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SHORT COMMUNICATION

Paratuberculosis in Holstein-Friesian cattle farms in Central Iran

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Abstract Paratuberculosis is an important disease of ruminants with a worldwide distribution. In developing countries where funding constraints challenge establishment of control schemes, large losses are incurred on cattle farmers due to paratuberculosis. In this study, faecal specimens from Holstein-Friesian cows with progressed and moderate clinical paratuberculosis (N=223) from 13 dairy farms in Isfahan, Central Iran, were subjected to bacterial culture. Culture growth diagnostic for *M. avium* subsp. *paratuberculosis* was found in cattle from nine of the 13 farms and in 71 of the cattle studied. These results illustrate the emergence of PTb in this region, and they imply that PTb should be given a higher priority for veterinary measures.

Keywords Epidemiology · Paratuberculosis · *Mycobacterium avium* · Farm animals · Holstein-Friesian

Introduction

Paratuberculosis (PTb) is an emerging chronic and degenerative enteritis in domestic and wild ruminants. The causative agent of PTb is *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) (Johne and Frothingham, 1895, Hermon-Taylor et al., 1998) with a worldwide distribution (OIE, 2004). Diagnostic identification of *M. paratuberculosis* requires costly field and laboratory tests and lengthy growth in microbiological culture that must include a background decontamination stage. More generally, *M. paratuberculosis* can survive in diverse environments outside hosts, including groundwater (Whan et al., 2005), environmental protozoa (Whan et al., 2006) and flies (Fischer et al., 2005), making cattle farms vulnerable to diverse sources of infection.

In Asia PTb has been reported in farm animals from Japan (Goto et al., 1972), Korea (Park et al., 2006), India (Singh et al., 2007), Iraq (Bashir et al., 1969), Turkey (Erol, 1968), Saudi Arabia (Al Hajri and Alluwaimi, 2007) and Iran (Talatchian, 1965). In Iran, PTb was observed initially in 1957 in Jersey and Sindhi cattle in Southern Iran. Talatchian reported isolation of *M. paratuberculosis* from sections of intestine and mesenteric lymph nodes of a dead Jersey cow with a history of chronic diarrhoea and progressed emaciation lasting for several months (Talatchian, 1965). In 1970, *M. paratuberculosis* was isolated from sheep and goats in the suburb of Tehran (Baharsefat et al., 1972).

The principal method of infection in PTb (ingestion of infected milk, food, water, etc.) is different to that of bovine tuberculosis (BTb) (respiration) (OIE, 2004) nevertheless, there are convincing grounds that tentatively support the view that today's abundance of

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PTb in Iran is likely to have resulted from the same epidemiological factors that had led to dispersion of BTb over the last half a century (Figure 2): Pure and crossed Holstein-Friesian (HF) cattle comprise some 40% of the 9.2 million head national herd and appear to be vulnerable to PTb infection because they account for most clinically-identified cases of PTb (The Iranian Veterinary Organisation, unpublished material). In contrast with bovine tuberculosis where a national test-and-slaughter programme operates, there is essentially no systematic control measure in place to eliminate PTb in cattle farms. Cattle farmers are not officially obliged to provide PTb-free certificate to sell their animals thus introduction of infected animals can easily transmit PTb between farms.

Interestingly, a number of tuberculinated cattle in Iran are reported to show relatively large skin reactions against avian tuberculin in the single intradermal comparative test (SCITT) which is the official test used in the test-and-slaughter programme. On average, skin reactions measure almost 7 mm but can be as large as 50 mm (The Iranian Veterinary Organisation, unpublished material). It is believed that infection with *M. paratuberculosis* or other environmental mycobacteria might explain skin reactions in cattle against avian tuberculin in the tuberculin test but the frequency of these mycobacteria in Iran is poorly known (Khavari Khorasani, 1999). These considerations suggest that PTb might be more widely distributed in Iran than is presently assumed.

The goal of the present study was to achieve a better epidemiological understanding of PTb in farms with HF cattle in Isfahan, the largest city in Central Iran, using a faecal culture strategy to isolate *M. paratuberculosis* from cattle with progressed and moderate clinical symptoms of the disease.

Materials and methods

Cattle farms and animals The regional state veterinary service at Isfahan was consulted for the sampling plan. A total of 78 cattle farms with approximately 22,500 cattle were identified in the city of Isfahan and its suburbs of which 13 farms within a radius of 10 km from the city centre were chosen according four criteria: farming of HF milking cows with intensive herd management, experience of breakdowns of paratuberculosis, active supply of cattle to other farms in the region, and owner's consent to participate in the study (Table 1).

Faecal specimens Cows with clinical symptoms of PTb were identified by reviewing their records (N= 223), and fresh faeces was collected on site from the rectum using disposable plastic gloves and small plastic containers. One faecal specimen was collected from each cow. These were transferred at ambient temperature to the laboratory, kept at 4°C over night and then, if required, at −20°C pending examinations.

