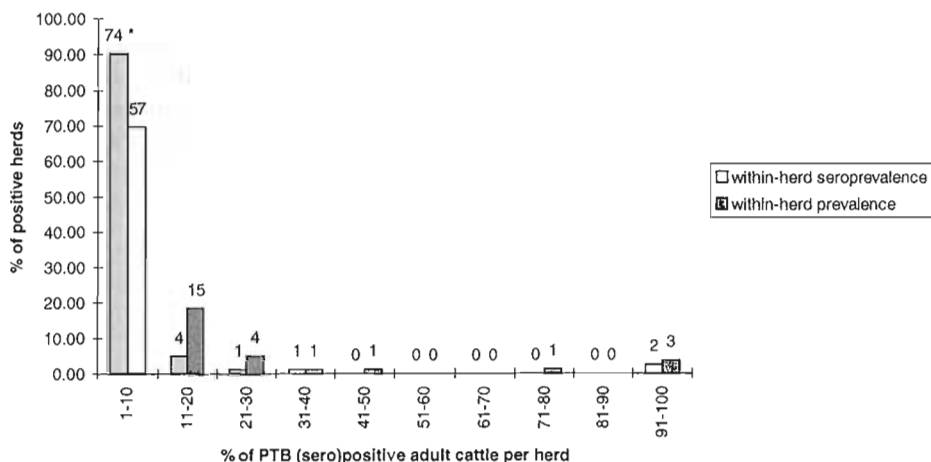


Fig. 1. Frequency distribution of the paratuberculosis within-herd seroprevalence and within-herd prevalence of adult cattle in Belgium, 1998, assuming a test sensitivity of 45%, and a test specificity of 99%. Asterisk (*) denotes number of positive herds.

Table 3
National seroprevalence of paratuberculosis in non-vaccinated herds per herd type in Belgium, 1998

Herd	Within-herd seroprevalence (%)		Individual-animal seroprevalence			Herd seroprevalence		
	Median (quartiles)	Average	N	N _{pos}	%	N	N _{pos}	%
Dairy	2.2 (1.7–4.9)	3.3	4497	52	1.16	98	31	32
Mixed	2.9 (1.7–4.7)	4.4	4643	40	0.86	101	30	30
Beef	4.2 (1.1–17.5)	18.4	4010	21	0.52	259	18	7

3.3. PTB true prevalence

As a randomized survey design was followed, and adult cattle of 24 months of age or older were sampled, a test sensitivity of 45% and a test specificity of 99% were assumed, according to Sweeney et al. (1995) and Sockett et al. (1992).

The overall median (quartiles) and average within-herd prevalence were, respectively, 7% (4–12) and 13%. The overall frequency distribution of the PTB true within-herd prevalence is shown in Fig. 1; of the positive herds, 93% had a maximum true within-herd prevalence of 30%. The true overall individual-animal prevalence was 2%.

Based on the aforementioned parameters, the overall IHD and the overall HSPEC were both 83%. Consequently, the overall true herd prevalence was 0.8%.

Fig. 2 depicts the calculated IHD, HSPEC, and herd true prevalences according to a range of test sensitivities and specificities of, respectively, 25–55 and 99.0–99.9%, a median adult herd sample size of 19 animals, and a PTB overall true within-herd prevalence of 7%. It shows that, for a test sensitivity of 45%, the true herd prevalence estimation decreased from 36 to 0.8% if the test specificity decreased from 99.9 to 99%, respectively.

The alternative approach consisting in using the cut-off of two positive test results, led to a true herd prevalence estimation of 4.7% (22/464) (Table 2). In the group of 60 herds with only one positive test result there was one herd with historical evidence of PTB, and five other herds with small adult herd size (≤ 5). Consequently, the estimated true herd prevalence is 6% (28/464). The herd-type-specific true prevalence parameters are summarized in Table 4.

4. Discussion

4.1. PTB seroprevalence

The present survey aimed to provide an unbiased estimate of the true national PTB herd prevalence by random selection of herds to sample. Because the percentage of non-responding herds was low (14%), this random sample of herds can be considered to be representative for the Belgian cattle population (Thrusfield, 1995). This was also evidenced by the fact that there was no difference in the average herd size of the target and study population.

