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Original Scientific Article

SEROPREVALENCE OF CATTLE PARATUBERCULOSIS IN SLOVENIA IN 2008 AND A COMPARISON OF DATA FROM CURRENT AND PREVIOUS STUDIES

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Summary: Paratuberculosis is caused by *Mycobacterium avium* subsp. paratuberculosis (Map) and affects many animal species, with ruminants as usual hosts. Due to the lack of successful treatment, infection leads to chronic wasting and death of the affected animals, causing serious economic losses in addition to the spread of public fear connected to the possible role of Map in Crohn's disease. It is also a common disease of ruminants in Slovenia. Because of the lack of prevalence data since 2001, the purpose of this work was to estimate the seroprevalence of paratuberculosis in cattle herds in Slovenia. Animals older than two years in 20% of herds, originating from all different areas of Slovenia were tested in 2008 for the presence of antibodies against Map. A total of 38,374 sera from 6,779 cattle herds were initially examined by the in-house ELISA screening method, and positive or suspect sera were subjected to second screening and final confirmation by applying the Pourquier ELISA Paratuberculosis kit (Institut Pourquier, France). A positive result was obtained in 228 (0.59%) animals from 188 (2.77%) herds, resulting in true-prevalence (TP) estimates of 3.96% at animal and 18.49% at herd level. Currently, TP of paratuberculosis in cattle in Slovenia is lower than at the time of monitoring in 1999, when a comparable number of animals were tested but has remained similar at the herd level. Compared to many European countries, both the animal and the herd prevalences in Slovenia are fairly low, which can be partly attributed to the existence of numerous "family" farms, with a small number of animals per herd, since Map more easily spreads through an infected herd than among different herds. If the breeding strategy changes and animal trade with other countries increases, the present favorable situation in Slovenia will probably also change.

Key words: paratuberculosis; *Mycobacterium avium* subsp. *paratuberculosis;* cattle; seroprevalence; ELISA

Introduction

Paratuberculosis is a chronic infectious disease of ruminants but it also affects many other domestic and wild animals. It is caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). Map infection is acquired when animals feed on contaminated pastures but the most important mode of transmission is when newborns ingest Map from the faeces of paratuberculous cows; the disease can also be acquired *in utero* and when newborns ingest colostrum or milk from infected cows (1,2). The infection spreads from the intestinal tract to other organs and progresses through three clinical stages: asymptomatic with undetectable Map excretion, asymptomatic but excretory with a gradual increase of Map concentration in the intestinal mucosa and lumen, and symptomatic with heavy shedding of Map (2). The last stage of the disease is characterized by chronic diarrhea and symptoms of generalized infection, such as emaciation, decreased milk production, infertility and death. In cattle herds, animals with the asymptomatic form of paratuberculosis greatly outnumber those with the clinical disease (3,4).

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Fighting paratuberculosis is important in cattle breeding because of the major economic losses it causes (5), in addition to possible transmission of Map to humans in connection with Crohn's disease (6). In the USA, losses are estimated to over 1.5 billion dollars per year (2). Paratuberculosis is also a common disease of ruminants in Slovenia (7). The first case was detected in 1961 in imported Jersey cows. No other cases were reported until 1993, when paratuberculosis was found in a sheep flock; since then, several outbreaks of the disease in cattle, goats and sheep have been documented (8).

An estimation of prevalence is needed for taking the right measures to control the disease. Systematic screening of paratuberculosis in Slovenia, based on the complement fixation test, therefore began in 1995. In order to estimate the prevalence and geographic distribution of paratuberculosis in Slovenia, 20% and 5% of cattle in all herds were tested in 1995 and 1996, respectively (7). In the following two years, 3% and 5% of cattle in all herds were tested by ELISA, which became the most commonly used assay for Map antibody testing (7). In 1999, 10-15% of cows and pregnant heifers were tested, while in 2000 and 2001, the survey was limited to bulls' mothers herds because of their significant involvement in selection for reproduction (9).

