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Short Paper

The prevalence of *Mycobacterium paratuberculosis* infection in ileocecal valve of cattle slaughtered in Ahvaz abattoir, southern Iran

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Summary

To investigate the prevalence of *Mycobacterium paratuberculosis* (*M. avium* subsp. *paratuberculosis)* infection among cattle slaughtered in Ahvaz abattoir, samples were taken from 250 cattle. Before slaughter, sex and age were assessed. Thereafter, samples were taken from ileocecal valve, rectum mucosa, ileocecal lymph node and stool. All smears which taken from these samples were stained by Ziehl-Neelsen method and examined by light microscopy. At first, ileocecal samples were examined; other samples were examined when ileocecal sample was positive. Out of the 250 cattle, 5 (2%) were positive. With the exception of one sample in which the organism was observed in both ileocecal valve and ileocecal lymph node, in other positive samples the bacteria was observed in only ileocecal valve smear.

Key words: *Mycobacterium paratuberculosis,* Cattle, Ahvaz

Introduction

Johne's disease is a chronic infection of ruminants caused by infection with *Mycobacterium paratuberculosis* (*M. avium* subsp. *paratuberculosis*) (Reichel *et al*., 1999; Boelaert *et al*., 2000; Eamens *et al*., 2000). Cattle that are infected with *M. paratuberculosis* are considered to have paratuberculosis. Johne's disease is a clinical disease that is characterized by progressive cachexia and chronic diarrhea. Most instances of the clinical disease develop in cattle aged 3–5 years. Cattle with paratuberculous may be categorized into three groups: infected which do not shed *M. paratuberculosis* in the faeces and have no clinical disease; infected which shed *M. paratuberculosis* in the faeces but have no clinical disease; and infected which shed the organism in the faeces and have clinical disease (Merkal *et al*., 1987).

Effective detection of subclinical cases of bovine Johne's disease is a critical step in the reduction of disease prevalence in infected herds. To date, few diagnostic tests have been developed to estimate the prevalence of paratuberculosis at the regional or national levels. These tests for serum antibody and excretion of bacteria in faeces can be applied using a range of methodologies (Boelaert *et al*., 2000; Eamens *et al*., 2000). Faecal culture is presently recognized as the gold-standard test for diagnosis of infection in live cattle. Microscopic study of Ziehl-Neelsen stained smear of faeces and intestinal mucosa for the typical clumps of acid-fast bacteria has been an attractive alternative to faecal culture since the results are available within an hour (Radostits *et al*., 2000). Currently available serological tests for Johne's disease including the complement fixation test (CFT), the gel diffusion test (GDT) and various forms of enzyme-linked immuno-sorbent assays (ELISA) have nonetheless, significant limitations (Reichel *et al*., 1999;

Radostits *et al*., 2000).

Materials and Methods

This study was carried out on 250 (124 male and 126 female) cattle in the slaughter-house in Ahvaz. Sex and age of these animals were determined before slaughter. These animals were divided into five groups according to their age; <2 (n = 140), 2–3 (n = 38), 3–4 (n = 18), 4–5 (n = 25) and >5 (n = 29) years old. After slaughter, ileocecal valve, ileocecal lymph node, rectum and faecal specimen were obtained. Smears were taken from the mucosa of various parts of the intestine, ileocecal lymph node and faeces. Ziehl-Neelsen staining method was applied for smears and examined by light microscopy (Quinn *et al*., 1994). At first, the ileocecal valve samples were examined; other samples were examined when clumps of *M. paratuberculosis* were seen in ileocecal valve.

Statistical analyses were carried out using SPSS software. Fisher exact test was used to analyse the association between sex and infection. Spearman's correlation coefficient was used to determine the correlation between age groups and infection. A p value <0.05 was considered statistically significant.

Results

Clumps of *M. paratuberculosis* were observed in 5 (2%) of 250 samples. These included 2 males and 3 females. The prevalence of infected cattle in different sex and age groups are shown in Table 1.

With the exception of one sample in which *M. paratuberculosis* was observed in both ileocecal valve and ileocecal lymph node, in other positive samples, the bacterium was observed only in ileocecal valve smear. All of the infected cattle had normal faeces and various parts of their intestine did not show any gross lesions of paratuberculosis or Johne's disease.

Statistical analyses showed no association between infection and sex (P = 1). No correlation was observed between infection and age groups in both females (P = 0.369) and males (P = 0.429).

