

# Seroprevalence of bovine paratuberculosis infections in Poland<sup>\*)</sup>

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### Summary

Paratuberculosis (JD) is a chronic contagious inflammation of the intestines of ruminants that causes considerable economic losses. The diagnosis of MAP infections could be the basis for the development of a JD eradication programme. The aim of the study was to determine the seroprevalence of JD in cattle herds in two districts of north-eastern Poland. Serum samples taken from 1,203 cattle herds were subjected to a commercial ELISA test (ELISA paratuberculosis screening test<sup>®</sup>, Institut Pourquier). Animals with positive reactions to paratuberculosis were detected in 76 herds. Considerable differences between the two districts were observed in the results obtained: in District I the seroprevalence of JD amounted to 3.2%, whereas in District II it was 0.8%. The differences in seroprevalence were related to the size of the herds.

**Keywords:** seroprevalence, cattle, *Mycobacterium avium* subsp. paratuberculosis

Johne's disease (paratuberculosis) is a chronic contagious inflammation of the intestines of both domestic and wild ruminants. An etiological factor of this disease is *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which is a Gram-positive, slowly growing, acid-resistant mycobacterium. MAP is also insusceptible to a number of environmental factors as well as disinfecting and therapeutic agents. It is transmitted primarily through the fecal-oral route, especially from manure and environment contamination by infected adult cattle, whereas young cattle are reported to be the most susceptible to MAP infection (18). Cases of paratuberculosis (JD) have currently been recorded in most European countries (10), in both Americas (8, 9), Asia (17) and Australia and New Zealand (7, 16). As shown in scientific reports, paratuberculosis causes considerable economic losses (1, 11), especially in herds of dairy cattle, in which its incidence is statistically higher than in herds of meat-type cattle (2). Given the extent of economic losses, MAP occurrence in milk (including pasteurized milk) (5), and the insufficiently elucidated role of the mycobacterium in Crohn's disease in humans, it is necessary to undertake research into the dissemination of JD as a potential zoonosis. Diagnosis of the disease is difficult because of its long period of incubation (3,

9). Depending on the severity of clinical symptoms, potential excretion of the mycobacterium to the environment, and the possibility of detecting the disease by known methods, four forms of the disease may be distinguished: latent, sub-clinical, clinical and clinically-advanced (21). Studies have shown a considerably higher incidence of the sub-clinical and latent forms in herds of dairy cattle (4). In Poland, no programme for JD eradication has been developed to date, owing to the long-established belief that paratuberculosis has been eradicated in the country. This view is based on the programme for the eradication of bovine paratuberculosis implemented in the 1960-70s, when all animals testing positive in a nationwide tuberculin skin test were culled. Randomized serological analyses conducted in 2004 in herds of dairy cattle from north-eastern Poland demonstrated, however, the presence of MAP-specific antibodies in 25% of herds selected for analyses (19). The incidence of the clinical form of paratuberculosis was confirmed by authorities in a report submitted to the OIE in 2006.

In view of the above, this study was undertaken to determine the seroprevalence of JD in cattle herds from two districts of north-eastern Poland characterized by different herd sizes, as well as land and climatic conditions. Oriented serological surveys conducted to detect MAP infections in animals provide a basis for evaluating the existing epizootic threat and elabora-

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ting a programme for the monitoring and eradication of the disease.

### Material and methods

The material to be examined originated from two districts of north-eastern Poland, located in the area of Żuławy Wiślane (District I) and on a morainic plateau (District II). Blood samples were collected from 1,203 herds of dairy cattle registered in 2009 in the Central Database for Animal Identification and Registration managed by the Agency for Restructuring and Modernisation of Agriculture.