Since 2001, no data on paratuberculosis prevalence in Slovenia have been available. The purpose of this study, therefore, was to estimate the current seroprevalence of paratuberculosis in cattle herds in Slovenia and to compare data from current and previous studies.

Materials and methods

Animals older than two years were tested in 2008 for the presence of antibodies against Map in 20% of cattle herds, randomly selected from all areas of Slovenia (Table 1). A total of 38,374 sera from 6,779 herds were tested. After the initial lower-cost screening, performed by in-house ELISA (10), with similar sensitivity but lower specificity as demonstrated in a preliminary study comparing the in-house and the subsequently employed commercial ELISA kit, all positive or suspect sera were subjected to additional screening using the Pourquier ELISA Paratuberculosis – Paratub.Serum-S kit (Institut Pourquier, France) according to the manufacturer's instructions. Confirmation of positive samples was performed by using the Pourquier ELISA Paratuberculosis – Paratub.Serum-B antibody verification kit by the same manufacturer. Only animals testing positive using the verification kit and herds with at least one test-positive animal were considered Map-seropositive.

In addition to estimating the current seroprevalence of paratuberculosis in Slovenian cattle, a comparison of the prevalence data from the present study with data collected in previous studies was made (Table 2). The apparent prevalence (AP) was calculated as defined by Nielsen and Toft (11). The true prevalence (TP) was estimated from the AP by correction with the Rogan-Gladen estimator (12) according to the following formula: $TP = (AP + Sp - 1) \times (Sp + Se - 1)^{-1}$; the previously reported most likely test accuracies for cattle serum ELISA by Idexx Laboratories Inc. (USA) and Institut Pourquier (France) were employed: a sensitivity (Se) of 0.088 and 0.15, and specificity (Sp) of 0.976 and 1.0, respectively (11). At the herd level, the parameters AP, TP, Sp and Se were termed HAP, HTP, HSp and HSe; animal- and herd-level parameters were considered similar (11).

Results

A total of 228 (0.59%) animals from 188 (2.77%) herds tested positive for paratuberculosis (Table 1), resulting in calculated true-prevalence estimates of 3.96% (TP) and 18.49% (HTP) (Table 2). The majority of the positive herds originated from areas of NM and MB located in the south-eastern and north-eastern parts of Slovenia, respectively. In herds with more than one seropositive animal, almost half (47%) of the positive animals were of the Black-and-White (Holstein-Friesian) breed (*data not shown*).

Comparison of data from the present and previous studies on paratuberculosis seroprevalence in cattle in Slovenia is shown in Table 2. The estimates of TP at animal and herd level were highest in 1999 (TP 15.50%) and 1998 (HTP 63.74%) respectively. In 2000-2001, HTP was also high (77.26%) but reflected prevalence in bulls' mothers herds only. In view of the comparable number of cattle sampled in 1999 and 2008, albeit originating from a different number of herds, estimates of TP from these two periods were compared: in 2008, the prevalence of paratuberculosis at the animal level decreased (15.50% in 1999 vs. 3.96% in 2008) but remained similar at the herd level (25.69% in 1999 vs. 18.49% in 2008).

Table 1: Distribution of collected and positive samples and number of investigated and infected herds according to different areas of Slovenia

Area*	No. of samples		No. of herds	
	Collected	Positive (%)	Investigated	Infected (%)
LJ-CE	12692	71 (0.56)	2242	64 (2.85)
MB	6470	37 (0.57)	1143	35 (3.06)
PT	2900	8 (0.28)	512	8 (1.56)
KR	3712	28 (0.75)	656	17 (2.59)
NM	5934	53 (0.89)	1048	38 (3.63)
MS	3684	19 (0.52)	651	17 (2.61)
GO	2982	12 (0.40)	527	9 (1.71)
Total	*38374*	*228 (0.59)*	*6779*	*188 (2.77)*