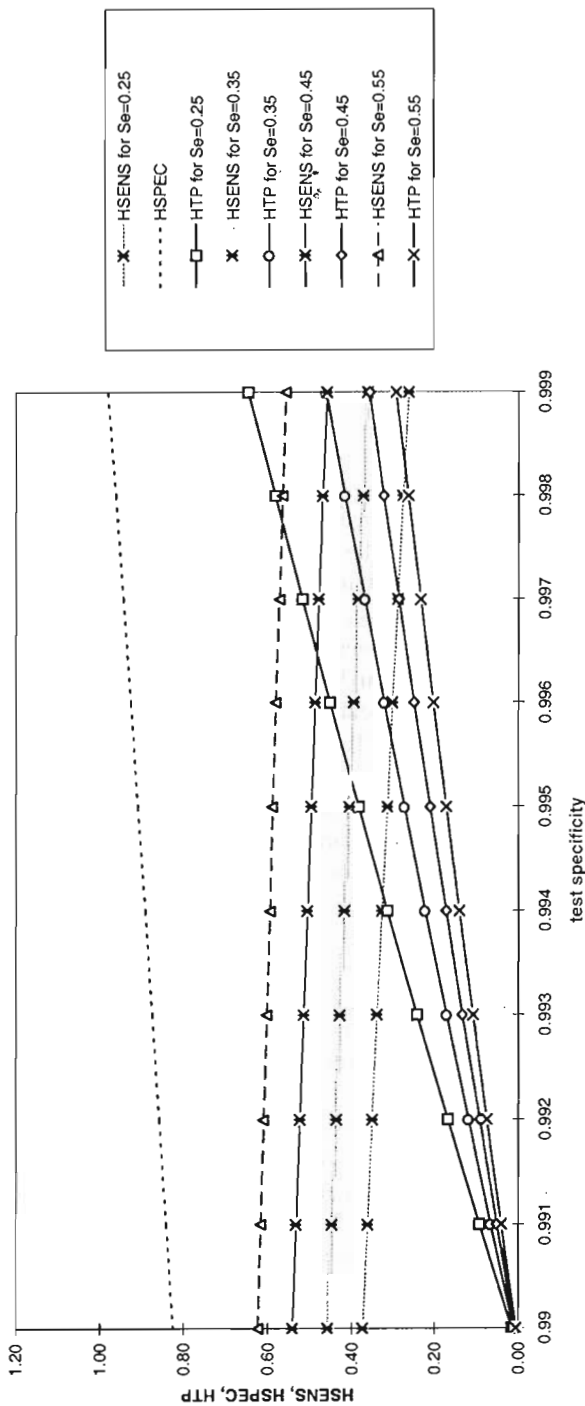


Fig. 2. Sensitivity analysis of the paratuberculosis infected herd detectability, herd specificity and herd true prevalence in non-vaccinated herds in Belgium, 1998, assuming a true within-herd prevalence of 7%.

Table 4
Estimates of the national prevalences of paratuberculosis in non-vaccinated herds per herd type in Belgium, 1998

Herd	Within-herd prevalence (%) ^a		True individual-animal prevalence ^a			True herd prevalence ^b		
	Median (quartiles)	Average	<i>N</i>	<i>N</i> _{pos}	%	<i>N</i>	<i>N</i> _{pos}	%
Dairy	5 (4–11)	8	4497	209	5	98	10	10
Mixed	7 (4–10)	10	4643	159	3	101	11	11
Beef	9 (3–36)	27	4010	76	2	259	7	3

^a Assuming (1) a median sample size of 46 for dairy herds, 39 for mixed herds, and 6 for beef herds; (2) a test sensitivity of 45%, and a test specificity of 99% and (3) non-reactor herds non-infected.

^b Including herds with ≥ 2 positive animals, herds with one positive test result if there was historical evidence of PTB (previous diagnosis and/or clinical signs) on the farm, and herds with small adult herd size (≤ 5) with one positive test result.

When comparing the apparent prevalences to published figures of seroprevalence, the following observations can be made. The average Belgian PTB within-herd seroprevalence of dairy herds, 3.3% was lower than the average figure of 20% found by Collins et al. (1994). At the individual-animal level, the Belgian figures for dairy and beef cattle, 1.16 and 0.52%, respectively, are lower than analogue figures for other countries published so far, ranging from 7.3% in Wisconsin (Collins et al., 1994) to 17.1% in Florida (Braun et al., 1990) for dairy cattle and from 1.2% in Finland (Hintikka and Seuna, 1998) to 25.2% in beef cattle in Texas (Alexander et al., 1993). Also the Belgian dairy and beef herd seroprevalences, 32 and 7%, respectively, are lower than analogue figures for other countries published so far, ranging from 50% in Wisconsin (Collins et al., 1994) to 74% in Missouri (Thorne and Hardin, 1997) for dairy herds and from 30% in Louisiana (Turnquist et al., 1991) to 40% in Missouri (Thorne and Hardin, 1997) for beef herds.

4.2. PTB true prevalence

The aforementioned calculations assume a perfect test sensitivity and specificity of 100%. Because no test is perfect, the testing procedure could also have been a source of information bias.

