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Short communication: Seroprevalence of paratuberculosis in Italian water buffaloes (*Bubalus bubalis*) in the region of Campania

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ABSTRACT

Paratuberculosis is a chronic enteric disease affecting virtually all ruminants, but only anecdotal information is currently available about the occurrence of this disease in water buffaloes (*Bubalus bubalis*). We carried out a survey study aimed at determining the prevalence of paratuberculosis in 2 provinces in the region of Campania, Italy, where about half of all Italian buffaloes are reared. From May 2017 to December 2018, we collected 201,175 individual serum samples from 995 buffalo herds. The sera were collected from animals over 24 mo old and were tested using a commercial ELISA test. The herd-level apparent prevalence result was 54.7%, and the animal-level apparent prevalence was 1.8%. The herd-level true prevalence was estimated using a Bayesian approach, demonstrating a high herd-level prevalence of paratuberculosis in water buffaloes from the Campania area. These findings suggest that the urgent adoption of paratuberculosis herd-control programs for water buffaloes in this area would be beneficial.
Key words: paratuberculosis, herd-control program, buffalo

Short Communication

Paratuberculosis, also known as Johne's disease (**JD**), is an infectious disease caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), affecting both domestic and wild ruminants (Anonymous, 2014). Although water buffaloes (*Bubalus bubalis*) are generally considered to be more resistant compared with cattle and other ruminants (Sivakumar et al., 2006), paratuberculosis can also affect this species, showing gross and histological lesions very similar to those observed in cattle (Sivakumar et al., 2006). Notably, the clinical signs are also similar, including diarrhea, decreased milk production, and progressive emaciation that leads to cachexia and eventually premature death (Dalto et al., 2012). However, little information is currently available about the age of the animals at the onset of the disease. In previous papers, affected buffaloes were 3.5 to 5 yr old (Lillini et al., 1999; Dalto et al., 2012), but positive-serum ELISA results were also recorded in younger animals (Singh et al., 2008). Among the currently available tests, the ELISA methods are widely used for the detection and control of JD (Anonymous, 2014). In fact, several programs (Weber et al., 2008; Nielsen and Toft, 2009; Sardaro et al., 2017), including the Italian guidelines for JD control and herd certification in both cattle and water buffaloes (Italian government, 2013), have been based on such tests. In water buffaloes, different ELISA have been used in surveys carried out in India (Sivakumar et al., 2005; Sivakumar et al., 2006; Singh et al., 2008; Khan et al., 2010; Chaubey et al., 2019), where approximately 55% of the world's buffalo population lives (Sivakumar et al., 2006), Pakistan (Rehman et al., 2018), Philippines (Uy et al., 2018), and Brazil (Dalto et al., 2012; de Albuquerque et al., 2018).

In Italy, the first case of paratuberculosis in water buffaloes was described in the Latium region in 1999 in a 5-yr-old animal (Lillini et al., 1999). A further study, carried out in the same region using 2 ELISA assays, revealed an animal-level apparent prevalence (**AP**) of 0.2% (Sezzi et al., 2010). Finally, in 2 preliminary studies in the Campania region, carried out on 56 and 48

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buffalo herds (1,350 and 1,888 sera, respectively) using ELISA commercial kits, herd-level AP estimates of 32% and 27% were reported, respectively (Desio et al., 2013; Pesce et al., 2014). Because paratuberculosis diagnostic tests generally have poor sensitivity (**SEN**; Nielsen and Toft, 2008), estimation of the true prevalence (**TP**) through Bayesian approaches is often used (Branscum et al., 2004; Nielsen and Toft, 2008, 2009).

In the present paper, we report data about the JD prevalence in water buffaloes of 2 provinces (Salerno and Caserta) in the Campania region (southern Italy). The buffalo heritage of the provinces of Caserta and Salerno represents 63.5% (187,620 buffaloes) and 34.4% (101,692 buffaloes) of the Campania region respectively, which in turn represents almost 50% of the Italian buffalo population. Buffalo farming in southern Italy could be defined as semi-intensive, with about 2.5 animals per hectare (0.95 animal per acre). This breeding represents a major economic resource, both for the number of animals bred and for the importance of mozzarella cheese production (Borghese, 2005). Although buffalo farming was based on traditional techniques characterized by the use of environmental marshes in the past, following the increase in the importance of buffalo production, there has been a shift to intensive farming systems without access to pasture areas and wallowing waters in the majority of the herds.