* LJ-CE, area of Ljubljana and Celje; MB, area of Maribor; PT, area of Ptuj; KR, area of Kranj; NM, area of Novo mesto; MS, area of Murska Sobota; GO, area of Nova Gorica

Table 2: Animal and herd level apparent and true prevalence of paratuberculosis in cattle in Slovenia obtained from the present and previous studies

Study period	Animals				Herds				Test	Ref.
	All[a]	Pos[b]	AP[c] [%]	TP[d] [%]	All[a]	Pos[b]	HAP[c] [%]	HTP[d] [%]		
1997	11513	47	0.41	-31.12	1690	48	2.84	6.89	A	7
1998	12082	140	1.16	-19.39	2423	157	6.48	63.74	A	7
1999	38469	1305	3.39	15.50	26088	1055	4.04	25.69	A	9
2000-2001	9388	41	0.44	2.91	302	35	11.59	77.26	B	9
2008	38374	228	0.59	3.96	6779	188	2.77	18.49	B	This study

Note: The study period 2000-2001 contained cattle sera from bulls' mothers herds only. The employed paratuberculosis ELISA kits for cattle sera were manufactured by Idexx Laboratories Inc., USA (A) and Institut Pourquier, France (B).

[a] No. of collected samples (animals) and investigated herds (herds); [b] No. of positive samples (animals) and infected herds (herds); [c] Apparent seroprevalence of paratuberculosis at animal (AP) and herd (HAP) levels according to Nielsen and Toft (11); [d] True seroprevalence of paratuberculosis at animal (TP) and herd (HTP) levels according to Nielsen and Toft (11); negative TP should be considered as 0%

Discussion

The slow progression of paratuberculosis, the non-specific clinical signs, irregular faecal shedding of Map, the long incubation period of the disease and slow growth of Map on culture media make reliable diagnosis a difficult task, especially due to the lack of highly sensitive and specific diagnostic tests (2,13). Enzyme immunoassays are very suitable for the detection of Map antibodies but have to be adapted to remove cross-reacting antibodies (14). However, the faecal shedding of large quantities of Map provides a pressing reason for early and reliable diagnosis in order to limit the spread of paratuberculosis within and among cattle herds. To control the spread of the disease, farms rearing animals for reproduction must be free of paratuberculosis or, under Slovenian national legislation, they lose their status of a farm with a permit for ova and embryo donation (9). In addition, an annual governmental decree on the general monitoring of paratuberculosis in Slovenia was passed from

1995 to 1999, but has not been renewed since then. Due to the lack of financial support, data on the seroprevalence of paratuberculosis in cattle herds in Slovenia has been lacking for the past several years.

The present study showed that the herd prevalence in Slovenia has remained at almost the same level as it was about ten years ago and it is fairly low compared to many European countries (11). This can be partly attributed to the small number of animals per herd, *i.e.*, family-farm breeding, which results in the limited spread of Map between different herds (9). To some extent, differences in prevalence on both animal and herd levels observed over the years in Slovenia, reflect not only the actual prevalence but also the different populations tested, the number of animals and herds included in the test and the use of ELISA kits with different sensitivities. However, comparing data from the present study with previous data leads to interesting conclusions. For example, a marked increase in prevalence at the herd level was observed for the period from 1997 to 1998 (7). In those two years, mostly older animals were selected for testing, since paratuberculosis has slow progression and ELISA tests will usually not detect infected animals aged less than two years (15). In 1998, a larger number of herds were selected for monitoring in comparison to 1997 and more older animals were tested. A higher HTP was therefore not surprising, and the extent of the increase may also be partially explained by the improvement of the Idexx ELISA test reported by the manufacturer for 1998. In 1999, a marked increase in prevalence at the animal level was observed (9). More animals were tested in comparison to 1998, leading to a higher TP estimate. In contrast, HTP in 1999 decreased, possibly because many more cattle herds were inspected, with an average of 1.47 animals per herd selected for testing; with fewer animals tested per herd, the probability of detecting Map-positive herds was lower. Due to a change in the selection criteria for animals and herds (*i.e.*, all animals over the age of two years from bulls' mothers herds) and in the ELISA kit for testing (9), the results from the period 2000-2001 are difficult to compare with the previous ones; since 2000, the ELISA kit manufactured by Institut Pourquier (France), with higher specificity and sensitivity (11), has been used for Map-serological testing in Slovenia.