Considering that in the selected herds all adult animals were tested, the reactor herds provided data without sampling bias for estimation of the true within-herd prevalence, compared to studies with a within-herd sample-based design. The median true within-herd prevalence of PTB seropositive herds was 7%. This estimation assumed non-reactor herds to be non-infected, which is a potential bias, because the use of tests of poor sensitivity to attempt to substantiate freedom from diseases of low within-herd prevalence is extremely difficult (Cameron and Baldock, 1998). Consequently, the present estimated PTB within-herd prevalence, based on seropositive herds, could be an overestimation.

Corrected for testing procedures the overall true within-herd prevalence and the overall true individual-animal prevalences increased to 7 and 2%, respectively. The Belgian true

PTB within-herd prevalence of dairy herds, 5% was comparable with the figure of 5% found by Obasanjo et al. (1997) based on whole herd examination by fecal culture. The Belgian true individual-animal prevalence in dairy cattle, 5%, was in line with the estimation of 4.8% in Wisconsin (Collins, 1994) and of 6.1% in Ontario (NcNab et al., 1991).

When true herd prevalence calculations were applied to PTB, problems arose because of the poor sensitivity of the available diagnostic tests, the low within-herd prevalence of infection, and clustering of false positives within a herd (Jordan, 1996). In the case of PTB, animals usually become infected as calves and develop clinical disease as adults several years later (Chiodini et al., 1984). Antibodies to *M. paratuberculosis* appear to occur late in the course of the infection, albeit before the onset of clinical signs. Thus, the pathobiology of PTB somewhat limits the ability of tests for serum antibodies to detect animals in the early stages of a *M. paratuberculosis* infection (Collins, 1996). The absorbed ELISA is, at present, the most sensitive and specific test for serum antibodies to *M. paratuberculosis* (OIE, 1996). Ridge et al. (1991) found an absorbed ELISA to have a sensitivity of 88.3% in clinical cases, and 48.8% in subclinical cases; whereas the specificity was 99.8%. Sweeney et al. (1995) showed that the sensitivity in low-level fecal shedders could be as low as 15%. Although no published data of sensitivity and specificity of the absorbed ELISA kit, used in this survey, exist, the overall sensitivity and specificity of absorbed ELISAs are considered to be, respectively, 45% and greater than 99% (Collins, 1996). The probability of false positives created problems in classifying seropositive herds as being infected herds, especially those with only one single positive test result. This classification problem was particularly important in this study because 73% of herds testing positive had only one single positive test result. The lack of test specificity has a dramatic effect on the estimation of the true herd prevalence; some decimal changes in test specificity result in a true herd prevalence being two, three or more times higher or lower, for constant test sensitivity, true within-herd prevalence and sample size. For instance, assuming a test sensitivity of 45% and a true within-herd prevalence of 7%, the true herd prevalence estimation decreases from 36 to 0.8% if the test specificity of the absorbed ELISA decreases from 99.9 to 99%, respectively. Lack of test sensitivity leads to higher estimations of the true herd prevalence, with a greater impact at higher specificity levels. The true herd prevalence calculations revealed the implications of the aforementioned parameters, as depicted in Fig. 2. This sensitivity analysis showed that the practical limits of the accuracy of the used screening test jeopardize the estimation of the true herd prevalence within reasonable confidence intervals.

For this reason we used an approach that increased the herd specificity. If the herd specificity was less than 95%, we raised the cut-off number of positive cattle required, as described by Jordan (1996), and we included herds with one positive test result if there was historical evidence of PTB (previous diagnosis and/or clinical signs) on the farm. The latter was an attempt to use available information from the herds to correctly identify herds that have only one positive test as truly positive herds. If the herd specificity was at least 95%, i.e. if the adult herd size was 5 (maximum), we assumed that herds were truly infected even if they had only one positive test result. Consequently, our best estimate of the true herd prevalence of *M. paratuberculosis* infection is 6%. The Belgian true dairy

herd prevalence, 10%, is higher than in England, 1.3% (Cetinkaya et al., 1998) and lower than in Wisconsin, 34% (Collins, 1994).

This pilot study provides estimates regarding the PTB prevalence in the Belgian dairy, mixed and beef cattle population. A risk factor study considering all herd and management characteristics possibly associated with the PTB herd prevalence would be extremely beneficial.

