

Short Paper

Bacteriological and serological studies on *Mannheimia haemolytica* infection in cattle slaughtered at Ahvaz (southwestern Iran) abattoir

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Summary

In order to investigate the prevalence of *Mannheimia haemolytica* infection in cattle, nasal and nasopharyngeal swabs and blood samples were obtained from 250 cattle after slaughter at Ahvaz (southwestern Iran) abattoir. Nasal and nasopharyngeal swabs were cultured on blood agar and incubated at 37°C for 24-48 h. The suspected bacterial cultures were processed for isolation of *M. haemolytica* following routine bacteriological techniques. Sera were tested by indirect hemagglutination test (IHA) to reveal antibodies against the organism. *M. haemolytica* was isolated from 1.6% of the sampled cattle. Statistical analysis showed that there was no relationship between age and sex with bacterial infection. Serological studies showed that 71.6% of tested sera contained antibody (titer \geq 1/16) against *M. haemolytica*. There was no association between age and sex with serological results.

Key words: *Mannheimia haemolytica*, Cattle, Ahvaz, Iran

Introduction

Pneumonic pasteurellosis of cattle is a major cause of economic loss in the feedlot industry. In addition to the death losses, the cost of treatment is considerable (Radostits *et al.*, 2007). *Mannheimia haemolytica* biotype A serotype 1 is the most common cause of pneumonia. Eleven serotypes have been demonstrated within *M. haemolytica*. *Mannheimia haemolytica* serotypes 6, 2, 9 and 11 and untypable serotypes have been found in lesions of pneumonic pasteurellosis (Angen *et al.*, 2002; Jaramillo-Arango *et al.*, 2008)

Mannheimia haemolytica is carried in the nasopharynx and tonsils of apparently healthy animals where, interestingly, serotype A2 is most commonly isolated

from both sheep and cattle (Rowe *et al.*, 2001). The aim of this study was to determine the prevalence of *M. haemolytica* infection in slaughtered cattle at the Ahvaz abattoir (southwestern Iran).

Materials and Methods

This study was carried out on 250 apparently healthy slaughtered cattle (130 male and 120 female) at the Ahvaz abattoir in Khuzestan province, southwestern Iran from February to July 2005. Sex and age of the sampled cattle were recorded before slaughter. Age was classified into four groups of <2, 2, 3, and \geq 4 years old, according to dental formula.

Nasopharyngeal and nasal swabs and 10 ml of blood samples were collected

immediately after slaughter. The swabs were streaked on 5% sheep blood agar plates and incubated at 37°C for 24 h. The plates were examined for colonies resembling *M. haemolytica* and suspicious colonies were examined microscopically and biochemically (Quinn *et al.*, 1994; Carter and Wise, 2004).

The blood samples were allowed to clot and were centrifuged for 10 min at 2500 g. After centrifugation, the sera were collected and stored at -20°C until analysis. Sera were tested for the presence of antibody against *M. haemolytica* by indirect haemagglutination (IHA) test. The IHA test was performed in two-fold serial dilutions of serum, beginning at 1:2 to 1:256. Sera with a titre of $\geq 1:16$ were considered positive (Wijewanta and Karunaratne, 1968).

The results were analyzed statistically using Chi-square and Fisher's-exact tests with a 5% significance level.

Results

The bacteriological investigations on the nasopharyngeal and nasal samples of the slaughtered cattle resulted in the isolation of *M. haemolytica* from 4 (1.6%) of these animals (Table 1). The percentage of *M. haemolytica* carriers in female and male were 0.8 and 2.3%, respectively. There was no significant difference between female and male ($P = 0.62$) or age groups ($P = 0.24$) for *M. haemolytica* status.

Indirect haemagglutination test revealed the titers of $\geq 1:16$ of *M. haemolytica* antibody in 178 (71.2%) cattle (Table 1). Statistical analysis showed that IHA titers and the age distribution of seropositivities were not significantly different between the female and male groups ($P = 0.20$) (Table 2).

Among 4 cattle recognized as carriers of *M. haemolytica*, 3 animals were seropositive and had titers of 1:16 and 1:128.

