Study on the prevalence of *Pasteurella multocida* carriers in slaughtered cattle and relationship with their immunity status at Ahvaz abattoir

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Abstract: **This study was carried out on 250 slaughtered cattle at Ahvaz abattoir in Khouzestan province of Iran to investigate the occurrence of *Pasteurella multocida* carriers and relationship with their immunity status. Nasopharyngeal swabs and 10 ml blood samples were taken immediately after slaughter. The swabs were streaked on 5% sheep blood agar plates. Cultures were incubated at 37°C for 24h and the plates were examined for colonies resembling *P. multocida*. Suspicious colonies were further subcultivated and examined microscopically and biochemically. The isolates were serotyped serologically and their pathogenicity in mice was carried out. Sera samples were tested for the presence of antibody against *P. multocida* by indirect haemagglutination (IHA) test and sera with a titer of ≥1:16 were considered as positive. *P. multocida* was isolated from the nasopharynx of 6 (2.4%) out of 247 healthy cattle examined. There was no relation between infection and sex or age. All of 6 isolates belonged to type B. They were pathogenic for mice and caused death in injected mice within less than 24h after injection. Indirect haemagglutination test revealed the titers of ≥1:16 of *P. multocida* antibody in 212 (84.8%) cattle. Among 6 cattle recognized as the carriers of *P. multocida*, 5 were positive serologically and 2, 2, and one of them had titers 1: 128, 1: 64, and 1: 32, respectively.**

Key words: *Pasteurella multocida*, carrier, cattle, Ahvaz, Iran.

Introduction

Pasteurella multocida is a primary or, more frequently a secondary invader in pneumonia of cattle swine, sheep, goats and other species (Carter and Wise 2003). Two serotypes (B, E) of *P. multocida* causes hemorrhagic septicemia, an acute disease principally of cattle and water buffalo in tropical and subtropical regions. The disease is an acute septicemia characterized by a rapid course, high fever, depression swollen and hemorrhagic lymph nodes, numerous subserous petechial hemorrhages, hypersalivation, diarrhea, and sudden death. The morbidity rate varies considerably, but the mortality is high (Carter and Wise, 2003; Hirsh *et al.*, 2004). The disease occurs in Africa, Asia, central and south America and Europe (Benkirane *et al.*, 2002; Borkowska- Opacka *et al.*, 2003; De Alwis *et al.*, 1992; Mohan *et al.*, 1968; Mustafa *et al.*, 1978; Wijewanta *et al.*, 1968).

In Iran, the incidence of haemorrhagic septicemia

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varies greatly in different agro-climate zones and enzootic and non-enzootic areas can be mapped out clearly. There are annual reports of the incidence of the disease in cattle and buffaloes from Iran by OIE. For example, according to these reports, the numbers of outbreaks of hemorrhagic septicemia in Iran in cattle in the years of 1997, 1999, 2000, 2002, 2003 and 2004 were 42, 11, 20, 40, 87 and 11, respectively. Haemorrhagic septicemia is endemic in some areas of Iran especially in Khouzestan province where cattle and buffaloes are commonly kept together.

The occurrence of healthy carrier of *P. multocida* in cattle, buffaloes, other animals and in human have been reported from many countries (De Alwis *et al.*, 1992; Chandrasekaran *et al.*, 1981; Hiramune *et al.*, 1982; Mohan *et al.*, 1968; Mustafa *et al.*, 1978). So far, there has not been any report of *P. multocida* carrier state among cattle in Iran. Therefore, the present work was undertaken for the first time in Iran to determine the rate of cattle carrier of *P. multocida* and its relationship with immunity status.

Materials and Methods

This study was carried out on 250 slaughtered cattle at Ahvaz abattoir in Khouzestan province, south-west of Iran from February to July 2005. Before slaughter, sex and age of cattle were documented. Cattle were divided into two sex groups (130 male and 120 female). Each sex group was also divided into four age groups of <2, 2, 3, and ≥4 years old, according to dental formula.