Table 1 Prevalence of paratuberculosis in studied farms†: Each bovid provided a single faecal specimen

Farm	Herd size (bovid)	№ of bovids with clinical symptoms †	Prevalence (%) (clinical symptoms)	№ of culture positive specimens	Prevalence (%) (bacterial growth)
1	4,800	95	2	33	0.6
2	1,360	21	2	10	0.7
3	2,500	18	1	3	0.1
4	700	13	2	10	1.5
5	520	12	2	3	0.5
6	1,200	11	1	5	0.4
7	1,500	10	1	0	0
8	1,800	9	1	5	0.2
9	100	9	9	0	0
10	50	8	16	0	0
11	200	6	3	0	0
12	300	6	2	1	0.3
13	600	5	1	1	0.2
Total	15,630	223	1.5	71	0.5

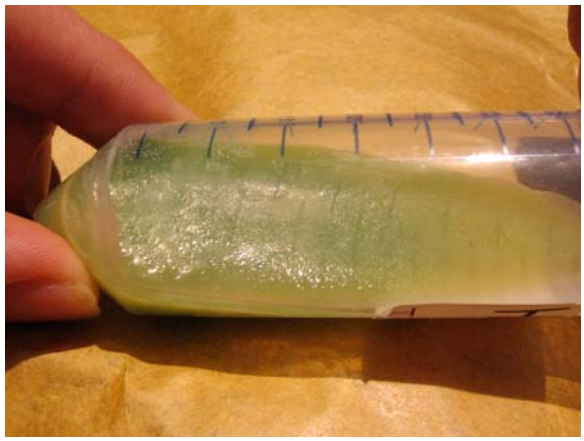

Fig. 1 *Mycobacterium paratuberculosis* growth on mycobactin J-supplemented Herrold's egg-yolk medium

Acid-fast staining microscopy The microscopic smears were produced in two stages; direct smears from fresh faeces and smears from bacterial cultures after incubation. In both cases, smears were prepared using filtered distilled water, air-dried and then acid-fast stained (de Kantor et al., 1998).

Bacterial culture Culture is the gold standard methodology for diagnosis of paratuberculosis (Muskens et al., 2003; Singh et al., 2007). It is definitive with virtually no false positives, and able to detect *M. paratuberculosis* bacilli from as few as 10–100 CFU in specimens (Pavlik et al., 2000). The OIE guidelines were followed with a few modifications. Briefly, 2–5 g of faeces was placed in a 50 ml test tube containing 20 ml of sterile distilled water. A sterile swab stick was used to break up the contents; the tube was vortexed for 10 minutes and left to stand for 30 minutes at room temperature. The content was then filtered through gauze into a new tube and centrifuged at 3,000 *g* for 30 minutes. The supernatant was discarded and for decontamination of the background microorganisms, the sediment was resuspended in 20 ml of 0.75% hexadecylepyridinium chloride (Merck. Darmstadt, Germany) and left undisturbed for 18 hours at 37°C. The tube was then centrifuged at 1,000 *g* for 30 minutes. The sediment was collected and resuspended in 1 ml of sterile distilled water. About 0.2 ml of the suspension was used to inoculate each of three replicate slants of Herrold's egg yolk medium supplemented with mycobactin J (Merck. Darmstadt, Germany) and one slant of Herrold's medium without mycobactin J. This was intended to display the characteristic mycobactin J-dependant growth of the bacterial culture, which is diagnostic for PTb. The inoculum was distributed evenly over the surface of the slants. The tubes were placed in a rack, maintained in a slanted position and incubated for 7 days with loose caps to release extra moisture. They were then put in vertical position with their caps tightened and incubated for 20 weeks at 37°C. The bacterial growth was inspected weekly. In total 892 tubes (4×223) were cultured.

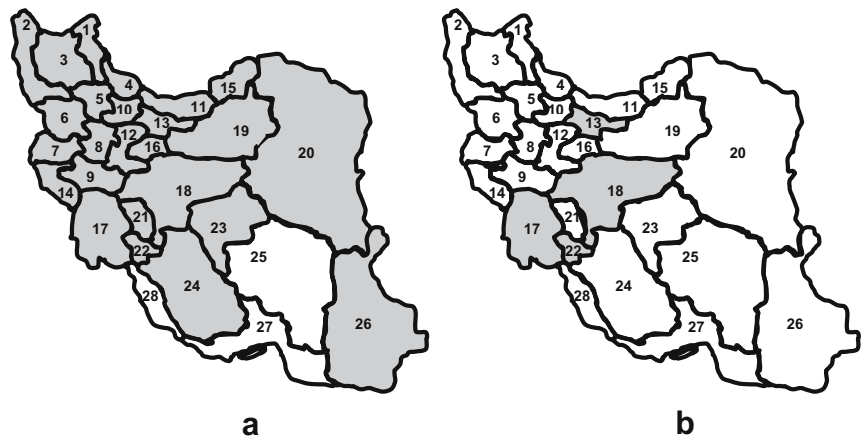

Fig. 2 Geographical occurrence of (**a**) bovine tuberculosis and (**b**) bovine paratuberculosis in provinces of Iran based on the confirmed (skin test and/or bacterial culture) (gray) and non-confirmed (white) reports (extracted from IVO records)