From May 2017 to December 2018, a survey on paratuberculosis seroprevalence was carried out using individual serum samples collected in the framework of the National Brucellosis Eradication program. This program is compulsory in Italy; therefore, all buffalo herds and all buffaloes older than 24 mo in the 2 provinces were included in the survey. A total of 995 different herds were included in the survey, of which 315 (31.7%) were buffalo herds mixed with bovines. In each herd, all buffaloes over 24 mo of age, totaling 201,175 animals, were tested for antibodies against paratuberculosis, though cattle were not tested. For each herd tested, we also collected the total number of reared animals (buffaloes and bovines) from the National Cattle Database (www.vetinfo.it).

Blood samples were drawn by the caudal vein, then refrigerated and delivered to the laboratory within 72 h. After arrival at the laboratory, blood samples were immediately centrifuged (3,000 × *g* for 5 min at 4°C) and the sera were aliquoted and frozen at −20°C. Frozen samples were thawed and tested using a commercial kit (ELISA ID Screen Paratubercolosis Indirect Screening, ID-Vet), following the manufacturer's instructions. Sample results were classified, according to the manufacturer's instructions, as negative if the sample-to-positive ratio was lower than 0.6 and positive if the sample-to-positive ratio was higher than 0.7. Notably, inconclusive results (sample-to-positive ratio between 0.6 and 0.7) were considered as positive.

For the TP estimation at herd level, we used the Bayes free calc software (https://cadms.vetmed.ucdavis.edu/diagnostic/software). This software computes, for each herd sampled, the posterior probability that the within-herd prevalence is not larger than a specified threshold, and the posterior median of within-herd prevalence with a 95% probability interval, following the hypergeometric sampling approach proposed by Branscum et al. (2004). We chose a very low prevalence threshold (0.0001), and considered 1 minus the posterior probability obtained with the Bayes free calc software as the posterior probability of the herd to be infected. As prior information about SEN and specificity (**SP**) of the ELISA test, we input the same values used for estimating the TP in dairy cattle in Lombardy and Veneto regions (Pozzato et al., 2011), which in turn were inspired by a review of the accuracies of the tests available for paratuberculosis diagnosis (Nielsen and Toft, 2008). Specifically, the SEN value used was 0.15 with 95th percentile of 0.30 [SEN~beta (5.04, 23.9)], and the SP was 0.99 with 5th percentile of 0.98 [SP~beta (163.44, 1.16)]. We also used a diffuse prior for SEN [SEN~beta (1, 1)]. Moreover, to estimate paratuberculosis prevalence at animal level we used the "estimated true prevalence using one test with a Gibbs sampler" module of Epitools (Sergeant, 2018), imputing the same priors for SEN and SP used for herd-level prevalence estimation.

Overall, 54.7% (CI 95%: 51.5–57.8) of tested herds and 1.75% (CI 95%: 1.70–1.81) of all buffaloes tested positive to the ELISA test. The AP of herds breeding only buffaloes was not significantly different from that of mixed herds (buffaloes and cattle), being 55.6% (CI 95%: 51.8–59.4%) and 52.7% (CI 95%: 47.0–58.3), respectively.

The herd-level AP seemed directly related to herd size, rising from 19.3% in the smallest (1–50 heads) to 86.9% in the largest herds (over 500 heads; Table 1). Animal-level AP was on average 1.8% (from 1.2–2.0%) in all classes, except in the smallest herds (1–50 heads), where a value of 3.2% was recorded (Table 1). In Figure 1, the distribution of within-herd AP for the different herd sizes is reported. Notably, only 32 herds (3%) showed a within-herd AP higher than 10%.