Discussion

In this study, the apparent prevalence of *M. paratuberculosis* infection was 2% in Ahvaz. In similar studies, Sadeghi-Garmarodi (1993) and Yousof-Beygi *et al*., (2003) reported prevalence rates of 3.07% in Tehran and 1.9% in Urmia abattoirs. Merkal *et al*., (1987) reported a prevalence of 1.6% (2.9% dairy and 0.8% beef) in cattle as determined by ileocecal lymph node biopsy from 7454 cull cattle from 76 slaughter-houses in the USA and Puerto Rico. The prevalence of paratuberculosis as determined by ileal and/or ileocecal lymph node culture from Canadian slaughter-house was 5.5% (McNab *et al*., 1991). Overall, the seroprevalence (by ELISA) of paratuberculosis in Missouri, USA cattle was reported to be 8 ± 3% in dairy cattle and 5 ± 2% in beef cattle, whereas 74 and 40% of dairy and beef herds were positive, respectively (Thorne and Hardin, 1997). In Belgian cattle, the median seroprevalence rates of paratuberculosis in herd and individual animal, were 2.9 and 0.87%, respectively. The seroprevalence of paratuberculosis in herd was 18% (Boelaert *et al*., 2000). In three South Wales dairy cattle herds with endemic Johne's disease, the prevalence rate as determined by faecal culture were 12,

Table 1: Prevalence of *M. paratuberculosis* infection in different age and sex groups of cattle in Ahvaz abattoir

Age (Yrs)	Male	Infected	Female	Infected	Total	Infected
<2	94	2 (2.13%)	46	0	140	2 (1.43%)
2–3	18	0	20	1(5%)	38	1 (2.63%)
3–4	3	0	15	1 (6.67%)	18	1 (5.56%)
4–5	4	0	21	0	25	0
>5	5	0	24	1 (4.17%)	29	1 (3.45%)
Total	124	2 (1.61%)	126	3 (2.38%)	250	5 (2%)

18 and 22%; as determined by whole herd faecal culture was 16.5%; and using ELISA, the prevalence was 13.5% of all cattle and 37% of the cattle with positive culture (Eamens *et al*., 2000).

Because ileocecal valve and distal part of ileum were the best tissue specimens for observation of *M. paratuberculosis* in sheep and cattle (Khaneghahi-Abianeh, 1993; Sadeghi-Garmarodi, 1993), we selected ileocecal valve smears for observation of this bacterium. In the two latter studies, specimen from faeces and four parts of intestine such as ileocecal lymph node, ileocecal valve, distal part of ileum and rectum were obtained from each animal. *M. paratuberculosis* was observed from the ileocecal valve and ileum of all the cattle and sheep with paratuberculosis but it was observed in the 85% of ileocecal lymph nodes and 57.1% of faecal samples of cattle with paratuberculosis (Khaneghahi-Abianeh, 1993; Sadeghi-Garmarodi, 1993). In the current study, *M. paratuberculosis* was only observed in one specimen of ileocecal lymph node (20% of infected cattle).

Although there are not large numbers of clinical cases with paratuberculosis in Ahvaz, our estimation indicates that there should be a considerable exposure to paratuberculosis in cattle. If control procedures are not instituted, the prevalence of paratuberculosis will be expected to increase and thus, more clinical cases will be detected (Thorne and Hardin, 1997). The animals that are infected with *M. paratuberculosis* but have no clinical disease may lead to losses that are much more difficult to calculate. Problems such as mastitis, infertility and decreased milk production are subtler than the clinical disease and the cost to the owners is difficult to determine. Producers involved in the sale of semen or purebred cattle can encounter substantial losses, resulting from restrictions that prevent the sale of their product when cattle become infected. *M. paratuberculosis* infection represents a lifetime infection, these animals may be shedding *M. paratuberculosis* in the faeces, which would contaminate the environment and possibly lead to the infection of other animals (Merkal *et al*., 1987). Therefore, establishment of control programs such as faecal culture, intradermal tests, serological tests and vaccination against Johne's disease is recommended so that the rate of infection will significantly reduce in long-term (Radostits *et al*., 2000; Yousof-Beygi *et al*., 2003). Worldwide reports indicate that test and culling controlling measures have influentially reduced the prevalence of the disease in herds from 2.6 to 0.5%, from 9 to 2% and from 7.8 to 1.8% (Yousof-Beygi *et al*., 2003).

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