# Study on the Prevalence of Subclinical Cattle Johne's Disease in the Urmia Abattoir

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#### Summarv

The prevalence of subclinical Johne's disease was investigated in 293 (97 male and 196 female) cattle slaughtered in Urmia abattoir during year 2001. Samples were included both faces and intestinal epithelial tissues which collected from the area between ileum and cecum. Zeihl Neelson staining method was applied to diagnosis the acid-fast microorganism (AFM) as an indication of subclinical Johne's disease. AFM was found in 9 of 293 samples (3.07%). Females with AFM (3.57%) were aged over 2 and males (2.06%) over 3 years old. There were no differences between sex and age variation. Distribution of subclinical Johne's disease in spring, summer, autumn and winter were 3, 1, 1 and 4 cases, respectively indicates that the difference (P<0.05) in seasons was statistically significant. The results indicate that subclinical Johne's disease could be a problem in cattle either male or female.

Keywords: paratuberculosis, subclinical, Johne's disease, cattle, Zeihl Neelson, Iran

### Introduction

Paratuberculosis which is known, as Johne's disease is a chronic, progressive enteric disease of ruminants caused by *Mycobacterium paratuberculosis* characterized by hypertrophic enteritis, chronic diarrhea and loss of condition in ruminants (Stabel 1998). Although cattle with clinical disease are often culled from the herd, but it is

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predominantly subclinical (Cetinkaya et al 1997) and causes economic losses because of reduced milk yields and poor reproductive performance (Wilson et al. 1993). The worldwide prevalence differed from 0% (Ellis et al 1998) to 1.7% (Kim Taejong et al 1997), 2.6% (Cetinkaya et al 1996), 5.99% (Meylan et al 1995) and 18% (Guy et al 1991). The disease reported to occur in over two years old calves (Stabel 1998) but differences among age groups were also not significant (Cetinkaya et al 1996) and season or months of year had no effect on prevalence of disease (Radostities et al 1996). Rakesh-Sisodia et al (1995) reported that the prevalence was greater in male than in female. In spite of the extensive measures of control implicated in many developed countries it is widely spread throughout the cattle population (Ogawa et al 1995). No report has been found in relation to the either rate of clinical or subclinical form of disease among the ruminants in Iran. Thus, understanding the circumstances of the disease in relation to rate of incidence, sex and age effects could be useful to reduce the economic losses when vaccination and control procedures are fairly hard to application. The aim of this study was to establish subclinical Johne's disease prevalence rate and the effects of sex and age on it.

#### Materials and Methods

293 samples included faces and intestinal epithelial tissues obtained from 97 males and 196 females were directly prepared from the area between ileum and cecum of slaughtered cattle in the Urmia abattoir. Number of samples in spring, summer, autumn and winter were 56, 60, 90 and 87, respectively. 10 to 12 samples were prepared once in time and collected into 10ml sterile glass tubes for laboratory examination. Age of the animals was estimated by teeth formula method and sex, age and date of sampling were also recorded. Samples were grouped according to the age up to <2, 2-3, 3-4 and >4 years old and the distribution was 82, 65, 60 and

86, respectively. Faces and intestinal epithelial tissues were completely mixed and at least four smears were prepared from each sample. Ziehl Neelsen staining method was applied for smears and AFM with characterization of Johne's diseases (clumb and thick form) was considered as an indication of subclinical paratuberculosis. Statistical programs of RBYC and Chi-Square method were applied to analyze the data.

#### Results

The frequency and percentage of infected cattle in sex and age groups were shown in table 1. AFM was observed in 9 of 293 samples (3.07%) as in which 2 (2.06%) cases were male and 7 (3.57%) cases were female.

Table 1. Frequency and percentage of subclinical Johne's disease in different age and sex groups of cattle in the Urmia abattoir

Age groups	Sex						_ ا
(year)	No. male	No. Infected	%	No. female	No. infected	%	Total
<2	36	0	0	47	0	0	83
2-3	34	0	0	85	3	2.53	119
3-4	7	1	2.1	38	2	4.4	45
>4	20	1	2.1	26	2	4.34	46
Total	97	2		196	7		293

Chi-Square showed no differences between infected male and female. No AFM was found among 83 cases with less than 2 years old. There was no difference between age of infected cattle from over 2 years to >4 years old. Distribution of subclinical Johne's disease in spring, summer, autumn and winter were 3, 1, 1 and 4 cases, respectively. A difference (P<0.05) was observed among seasons in occurrence of the disease.

## Discussion

As noted on *Introduction*, the prevalence of Johne's disease in individual herds reported from 0% to 18%. The economic impact of the disease on cattle industry has not been exactly determined but it is estimated to exceed \$1.5 billion per year (Stabel 1998). The worldwide reports indicates that test and culling control measures have predominately reduced the prevalence of the disease in the herds from 2.6% to 0.5% (Belletti *et al* 1991), 9% to 2% (Breukink *et al* 1990) and 7.8% to 1.8% (Wentink *et al* 1994). Despite the extensive measures of control implemented, the disease spread throughout the Japanese herds (Ogawa *et al* 1995). However, use of calf management, test and culling methods (Collins & Morgan 1992, Wilson *et al* 1993) and vaccination (Breukink *et al* 1990) has been reported to provide the quickest means of controlling paratuberculosis.

In this study fecal and epithelial cells microscopic test (Ziehl Neelson staining) was applied to diagnosis of paratuberculosis. However, some clinicopathological tests including ELISA (Ellis et al 1998), FI and CFT (Kormendy 1992), PCR (Cetinkaya et al 1996), Ziehl Neelson staining (Plante et al 1996) and fecal culture (Ellis et al 1998) used to help in the diagnosis of Johne's disease. According to the reports, the number of seropositive animals and the antibody titer was so higher than by fecal microscopic test and fecal culture in fact, the true diagnosis is based on the results of fecal culture (Ellis et al 1998).

Four months old calves are more susceptible to Johne's disease and often do not develop clinical signs until 2 to 5 years old (Stabel 1998). In fact, it is subclinical but some contributing factors such as age of first offering hay, type of concentrate feed to calves and calving in individual pens (Cetinkaya et al 1997) may play important rules in switching subclinical infection to overt disease. However, in spite of culling cattle with clinical signs as chronic or intermittent diarrhea, emaciation and death (Radostitis et al 1996), animals with subclinical form may cause economic loss because of reduced milk yield and poor reproductive performance (Wilson et al

1993, Stabel 1998). In this study the susceptible age was found over 2 years old same as reported by Guy *et al* (1991) and Cetinkaya *et al* (1996).

In relation to sex susceptibility it occurs in both beef (Belletti et al 1991) and dairy herds (Collins & Morgan 1992) and there was no apparently difference between sex as shown in this study. Although the rate of infection was greater in female than in male but it was not statistically significant. There was no report on seasonal variation in occurrence of paratuberculosis. Susceptible animals can infect under appropriate condition. In this study there was statistically differences between season and the rate of infection but from the biological point of view due to long incubation period it is impossible to determine the time of infection. The highest rate of infection was observed in winter and spring. However, microorganism is relatively susceptible to sunlight and drying and that could be a reason for seasonal distribution.

The results of this study indicate that the subclinical paratuberculosis in the Urmia dairy and beef cattle could be a problem in relation to cause for financial losses attributable to reduced milk production and increased culling of infected animals. Therefore, it is recommended that the application of control programs as fecal culture, intradermal tests and vaccination against Johne's disease will significantly reduced the rate of infection in long period although it never reach to zero percent.

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