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References

- Alexander, J.L., Mandy, W., Glass, R., 1993. Results of the ELISA seroprevalence survey for antibodies to paratuberculosis in Texas dairy and beef herds. In: Proceedings of the Annual Meeting of the United States Animal Health Association, October 23–29, Los Vegas, Nevada, USA, Vol. 97, p. 307.
- Benedictus, G., Dijkhuizen, A.A., Stelwagen, J., 1987. Economic losses due to paratuberculosis in dairy cattle. *Vet. Rec.* 121, 142–146.
- Boelaert, F., Biron, P., Soumare, B., Dispas, M., Vanopdenbosch, E., Vermeersch, J.P., Raskin, A., Dufey, J., Berkvens, D., Kerkhofs, P., 2000. Prevalence of bovine herpesvirus 1 in the Belgian cattle population. *Prev. Vet. Med.* 45, 283–293.
- Braun, R.K., Buergelt, C.D., Littell, R.C., Linda, S.B., Simpson, J.R., 1990. Use of an enzyme-linked immunosorbent assay to estimate prevalence of paratuberculosis in cattle of Florida. *J. Am. Vet. Med. Assoc.* 196 (8), 1251–1254.
- Buergelt, C., Duncan, J.R., 1978. Age and milk production data of cattle culled from a dairy herd with paratuberculosis. *J. Am. Vet. Med. Assoc.* 173 (5), 478–480.
- Cameron, A.R., Baldock, F.C., 1998. A new probability formula for surveys to substantiate freedom from disease. *Prev. Vet. Med.* 34, 1–17.
- Cetinkaya, B., Erdogan, H.M., Morgan, K.L., 1998. Prevalence, incidence and geographical distribution of Johne's disease in cattle in England and the Welsh borders. *Vet. Rec.* 143, 265–269.
- Chiodini, R.J., Van Kruiningen, H.J., 1986. The prevalence of paratuberculosis in culled New England cattle. *Cornell Vet.* 76, 91–104.
- Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell Vet.* 74, 218–262.
- Collins, M.T., 1996. Diagnosis of paratuberculosis. *Vet. Clin. North Am.: Food Anim. Practice* 12 (2), 357–371.
- Collins, M.T., Sockett, D.C., Goodger, W.J., Conrad, T.A., Thomas, C.B., Carr, D.J., 1994. Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin. *J. Am. Vet. Med. Assoc.* 204 (4), 636–641.
- Hintikka, E.L., Seuna, E., 1998. Paratuberculosis — a review and survey in Finland. *Suomen-Eläinlääkärilehti* 104 (1), 12–16.
- Jordan, D., 1996. Aggregate testing for the evaluation of Johne's disease herd status. *Aust. Vet. J.* 73 (1), 16–19.
- 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.
- McNab, W.B., Meek, A.H., Duncan, J.R., Martin, S.W., Van Dreumel, A.A., 1991. An epidemiological study of paratuberculosis in dairy cattle in Ontario: study design and prevalence estimates. *Can. J. Vet. Res.* 55 (3), 246–251.
- Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State paratuberculosis control program. *Prev. Vet. Med.* 32 (34), 243–251. ϵ
- OIE, 1996. *Manual of Standards for Diagnostic Tests and Vaccines*. Paris, France.
- OIE, 1998. *International Animal Health Code*. Paris, France.
- Ridge, S.E., Morgan, I.R., Sockett, D.C., Collins, M.T., Condron, R.J., Skilbeck, N.W., Webber, J.J., 1991. Comparison of the Johne's absorbed EIA and the complement fixation test for the diagnosis of Johne's disease in cattle. *Aust. Vet. J.* 68, 253–257.
- Sockett, D.C., Conrad, T.A., Thomas, C.B., Collins, M.T., 1992. Evaluation of four serological tests for bovine paratuberculosis. *J. Clin. Microbiol.* 30 (5), 1134–1139.
- Sweeney, R.W., Whitlock, R.H., Buckley, C.L., Spencer, P.A., 1995. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. *J. Vet. Diagn. Invest.* 7 (4), 488–493.
- Thorne, J.G., Hardin, L.E., 1997. Estimated prevalence of paratuberculosis in Missouri, USA cattle. *Prev. Vet. Med.* 31 (12), 51–57.
- Thrusfield, M., 1995. *Veterinary Epidemiology*, 2nd Edition. Blackwell Scientific Publications, Cambridge, UK.
- Turnquist, S.E., Snider, T.G., Kreeger, G.M., Miller, J.E., Hagstad, H.V., Olcott, B.M., 1991. Serological evidence of paratuberculosis in Louisiana beef cattle herds as detected by ELISA. *Prev. Vet. Med.* 11 (2), 125–130.
- Vannuffel, P., Gilot, P., Limbourg, B., Naerhuyzen, B., Dieterich, C., Coene, M., Machtelinckx, L., Cocito, C., 1994. Development of species-specific enzyme-linked immunosorbent assay for diagnosis of Johne's disease in cattle. *J. Clin. Microbiol.* 32 (5), 1211–1216.