Nasopharyngeal swabs and 10ml blood samples were collected immediately after slaughter. Sterile cotton swabs were used in sampling the nasopharynx of each animal. 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 *P. multocida* and suspicious colonies were further subcultivated and examined microscopically and biochemically. Biochemical tests were performed according to the methods of Carter *et al* (1991) and Quinn *et al* (1994). Based on these methods, the following biochemical characters of *P. multocida* were assessed: fermentation of sugar (lactose, sucrose, manitol and maltose) in phenol red broth base at 37°C for 48h, catalase production, H_2S production in triple sugar iron agar (TSI) and indole, oxidase and urease production.

Table 1: Isolation of *P. multocida* from the nasopharynx of slaughtered cattle at Ahvaz abattoir (2005).

Sex	Age	positive numbers (%)	negative numbers (%)	Total
Male	<2	0 (0)	66 (100)	66
	2	3 (7.9)	35 (92.1)	38
	3	1 (6.25)	15 (93.75)	16
	>4	0 (0)	10 (100)	10
Female	<2	1 (2.18)	46 (97.9)	47
	2	0 (0)	14 (100)	14
	3	0 (0)	12 (100)	12
	>4	1 (2.1)	46 (97.9)	47
Total		6(2.4)	244(97.6)	250

The serotypes of the *P. multocida* isolates were determined serologically in Razi Vaccine and Serum Research Institute and pathogenicity was carried out in mice, as described by Namioka *et al* (1963).

The blood samples were allowed to clot and were centrifuged for 10 min at 2500g. After centrifugation, the sera were collected and stored at -20°C, until ready for test. Sera samples were tested for the presence of antibody against *P. multocida* 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 ≥1:16 were considered as positive (Wijewanta *et al.*, 1968).

The results were analyzed statistically using Chi-square and Fisher's-exact tests with confidence level 95%.

Results

The bacteriological investigations on the nasopharynx samples of the slaughtered cattle resulted in the isolation of *P. multocida* from 6 (2.4%) of these animals (Table 1).

The percentage of cattle carrier of *P. multocida*, was not significantly different (p=0.685) between female and male groups.

Distribution of carrier state between age groups of male and female cattle are shown in tables 1. The

Table 2: Detection of *P. multocida* antibodies by IHA in the serum of slaughtered cattle at Ahvaz abattoir (2005).

	< 1:16 (%)	1:16 (%)	1:32 (%)	1:64 (%)	1:128 (%)	1:256 (%)
Male	21 (16.15)	33 (25.38)	40 (30.77)	18 (13.85)	12 (9.23)	6 (4.61)
Female	17 (14.1)	28 (23.3)	30 (25)	30 (25)	13 (10.83)	2 (1.66)
Total	38 (15.2)	61 (24.4)	70 (28)	48 (9.2)	25 (10)	8 (3.2)

Table 3: Distribution of *P. multocida* antibodies by IHA between sex and age groups of slaughtered cattle at Ahvaz abattoir (2005).

Sex	Age	positive numbers (%)	negative numbers (%)	Total
Female	< 2	40 (85.1)	7 (14.9)	47
	2	12 (85.7)	2 (14.3)	14
	3	11 (91.6)	1 (8.4)	12
	≥4	40 (85.1)	7 (14.9)	47
Male	< 2	51 (77.3)	15 (22.7)	66
	2	35 (92.1)	3 (7.9)	38
	3	14 (87.5)	2 (12.5)	16
	≥4	9 (90)	1 (10)	10
Total		212(84.8)	38 (15.2)	250

results indicated that carrier rates of *P. multocida* between age groups of females (p=0.905) and males cattle (p=0.116) were not significantly different.

All of the 6 isolates of *P. multocida* belonged to capsular type B. They were pathogenic for mice and caused death in injected mice within less than 24h after injection.

Indirect haemagglutination test revealed the titers of ≥1:16 of *P. multocida* antibody in 212 (84.8%) cattle (Table 2). Statistical analysis showed that IHA titers and the age distribution of seropositivities were not significantly different between female and male groups (tables 3).