According to the Bayesian approach (Branscum et al., 2004), we estimated the posterior probability of each herd to be infected. As expected, when increasing the posterior probability of infection, the estimated prevalence decreased (Table 2). Considering a posterior probability higher than 85%, 93.7% of the herds were

Table 1. Seroprevalence of paratuberculosis recorded in water buffaloes in the Campania region from 2017 to 2018

	Herd			Animal		
Herd size	Tested	Positive	Apparent prevalence, %	Tested	Positive	Apparent prevalence, %
1–50	83	16	19.3	1,633	52	3.2
51–100	132	39	29.5	7,248	90	1.2
101–150	131	56	42.7	12,565	208	1.7
151–200	125	56	44.8	15,540	232	1.5
201–300	186	118	63.4	36,253	739	2.0
301–500	201	140	69.7	56,084	1,022	1.8
>500	137	119	86.9	71,852	1,188	1.7
Total	995	544	54.7	201,175	3,531	1.8

estimated to be infected with the first set of priors, and it resulted in 64.0% using the diffuse prior for SEN. With a posterior probability of 0.90 and 0.95, the estimates with both sets of priors were similar (around 55%; between 41% and 47%, respectively). Notably, at 90% posterior probability level, AP and estimated TP of infection assumed the same values (Table 2). Unfortunately, with the priors assigned to the SEN of the test, the model did not give reliable results, and it was not possible to obtain an estimate of animal-level TP.

This survey reported a high prevalence estimate of paratuberculosis in water buffaloes in the considered area. Although the zoonotic role of MAP has not yet been established, attention to its effect on human health is increasing, especially with regard to Crohn's disease (Chiodini et al., 2012). One of the sources of transmission to humans is considered to be the consumption of contaminated milk and dairy products (Gill et al., 2011); therefore, coupled with the growing demand for guarantees from several non-EU countries, such as the Russian Federation, India and China, the dairy industry is very concerned about the issue. Regarding water buffaloes, the demand of dairy products for export is growing rapidly, particularly for buffalo mozzarella. For these reasons, in 2013, the Italian government (2013) issued the National Guidelines for the adoption of control plans against paratuberculosis and for assigning a health ranking to bovine and buffalo herds. The objectives of the Italian Guidelines were as follows: (1) to collect data on the occurrence of clinical cases, (2) to provide a herd classification ranking based on their risk of infection, (3) to promote the implementation of voluntary herd-control programs, and (4) to define a transparent procedure for the trade of animals and their products. Notably, among all of the tests available, ELISA assays are currently the most widely used for the control of paratuberculosis and are those required in the National Guidelines for the attribution of health ranking to cattle and buffalo herds.

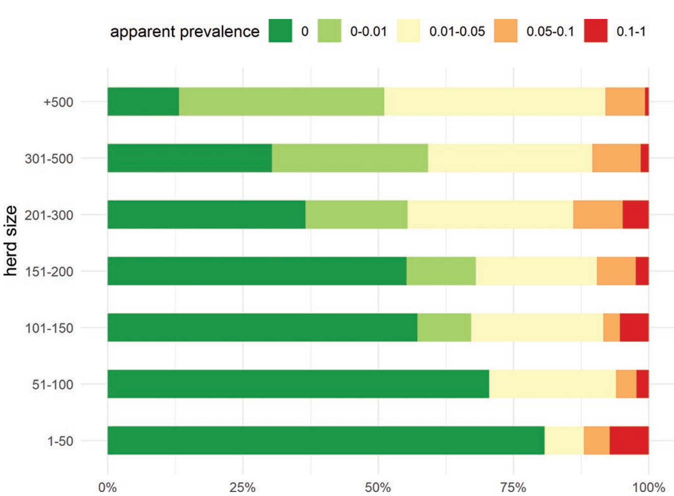

Figure 1. Distribution of within-herd apparent prevalence for the different herd sizes.

Our survey involved about 68% of water buffaloes reared in the region of Campania, and 50% of the Italian buffalo population. Previous studies (Desio et al., 2013; Pesce et al., 2014) have already reported relatively high herd-level AP in the same area. Our data suggested an increase of MAP infection in buffalo herds reared in the study area. In fact, 54.7% of herds had at least 1 seropositive animal, but the vast majority of infected herds showed a low within-herd AP (Figure 1). Notably, the herd prevalence is probably underestimated because of the poor SEN of the ELISA tests (Nielsen and Toft, 2008). On the other hand, almost all publications agree about the high specificity of the ELISA assays for the detection of infected animals (Nielsen and Toft, 2008), and thus the risk of false-positive results is considered to be very low.

