Estimated apparent and true prevalences of paratuberculosis in sheep herds of the Kars Region in Northeastern Turkey

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ABSTRACT: Paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis (Map)*, is one of the most prevalent and costly infectious diseases of livestock, particularly sheep and cattle herds. The aim of this study was to estimate true animal, within-herd, and between-herd prevalence of *Map* antibodies in sheep herds of the Kars Region in the Northeast part of Turkey. A seroprevalence study was carried out using a commercial ELISA kit. Twenty six sheep herds, non-vaccinated against *Map*, were randomly selected in different regions and in total 450 sheep aged 24 months and more were sampled. Herds were declared positive if one or more sheep in the herd tested positive for *Map* antibodies. The animal, within-herd, and between-herd apparent prevalences were calculated as 6.2% (95% CI = 4.3 to 8.8%), 10.2% (95 CI = 7.1 to 14.3%) and 57.7% (95% CI = 38.9 to 74.5%), respectively. True prevalences were estimated by conversion from apparent prevalences via the Rogan-Gladen estimator. True animal, within-herd, and between-herd prevalences were calculated as 8.3% (95% CI = 4.7 to 11.8), 14.6% (95 CI = 8.9 to 20.2) and 90% (95 CI = 59.8 to 120.1), respectively. The results provide useful information regarding the prevalence of *Map* infection in sheep herds in the Kars Region and will hopefully attract the special attention of veterinarians and promote the establishment of an efficient control programme.

Keywords: Johne's disease; extensity; ewe

Paratuberculosis is a chronic infectious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) (Twort and Ingram 1912). The disease is prevalent worldwide and has a significant financial impact on animal husbandry (Ott et al. 1999; Losinger 2006; Anna Rita et al. 2011). Although *Map*'s zoonotic potential is the subject of debate (Gitlin et al. 2012) the organism's ability to contaminate milk (Okura et al. 2012) plus its frequent detection in patients with Crohn's disease (Feller et al. 2007; Abubakar et al. 2008) implicate it as a potential public health hazard. The disease is also found in sheep in Turkey and has a progressive history with time (Hakioglu 1968; Ciftci and Hatipoglu 1991).

It is important to estimate valid and true prevalences of *Map* infection in order to determine whether the infection should be considered as important or not. Several direct and indirect detection methods are available for the diagnosis of *Map* infections. However, many of them lack specificity and sensitivity, due to the slow progress of infection and in particular, the often sub-clinical nature of *Map* infection (Whittington and Sergeant 2001). Therefore, it is imperative to estimate the true animal and herd-based prevalence of *Map* infection using a reliable diagnostic procedure. The enzyme-linked immunosorbent assay (ELISA) is the current method used in serological diagnosis of paratuberculosis; it can be conducted rapidly, results in reliable data and requires only limited expertise (McKenna et al. 2005).

Paratuberculosis is widespread in ruminant populations in almost all countries with dairy industry (Nielsen and Toft 2009). In several countries, disease-control programs have been developed to reduce *Map* prevalence in the participating dairy

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farms (Benedictus et al. 2000; Whittington and Sergeant 2001). Currently available *Map* control strategies include management measures to improve hygiene, the culling of serological- or faecal-positive animals, and vaccination. Although the first two control strategies have been reported to be effective in reducing the incidence of *Map* infection, the changes in herd management needed to conduct these control strategies require significant effort on the part of the dairy producer (Cho et al. 2012).

The last strategy is vaccination; it clearly prevents the appearance of clinical cases if done properly and is a highly cost-efficient strategy (Fridriksdottir et al. 2000). The main drawback to vaccination is that, since vaccines used in the field do not differentiate infected from vaccinated, it can interfere with serological diagnosis of paratuberculosis and tuberculosis infections (Bastida and Juste 2011).

Consequently, it has been suggested that herd and animal prevalence levels will influence *Map* control programs (Nielsen and Toft 2009). Control of clinical signs, with vaccination and management practices, should be considered in herds with high within-herd prevalence, management practices should be considered in herds with middle within-herd prevalence and surveillance should be considered in cases of the likely absence of *Map* infection (Mercier et al. 2010).

Currently, there is neither a specialised nationwide control programme in Turkey, nor a regional programme in Kars. Therefore, the data reported here provide a general indication of the true state of *Map* infection in sheep in the Kars Region, and suggest that a control program could be considered in the prevention of this disease.

Thus, the present study was conducted to estimate the true animal, within-herd, and between-herd prevalence of *Map* antibodies in sheep herds in the Kars Region.