1 / Ardebil, 2 / West Azerbaijan, 3 / East Azerbaijan, 4 / Gilan, 5 / Zanjan, 6 / Kurdistan, 7 / Kermanshah, 8 / Hamedan, 9 / Lorestan, 10 / Qazvin, 11 / Mazandaran, 12 / Markazi, 13 / Tehran, 14 / Ilam, 15 / Golestan, 16 / Qum, 17 / Khouzestan, 18 / Isfahan, 19 / Semnan, 20 / Khorasan, 21 / Chaharmahal, 22 / Kohgilouyeh, 23 / Yazd, 24 / Fars, 25 / Kerman, 26 / Sistan, 27 / Hormozgan, 28/ Boushehr

Results

In the direct smear tests, 46 specimens were positive. bearing the characteristic arrangements of three or more acid-fast bacilli in clumps. Cultures of specimens from a total of 71 cows produced visible growth on mycobactin J-supplemented slants, which again produced acid-fast bacilli in microscopy (Table 1). The colonies were very small and translucent with no pigments (Figure 1). Subculture of acid-fast positive cultures on Herrold's slants supplemented with mycobactin J was successful but growth on the plain medium was either unsuccessful or visibly poor. Culture was successful in isolation of the pathogen in 9 of the 13 farms. The highest recovery rate was achieved in farm 4 with 10 isolates (77%) out of 13 specimens followed by farm 8 with 5 (56%) isolates from 9 specimens (Table 1). Based on the culture results, the frequency of PTb in the studied farms ranged from 0 to 1.4% and was 0.5% on average (Table 1).

Discussion

In the present study culture of 223 faecal specimens led to recovery of 71 isolates representing a 32% success in bacterial isolation. This percentage should be regarded as a minimum for two reasons. First, chemical decontamination detrimentally affects the viability of *M. paratuberculosis*, making false-negative cultures more likely (Grant and Rowe, 2004). Second, the success of faecal culture depends on how many bacilli are shed from intestinal lesions (Perez et al., 1996, Whittington et al., 1999). Some low-shedder cows might therefore have been categorised as negative in this study. An annual percentage of clinical PTb cases of 2% is thought to imply a true herd prevalence of 80% (Kudahl *et al.,* 2007). In the present study the number of cows with clinical signs of PTb was 1–16 % (Table 1), which indicates a very high prevalence of PTb in these farms.

The losses incurred by PTb in Iran are estimated to be approximately several thousand Euros per year for an HF farm with 200 cows (Taghipour Bazargani et al., 2007), and this is consistent with losses estimated elsewhere (Lee et al., 2006). Kasravi and Nowrouzian showed HF cattle with clinical PTb in Iran are more likely to be culled because of having a left-displaced abomasum (LDA) than symptom-free cattle (Kasravi and Nowrouzian, 2004). This imposes an extra cost to farmers.

The findings of this study are consistent with a broad dispersion of PTb among cattle farms around Isfahan. Whether PTb also has a broad distribution across other areas of Iran is unknown. However, PTb has been reported from some other parts of Iran including Tehran (Bazrpour, 2001, Taghipour Bazargani et al., 2007), Ahvaz (Hajikolaei et al., 2006) and Kohgilouyeh (Pourjafar and Badiei, 2005) (Figure 2). All the 13 farms analysed in the present study were actively providing young calves and heifers to other farms in the region, which might explain the local transmission of PTb in the Isfahan region.

We hypothesise that any pattern of dispersion of PTb at the national scale in Iran is mainly due to the rapid, recent expansion of the HF herd. Farming of HF cattle was instituted on state farms in Tehran in the 1960s and was later expanded to other suitable regions of Iran. If this farming strategy also spread PTb then similar or identical strains of *M. paratuberculosis* should occur in a homogeneous pattern throughout Iran (this hypothesis is presently being tested by molecular typing of 31 of the *M. paratuberculosis* isolates from this study). A similar argument has been made for the origin and dispersion of *M. bovis* across Iran (Tadayon et al., 2006). Lastly, almost nothing is known about PTb prevalence in indigenous zebu cattle, which currently comprise the remainder (60%) of the national herd. Our knowledge of interaction between zebu and HF cattle in the epidemiology of PTB is also poor and in need of further study.

We believe PTb to be a neglected but serious threat to cattle farming in Isfahan, and in Iran as a whole. Urgent health measures are required to counter the economical losses caused by this mycobacterial disease.

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