In 2008, all animals older than two years were tested, originating from 20% of randomly selected cattle herds in Slovenia. These data were compared to those from 1999, as reflecting sampling groups of a similar size, although a lower number of herds were inspected in 2008, with a higher number of tested animals per herd (an average of 5.67 animals per herd in 2008 vs. 1.47 in 1999). Since the disease is less prone to spread between herds than within an infected herd, the lower number of inspected herds does not necessarily mean a markedly lower number of Map-positive animals, if more animals per herd are sampled. Due to the non-homogeneous distribution of Map-positive cattle herds in Slovenia, the random-sampling strategy was of utmost importance for generating reliable data on the current seroprevalence of paratuberculosis in cattle. Since the ELISA kit for Map-serological testing in 2008 had higher sensitivity and specificity than the kit employed in 1999 (11), the lower TP at the animal level probably reflects a more favourable present situation in Slovenia.

In many countries, herd level prevalences are likely to be >50% and estimates of animal level prevalences have been reported to be approximately 20% or at least 3-5% in several countries (11). Despite changes in the criteria for the selection of animals in Map-testing during previous years, Slovenia still ranks among countries with the lowest paratuberculosis prevalence at the animal and herd levels. The relatively good present situation could change rapidly in the near future, due to the unlimited trade of animals in the European Union. Moreover, in-country animal trade, including large dairy cattle herds with the Black-and-White breed, which currently represents 19% of cattle in Slovenia and is most commonly infected, may also contribute to the spread of paratuberculosis.

In general, our findings contribute to current knowledge on paratuberculosis prevalence in the European countries. Due to the increasing trade and changes in the animal breeding strategy, *i.e.*, reduction in the number of herds and increase in the number of animals per herd, the promising results for our country are an encouragement to policy makers to make a prompt decision and prepare effective measures for surveillance and control of the disease.

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References

1. Sweeney RW, Whitlock RH, Rosenberg AE. *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. Am J Vet Res 1992; 53: 477-80.

2. Cocito C, Gilot P, Coene M, De Kesel M, Poupart P, Vannuffel P. Paratuberculosis. Clin Microbiol Rev 1994; 7: 328-45.

3. Brugère-Picoux J. Le diagnostic de la paratuberculose chez les ruminants. Rec Med Vet 1987; 163: 539-46.

4. Whitlock RH, Buergelt C. Preclinical and clinical manifestations of paratuberculosis (including pathology). Vet Clin North Am Food Anim Pract 1996; 12: 345-56.

5. Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev Vet Med 1999; 40: 179-92.

6. Hermon-Taylor J. *Mycobacterium avium* subspecies *paratuberculosis* in the causation of Crohn's disease. World J Gastroenterol 2000; 6: 630-2.

7. Ocepek M, Posedi J, Pislak M. Prevalence of bovine paratuberculosis in Slovenia in 1997 and 1998. Zb Vet Fak Univ Lj 1999; 36: 111-9.

8. Juntes P, Prevorčnik J, Pogačnik M. Epidemiologija in diagnostika paratuberkuloze v Sloveniji. In: Zbornik referatov 3. problemske conference "Razvoj veterinarstva v tranziciji". Čateške toplice: Slovenska veterinarska zveza, 1995: 120-6.

9. Ocepek M, Krt B, Pate M, Pogačnik M. Seroprevalence of paratuberculosis in Slovenia between 1999 and 2001. Slov Vet Res 2002; 39: 179-85.