MATERIAL AND METHODS

Study design. A cross-sectional study approved by the local ethical committee of animal experiments at Kafkas University (Protocol no. KAU-HADYEK/2011-31) was carried out. Simple random sampling was conducted with the herd as the epidemiological unit of concern. The main criteria for the selection of herds and individual animals were the size of herds (> 100), clinical suspicion of paratuberculosis, and the use of animals over two years of age, respectively. Sample size was calculated as three hundred and eighty four using a confidence level of 95% and confidence interval (CI) of 5% and considering the sheep number of Kars Region as approximately five hundred and forty three thousand (data were obtained from the Kars Province of Food, Agriculture and Animal Husbandry Department).

Serum samples. In total, four hundred and fifty blood samples were randomly taken from 26 herds, although 384 samples would have been sufficient. Herds with no history of vaccination against *Map* consisted of female individuals over two consecutive years. The samples were collected from the villages of Kars and its counties between January and August 2013 (Table 1). Samples were submitted to the department of microbiology (Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey) within 24 h after bleeding. Serum samples were separated and stored at –20 °C pending analysis.

ELISA procedure. A commercial ELISA kit (IDEXX Laboratories, Inc., Westbrook, Maine) was used for detection of antibodies against *Map* in sheep serum samples. The test procedure and interpretation of the results were carried out according to the manufacturer's instructions. Results were expressed as sample to positive (S/P) ratio after correction with the negative control. Samples with S/P ratios equal to or greater than 55% were considered to be positive.

Statistical analysis. Statistical differences of ELISA results between Kars centre and county herds were evaluated using the Chi square test (Preacher 2001). *P*-values less than 0.05 were considered significant.

Data analysis

Case definitions. A sheep that tested positive for *Map* antibodies was considered infected. Herds were declared positive for *Map* if one or more sheep from the herd were positive for *Map* antibodies.

Calculation of apparent prevalence. The apparent animal, within-herd, and between-herd prevalences were calculated by dividing the number of test positive outcomes by the corresponding denominator (i.e., total number of sheep tested from all herds, total number of sheep tested within positive herds, and total number of herds tested, respectively) for each measure as described (Dohoo et al. 2003). The 95% CI for apparent and true prevalences were estimated using the Wilson binomial

approximation method as described (Brown et al. 2001).

Calculation of true prevalence. True animal, within-herd, and between-herd prevalences were calculated using the Rogan-Gladen estimator (Rogan and Gladen 1978) method using an ELISA kit with a sensitivity of 64% and specificity of 99%, as claimed by the manufacturer.

RESULTS

In total, 450 sheep from 26 herds were tested. With respect to individual animal numbers in herds, 240 sheep from 12 centre farms and 210 sheep from 14 county farms of Kars Region were tested. In total, 28 of the 450 sheep were positive for *Map* antibodies. The *Map*-positive animal distributions of

Table 1. Sample distribution among sheep herds and the results of the ELISA survey for paratuberculosis

Locality	Tested	Seropositive	Apparent prevalence		True prevalence	
			estimate (%)	95% CI	estimate (%)	95% CI
Counties of Kars	**210**	**10**	**4.8**	**2.6–8.5**	**6**	**1.4–10.5**
Sarikamis	**30**	**1**	**3.3**	**0.6–13.9**	**3.7**	**–6.5–13.9**
Boyali	15	–	–	–	–	
Center	15	1	6.7	1.2–29.8	9	–11–29–
Kagizman	**30**	**–**	**–**	**–**	**–**	**–**
Sagbas	15	–	–	–	–	–
Center	15	–	–	–	–	–
Arpacay	**30**	**2**	**6.7**	**1.8–21.3**	**9**	**–5.2–23.2**
Akcalar	15	–	–	–	–	–
Center	15	2	13.3	3.7–37.9	19.6	–7.7–46.9
Digor	**30**	**–**	**–**	**–**	**–**	**–**
Arpali	15	–	–	–	–	–
Center	15	–	–	–	–	–
Akyaka	**30**	**6**	**20**	**9.5–37.3**	**30.2**	**7.4–52.9**
Esenyayla	15	4	26.7	10.9–52	40.7	5.2–76.3
Center	15	2	13.3	3.7–37.9	19.6	–7.7–46.9
Selim	**30**	**–**	**–**	**–**	**–**	**–**
Darbogaz	15	–	–	–	–	–
Akyar	15	–	–	–	–	–
Susuz	**30**	**1**	**3.3**	**0.6–13.9**	**3.7**	**–6.5–13.9**
Yolboyu	15	1	6.7	1.2–2.8	9	–11–29
Center	15	–	–	–	–	–
Center of Kars	**240**	**18**	**7.5**	**4.8–11.5**	**10.3**	**5–15.6**
Tazekent	20	1	5	0.9–23.6	6.3	–8.8–21.5
Yucelen	20	1	5	0.9–23.6	6.3	–8.8–21.5
Hamzagerek	20	–	–	–	–	–
Tekneli	20	1	5	0.9–23.6	6.3	–8.8–21.5
Hasciftlik	20	2	10	2.8–30.1	14.3	–6.6–35.2
Kumbetli	20	1	5	0.9–23.6	6.3	–8.8–21.5
Aydinalan	20	3	15	5.2–36	22.2	–2.6–47.1
Yilanli	20	3	15	5.2–36	22.2	–2.6–47.1
Yalcinlar	20	2	10	2.8–30.1	14.3	–6.6–35.2
Oguzlu	20	1	5	0.9–23.6	6.3	–8.8–21.5
Karakas	20	3	15	5.2–36	22.2	–2.6–47.1
Center	20	–	–	–	–	–
Total	**450**	**28**	**6.2**	**4.3–8.8**	**8.3**	**4.7–11.8**