Table 2: Distribution of *M. haemolytica* antibodies by IHA between sex and age groups of slaughtered cattle at Ahvaz abattoir

Sex	Age (years)	No. positive (%)	No. negative (%)	Total
Female	<2	33 (70.2)	14 (29.8)	47
	2	12 (85.7)	2 (14.3)	14
	3	11 (91.6)	1 (8.4)	12
	≥ 4	35 (74.5)	12 (25.5)	47
Male	<2	39 (59.1)	27 (40.9)	66
	2	29 (76.3)	9 (23.7)	38
	3	12 (75)	4 (25)	16
	≥ 4	8 (80)	2 (20)	10
Total		178 (71.2)	72 (28.8)	250

Discussion

The results of this study showed that 1.6% of the examined cattle were carriers of the *M. haemolytica*. *Mannheimia haemolytica* isolation frequency was 18% (Jaramillo-Arango *et al.*, 2008) and 17% (Frank and Smith, 1983) in clinically healthy cattle and 34% (Jaramillo-Arango *et al.*, 2008) and 25% (Zanabria *et al.*, 2000) in pneumonic cattle. Angen *et al.* (2002) investigated 106 *M. haemolytica*-like strains isolated from pathological material from cattle, sheep, pigs and horses submitted to the Danish Veterinary Laboratory between 1994 and 1998. Out of 75 strains (71%) belonging to *M. haemolytica*, 57 were isolated from pneumonic lung tissue, 17 from the nose or trachea of animals suffering from respiratory distress, and one strain from arthritis in sheep (Angen *et al.*, 2002). Out of 584 lung samples of slaughtered sheep having clinical symptoms of pneumonia, 66 (11.35) *M. haemolytica* strains were isolated (Ilhan and Keles, 2007). Some studies have been investigated the percentage of carriers of *P. multocida*, and the results varied considerably, ranged from 0.4% to as high as 44.4% of the animals tested (Mohan *et al.*, 1968; Mustafa *et al.*, 1978; Ghandrasekaran *et al.*, 1981; Hiramune and De Alwis, 1982; Swada *et al.*,

Table 1: Determination of *M. haemolytica* antibodies by IHA in the serum samples of slaughtered cattle at Ahvaz abattoir

	<1:16 (%)	1:16 (%)	1:32 (%)	1:64 (%)	1:128 (%)	1:256 (%)
Male	42 (32.3)	51 (39.2)	23 (17.7)	9 (6.9)	3 (2.3)	2 (1.5)
Female	30 (25)	55 (45.8)	25 (21.7)	8 (6.7)	2 (1.7)	0 (0)
Total	72 (28.8)	106 (42.4)	48 (19.2)	17 (6.8)	5 (2)	5 (2)

1985; De Alwis *et al.*, 1990; Barbour *et al.*, 1997; Haji Hajikolaei *et al.*, 2006; Haji Hajikolaei *et al.*, 2008).

Although young animals are the most susceptible groups and respiratory diseases related to *M. haemolytica* occur most commonly in young growing cattle from 6 months to 2 years of age (Wray and Thompson, 1973; Pijoan *et al.*, 1999; Jaramillo-Arango *et al.*, 2008; Radostits *et al.*, 2007), in this study, statistical analysis showed that there was no relationship between age and sex with the serostatus of *M. haemolytica*.

The IHA titers of *M. haemolytica* antibodies were detected in 178 (71.6%) of the 250 cattle we tested. In a similar study, 84.8% of cattle and 27.12% of buffalo in Ahvaz had antibodies against *P. multocida* (Haji Hajikolaei *et al.*, 2006; Haji Hajikolaei *et al.*, 2008). In Khuzestan province, vaccination against *M. haemolytica* is not applied in cattle, therefore, all of the seropositive cattle might have acquired immunity by exposure to the organism.

In comparison to high seroprevalence (71.6%), the frequency of carrier (1.6%) of *M. haemolytica* was low. This may be due to loss of culturability of the organism on agar. Rowe *et al.* (2001) showed *M. haemolytica* cells lose culturability on agar, yet remain viable, although serotypes of *M. haemolytica* survive for long periods of time in relatively low-nutrition *in vivo* fluids and have survived for at least 244 days in ovine and 156 days in bovine trachobronchial washings (Rowe *et al.*, 2001). On the other hand, it is difficult to establish long term colonization of the nasal cavities of healthy, non-stressed calves with *M. haemolytica*. When calves were inoculated intranasally with infectious bovine rinotracheitis (IBR) or parainfluenza-3 (PI-3), the nasal cavity became much more susceptible to colonization with *M. haemolytica*, even in the presence of antibodies to the organism in the serum and nasal secretion. Over time, there may be an increase in the frequency of isolation of the bacteria from healthy calves that were moved to pens, held in low stress condition (Radostits *et al.*, 2007).

Our findings data support the hypothesis that *M. haemolytica* is carried in the nasopharynx of apparently healthy animals.

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