10. Pislak M. Primerjava učinkovitosti različnih seroloških metod za ugotavljanje paratuberkuloze pri drobnici = Evaluation of the effectiveness of different serological methods for the diagnosis of paratuberculosis in small ruminants: magistrsko delo. Ljubljana: Veterinarska fakulteta, 1997.

11. Nielsen SS, Toft N. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev Vet Med 2009; 88: 1-14.

12. Rogan WJ, Gladen B. Estimating prevalence from the results of a screening test. Am J Epidemiol 1978; 107: 71-6.

13. Nielsen SS, Toft N. Ante mortem diagnosis of paratuberculosis: a review of accuracies of ELISA, interferon-gamma assay and faecal culture techniques. Vet Microbiol 2008; 129: 217-35.

14. Bech-Nielsen S, Jorgensen JB, Ahrens P, Feld NC. Diagnostic accuracy of a *Mycobacterium phlei*-absorbed serum enzyme-linked immunosorbent assay for diagnosis of bovine paratuberculosis in dairy cows. J Clin Microbiol 1992; 30: 613-8.

15. Nielsen SS, Ersbøll AK. Age at occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in naturally infected dairy cows. J Dairy Sci 2006; 89: 4557-66.

SEROPREVALENCA GOVEJE PARATUBERKULOZE V SLOVENIJI V LETU 2008 TER PRIMERJAVA S PODATKI PREDHODNIH ŠTUDIJ

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Povzetek: Paratuberkulozo povzroča bakterija *Mycobacterium avium* subsp. paratuberculosis (Map) in prizadene mnoge živalske vrste, njeni običajni gostitelji pa so prežvekovalci. Ker uspešno zdravljenje ne obstaja, okužba vodi do kroničnega hiranja in pogina prizadetih živali. To povzroča znatne ekonomske izgube, poleg tega pa tudi širi strah, ki je povezan z možno vlogo Map pri kronovi bolezni (Chronova bolezen). Paratuberkuloza je pogosta bolezen prežvekovalcev tudi v Sloveniji. Zaradi pomanjkanja podatkov o prevalenci od leta 2001 naprej je bil namen našega dela oceniti seroprevalenco paratuberkuloze v govejih čredah v Sloveniji. Leta 2008 smo v 20 % čred z vseh področij Slovenije živali, starejše od dveh let, testirali na prisotnost protiteles proti Map. Z domačim ELISA presejalnim testom smo na začetku raziskave pregledali 38374 serumov živali iz 6779 govejih čred. Vse pozitivne ali sumljive serume smo pregledali z drugim presejalnim ter nato s končnim potrditvenim testom Pourquier ELISA Paratuberculosis kit proizvajalca Institut Pourquier, Francija. Pozitivnih je bilo 228 (0,59 %) živali iz 188 (2,77 %) čred, kar smo lahko ocenili kot 3,96 % in 18,49 % pravo seroprevalenco (PS) na nivoju živali in čred. Trenutno je PS paratuberkuloze goved v Sloveniji nižja kot leta 1999, ko smo testirali primerljivo število živali, vendar je ostala podobna na nivoju čred. V primerjavi z mnogimi evropskimi državami je prevalenca v Sloveniji tako na nivoju živali kot na nivoju čred precej nizka. To zaradi lažjega širjenja Map znotraj okužene črede v primerjavi s širjenjem med različnimi čredami lahko delno pripišemo dejstvu, da je v Sloveniji mnogo »družinskih« kmetij z majhnim številom živali v čredi. Če se bo način reje spremenil in se bo med državami povečalo trgovanje z živalmi, se bo verjetno spremenila tudi trenutno ugodna situacija v Sloveniji.

Ključne besede: paratuberkuloza; *Mycobacterium avium* subsp. paratuberculosis; govedo; seroprevalenca; ELISA