Table 2. Apparent and true animal, within-herd, and between-herd prevalence estimates

Prevalence type	Number tested	Number positive for *Map*	Apparent prevalence		True prevalence	
			estimate (%)	95% CI	estimate (%)	95% CI
Animal	450	28	6.2	4.3–8.8	8.3	4.7–11.8
Within-herd	275	28	10.2	7.1–14.3	14.6	8.9–20.2
Between-herd	26	15	57.7	38.9–74.5	90	59.8–120.1

centre and county farms were 18 and 10, respectively. The difference in terms of *Map* positivity was not significant between centre and county herds ($\chi^2 = 1.273$, $P = 0.259$). Among 26 herds tested, fifteen had at least one *Map* positive sheep (one sheep in seven herds and two or more sheep in eight herds), while eleven herds were found to be paratuberculosis-free. The numbers of individual animals in seropositive and seronegative herds were 275 and 175, respectively and these values were used to estimate animal, within-herd and between-herd prevalence (Table 1).

The animal, within-herd, and between-herd apparent prevalences were 6.2% (95% CI = 4.3 to 8.8%), 10.2% (95 CI = 7.1 to 14.3%) and 57.7% (95% CI = 38.9 to 74.5%), respectively. The true animal, within-herd, and between-herd prevalences were 8.3% (95% CI = 4.7 to 11.8), 14.6% (95 CI = 8.9 to 20.2) and 90% (95 CI = 59.8 to 120.1), respectively (Table 2).

DISCUSSION

The present study is the first conducted on the prevalence of *Map* infection in Kars sheep herds carried out using commercial ELISA. *Map* antibodies were found in 28 out of 450 sheep tested and accuracy was good (Sp of 99% and Se of 64%). The true individual prevalence was estimated to be 8.3% in the Kars Region (Table 1). The animal-level prevalence rate calculated here is similar to those reported in studies conducted worldwide in small ruminants; especially in sheep (Coelho et al. 2007; Liapi et al. 2011; Stau et al. 2012).

In this study, the positive reactor rates were compatible to that reported by Makav and Gokce (2013) who conducted a study in similar localities of Kars Region with a common ELISA kits both bovine and sheep and pointed that the seroprevalence of cattle paratuberculosis was 3.5% in animal-level. This may indicate that the infection is progressing in the area of the study. And the transmission of agent among different farm animal species need to be surveyed with future works.

The within-herd prevalence was calculated in serologically positive herds with one or more infected sheep. We found fifteen herds which included 275 individuals to be seropositive and accordingly the average estimate of within-herd true prevalence was calculated as 14.6% in this study (Table 1). These findings are similar to reports from Germany (21%; Stau et al. 2012) and Cyprus (24.6%; Liapi et al. 2011). The possible within herd transmission could occur by continuous new *Map* infection in adult animals and high seroprevalence with eventual contamination of the environment.

Out of 26 herds tested 15 were found to be positive for *Map* antibodies (at least one positive sheep). The estimated between-herd true prevalence of paratuberculosis was computed to be 90% (Table 1). Thus, the results of this study confirm that *Map* infection is widespread in sheep herds in the Kars Region. A recent study in Germany reported a between-herd prevalence of 65% (Stau et al. 2012). We suggest that the ongoing free and extensive movement of animals such as occurs during spring or holiday seasons together with slack border security practices contribute to elevating *Map* prevalence among herds.

In conclusion, it is our hope that the present study will attract the special attention of veterinarians and producers and will serve to promote the establishment of an efficient paratuberculosis control programme in cattle and sheep in the Kars Region.

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