Using a Bayesian approach for estimation of MAP infection, the majority of herds were infected at a posterior probability higher than 85%. A remarkable increase

Table 2. Estimated herd prevalence of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection in water buffaloes in the Campania region, 2017 to 2018

Priors		Posterior probability of MAP Infection		
Sensitivity	Specificity	>0.85	>0.90	>0.95
Beta(5.04, 23.9)[1]	Beta(163.44, 1.16)[2]	93.7%	54.7%	46.8%
Beta(1, 1)	Beta(163.44, 1.16)[2]	64.4%	54.8%	41.0%

[1]Sensitivity mode = 15%, 95% sure is lower than 30%.

[2]Specificity mode = 99%, 95% sure is greater than 98%.

from apparent to TP, irrespective of the mathematical model applied to evaluate it, is not surprising because all assays for the diagnosis of paratuberculosis show limited SEN, as previously reported (Pozzato et al., 2011; Desio et al., 2013; Lombard et al., 2013). Similar results were obtained in the United States (Lombard et al., 2013) in cattle, where the true herd-level prevalence after Bayesian modeling of environmental fecal cultures data was 91.1%, suggesting that the majority of dairy operations in the United States are infected with MAP.

According to the Italian guidelines for the control of paratuberculosis, farmers, advised by their veterinary practitioners, can voluntarily enroll in a control program aimed at gradually reducing the prevalence by adopting biosecurity measures coupled with an appropriate testing scheme, which is based on serological and fecal assays (PCR or culture). Culling test-positive animals is strongly advised, although not compulsory, with the exception of clinical cases. It is important to underline that test-and-cull programs are not successful if not coupled with the adoption of strict biosecurity measures (Collins and Morgan, 1992; Groenendaal et al., 2002; Garry, 2011; Camanes et al., 2018). Previous studies demonstrate that culling infected animals dramatically reduces the incidence of clinical cases (McAloon et al., 2017), contributing to the reduction of environmental contamination, and thus lowering prevalence (Lu et al., 2010), but biosecurity measures are necessary to prevent introduction and spreading of the infection within the herd, among young animals in particular (Garry, 2011).

To conduct a standard risk assessment and design a herd-specific MAP control program, the Italian guidelines include 2 manuals (1 for beef and 1 for dairy herds). This risk analysis helps to identify those practices that allow MAP infection to spread in the herd and suggests specific management practices to control the risk factors (Arrigoni et al., 2016). Interestingly, the main risk factors described by a previous paper (Desio et al., 2013) in buffalo herds of the same region were (1) poor hygiene of the calving area and (2) long calf-dam contact period. Other risks frequently occurring in this type of breeding are as follows: the frequent use of suckler cows or buffaloes, the seasonality of births (which increases the population density of animals in calving areas), the lack of application of colostrum heat treatment, the extensive use of natural breeding, the contact with animals of other susceptible species, the introduction of purchased animals without appropriate quarantine and testing, and the presence of elderly animals (over 10 yr of age). Moreover, some farms provide pools of stagnant water to guarantee welfare of the reared animals; in fact, buffaloes are animals with a low heat tolerance because of their dark coat and inadequately-dispersed sweat glands (Minervino et al., 2020). Finally, because of the lower susceptibility of buffaloes to clinical mastitis compared with cattle, the milking hygiene is often poor.

In conclusion, in the Campania region, where the herd-level prevalence of paratuberculosis is high, the adoption of control programs for MAP infection should be recommended. Efforts should be put in place by all stakeholders to improve both MAP control and the profitability of this kind of breeding. The final aim should be to sustain the export of milk derivatives, such as typical mozzarella Protected Designation of Origin cheese, a market accounting for around 20% of gross domestic product of the entire region with a value of 766 million Euros in 2019 (Minervino et al., 2020).

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