In total, 10,213 blood samples (ca. 10 ml per sample) from dairy cows were collected by selected veterinarians, who were simultaneously collecting samples for brucellosis analyses as part of a nation-wide programme for brucellosis monitoring ([http://www.wetgiw.gov.pl/files/3103\\_Programme-for-eradication-and-monitoring-of-bovine-brucellosis-in-Poland-in-2008](http://www.wetgiw.gov.pl/files/3103_Programme-for-eradication-and-monitoring-of-bovine-brucellosis-in-Poland-in-2008)). The samples were collected from February to June 2008. All samples were sent to a laboratory and centrifuged at  $3,220 \times g$  for 10 min. Samples of separated serum were frozen and kept at a temperature of  $-20^{\circ}\text{C}$  until serological analyses. Data for cattle identification, including the owner's name, age of the animal, herd seat number and ear tag number, were recorded and serum samples were coded. All samples were subjected to a commercial ELISA test according to the manufacturer's instructions (ELISA paratuberculosis screening test<sup>®</sup>, Institut Pourquier). This test measures serum antibodies against *Mycobacterium avium* subspecies *paratuberculosis* using an absorption step to remove non-specific antibodies. On each 96-well plate, 94 serum samples, as

well as a negative and positive control provided by the manufacturer, were tested in single wells. In accordance with the manufacturer's instructions, readings below 60% of the positive control serum OD were considered negative, and readings equal to or greater than 70% were considered positive, whereas readings between 60 and 70% were scored as doubtful. Positive and doubtful results were run into duplicate wells (one coated and one uncoated) with the same protocol. According to the manufacturer, the kit has a sensitivity of 50-65% and a specificity of over 99.5%.

The statistical analysis of the results of serological assays was conducted with the Statistica 9.0 PL software for Windows. Comparison of the seroprevalence level as affected by the age group, origin of the animals and size of the herd was carried out with an  $X^2$  test. Limits of confidence intervals were computed for seroprevalence using a precise binomial test with a 95% confidence interval (CL).

### Results and discussion

A total of 10,213 cattle from 1,203 herds were tested for antibodies against MAP. Analyses were carried out on 4,405 samples from 187 herds in District I and on 5,808 samples from 1,016 herds in District II. In total, 185 samples were positive as indicated by the ELISA kit (1.8%, 95% CL: 1.6-2.1). Animals with positive reactions to paratuberculosis were detected in 76 herds (6.3%, 95% CL: 5.0-7.8). Considerable differences between the two districts were observed in the results obtained: the level of seroprevalence in District I amounted to 3.2%, 95% CL: 2.7-3.7, whereas in District II it was 0.8%, 95% CL: 0.6-1.1. Differences in the results were also

Tab. 1. Seroprevalence of bovine paratuberculosis as affected by the age group in District I

Age group (years)	Number of animals examined (n)	Percentage of population (%)	ELISA-positive (n)	Prevalence (%) (p = 0.009)	95% CI (%)
< 2	429	9.7	1	0.2	0.0-1.3
2-4	1896	43.0	70	3.7	2.9-4.6
5-7	1678	38.1	57	3.4	2.6-4.4
8-10	315	7.2	9	2.9	1.3-5.4
> 10	87	2.0	2	2.3	0.3-8.1
Total	4405	100	139	3.2	2.7-3.7

Tab. 2. Seroprevalence of bovine paratuberculosis as affected by the age group in District II

Age group (years)	Number of animals examined (n)	Percentage of population (%)	ELISA-positive (n)	Prevalence (%) (p = 0.208)	95% CI (%)
< 2	970	16.7	2	0.2	0.0-0.7
2-4	2448	42.1	20	0.8	0.5-1.3
5-7	1450	25.0	15	1.0	0.6-1.7
8-10	554	9.5	5	0.9	0.1-2.1
> 10	386	6.6	4	1.0	0.3-2.6
Total	5808	100	46	0.8	0.6-1.1

observed as affected by the age of animals (tab. 1), and the highest seroprevalence level in District I was recorded in animals at the age interval of 2-4 years (at  $p = 0.009$ ). In District II, the highest seroprevalence was found in animals at the age interval of 5-7 years and over 10 years, but there were no significant differences between seroprevalence results depending on the age group of cattle ( $p = 0.208$ ) (tab. 2). In contrast, significant differences in seroprevalence occurred as affected by the size of the herd. The highest level of seroprevalence (4.2%, 95% CL: 3.4-5.1) was recorded in large herds (over 100 animals) from District I, whereas the herds in District II were considerably smaller (tab. 3 and 4). In District I, significant differences were also recorded in seroprevalence as affected by the country of origin. The differences occurred particularly in the seroprevalence level

**Tab. 3. Seroprevalence of bovine paratuberculosis as affected by the size of the herd in District I**

Herd size	Number of animals examined (n)	ELISA-positive (n)	Prevalence (%) (p = 0.0002)	95% CI (%)
1-10	293	2	0.7	0.1-2.4
11-100	1800	41	2.3	1.6-3.1
> 100	2312	96	4.2	3.4-5.1
Total	4405	139	3.2	2.7-3.7

**Tab. 4. Seroprevalence of bovine paratuberculosis as affected by the size of the herd in District II**

Herd size	Number of animals examined (n)	ELISA-positive (n)	Prevalence (%) (p = 0.102)	95% CI (%)
1-10	2120	10	0.5	0.2-0.9
11-100	3688	36	1.0	0.7-1.4
> 100	0	0	0.0	0.0-0.0
Total	5808	46	0.8	0.6-1.1

between the groups of animals originating from Poland (domestic) and those from other Member States of the EU (table 5). The seroprevalence level reported for animals originating from EU countries other than Poland was higher by 1.1 percentage points.

The first case of bovine paratuberculosis in Poland was described in 1970 (15). The programme for the eradication of bovine paratuberculosis implemented in the 1960-70s in Poland reinforced the belief shared by both veterinarians and breeders that paratuberculosis had been completely eradicated in the country. However, serological analyses of samples from selected herds of dairy cattle from north-eastern Poland confirmed the presence of MAP-specific antibodies (19). Serological surveys conducted in all herds in two districts of north-eastern Poland indicated seroprevalence in that area. Cattle herds bred in Poland differ significantly in size. Extrapolated data from the Main Statistical Office (GUS) for 2009 show that in 35.5% of all 2,688 cattle breeding farms the number of animals bred was 1-9, while in as few as 5.07% of the herds it was over 100. Despite the intermittent process of breeding concentration following Poland's accession to the EU, over 1/3 of farms still maintain small herds (of up to 9 cows). The number of large farms is

**Tab. 5. Seroprevalence of bovine paratuberculosis as affected by the origin of animals in District I**

Origin	Number of animals examined (n)	Percentage of population (%)	ELISA-positive (n)	Prevalence (%) (p = 0.000)	95% CI (%)
Domestic	9928	97.2	170	1.7	1.4-1.9
Imported (EU)	285	2.8	15	2.8	2.5-3.1
Total	10 213	100	185	1.8	1.6-2.1

gradually increasing, but not in all regions, with the greatest concentration of breeding being noted among others in north-eastern provinces. The process of concentration has been accompanied by the import of animals, originating in most cases from large herds. An example of these changes are two districts investigated for the seroprevalence of paratuberculosis in this study. In District II, there were no large herds (over 100 animals), and the percentage of imported animals was 0.3%, whereas in District I, herds of over 100 animals did occur, and imported cattle constituted 6% of the population. Such considerable differences are additionally affected by different land conditions in the two districts. Żuławy Wiślane are floodlands in the mouth of the Vistula river, whereas the other district is located on morainic plateaus crossed with sandur planes. Differences in landform features and the resulting diverse hydrological conditions in the two regions are likely to affect MAP survivability

in the environment (20). This may be one of the factors underlying the differences observed in JD dissemination.

The results of a survey by Nielsen and Toft (10) confirmed the incidence of JD in most European countries, including those neighbouring Poland (Germany, herd level apparent prevalence: 42%). Other studies show that in other European countries the percentage of herd population infected with MAP ranges from 3% to 68%. The mean seroprevalence determined on 114 farms in south-western England was 4.5% (22). In-depth analyses, carried out not only with serological methods, on the incidence of animal paratuberculosis and the dynamics of its dissemination were also conducted in the Czech Republic (12, 14). These analyses made it possible to develop an effective programme for its eradication (13). Investigations of herds from the Mecklenburg-West Pomerania region, in which 2,997 serum samples from dairy cows were analyzed using the Svanovir-ELISA, demonstrated the presence of antibodies in at least one sample in 84.7% of the herds examined (6). This study confirms the presence of seropositive animals (one or more) in 76 herds (6.3%, 95% CL: 5.0-7.8). The differences in the percentage of seropositive herds between District I (38 herds, 20.3%, 95% CL: 14.8-26.8) and District II (also 38 herds, 3.74%, 95% CL: 2.7-5.1) are significant.

The levels of JD seroprevalence in the examined districts of north-eastern Poland were significantly different: the seroprevalence was higher in the district with the higher number of cows per herd.

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