Among 6 cattle recognized as the carriers of *P. multocida*, 5 were positive serologically and 2, 2, and one of them had titers 1: 128, 1: 64, and 1: 32, respectively.

Discussion

In this study which carried out on cattle slaughtered at Ahvaz abattoir to determine the carrier rate of *P. multocida*, showed 6 (2.4%) of examined cattle to be nasopharyngeal carriers of the organism. In different studies investigating the percentage of carriers of *P. multocida*, the results have been considerably in variance and ranged from 0.4% to as high as 44.4% of the animals tested. The evidences available are suggestive of a relationship between the percentage of carrier animals and a recent exposure to the disease and the proportion of carriers is highest immediately after an epizootic and diminishes rapidly thereafter (Barbour *et al.*, 1997; De Alwis *et al.*, 1990; Chandrasekaran *et al.*, 1981; Hiramune *et al.*, 1982; Mohan *et al.*, 1968; Mustafa *et al.*, 1978; Swada *et al.*, 1985). Following an epizootic of haemorrhagic septicemia, surviving clinically normal in-contact animals carry in their nasopharynx virulent *P. multocida*. Such animals could excrete the organism in their nasal secretions and constitute a source of infection to susceptible animals (Hiramune *et al.*, 1982).

Mustafa *et al* (1978), found the carrier state in 44.4% of healthy cattle associated with an outbreak of haemorrhagic septicemia, while in herds unassociated with the disease the carriers percentage were 3.89%, 5.5% and nil.

De Alwis *et al* (1990) showed in most of buffaloes, experimentally infected or naturally exposed to haemorrhagic septicemia, *P. multocida* appeared in the nasopharynx for a short period initially and then disappeared. The organism reappeared intermittently and the longest observed period of reappearance was 215 days after exposure. On the other hands in the study of Hirammune and De Alwis (1982), of 589 cattle and buffaloes examined from enzootic area of Sri Lanka, 16 were found to be carriers (2.75%). No carrier could be found among 250 animals from non-enzootic areas. Thirteen of the 16 positive isolations of *P. multocida* from the nasopharynx were made from animals in 8 herds where the disease had occurred within the 6 weeks prior to examination. The highest carrier rate (22.7%) was observed in 4 herds where haemorrhagic septicemia had occurred a week previously and

diminished rapidly to 1.9% in herds where the disease had occurred 6 weeks previously. These observations indicate the percentage of carriers is related to the time of outbreak. The reduction in the number of carriers with time may reflect a total elimination of *P. multocida* or whether the organisms survive in site other than the nasopharynx.

All 6 isolates of *P. multocida* we obtained, were pathogenic for mice. They caused death to injected mice within 24h after injection. This is in accordance with the fact that healthy animals have been known to harbor virulent *P. multocida* in the respiratory tract. These apparently healthy animals could be the source of sporadic outbreak (Chandrasekaran *et al.*, 1981). So far, various serotypes (A, B, C, D and E) of *P. multocida* have been detected among the livestock populations (Borkowska- Opacka *et al.*, 2003; Ghandrasekaran *et al.*, 1981; Kedrak *et al.*, 2003; Kumar *et al.*, 2004; Namioka *et al.*, 1963). Serotypes B:2 an E:2 are two common serotypes of *P. multocida* associated with disease in animals in Asia and Africa, respectively (Benkirane *et al.*, 2002). In our study, all isolates of *P. multocida* belonged to Carter type B.

The IHA titers of *P. multocida* antibodies were detected in 212 (84.8%) of 250 cattle we tested. Although, not all instances of naturally acquired immunity to haemorrhagic septicemia may be explained by exposure to the disease and it is possible that other antigenically related pasteurella spp. be responsible in such instances (De Alwis and Sumandasa, 1982), but according to the reports of OIE, there were outbreaks of haemorrhagic septicemia in Iran in the years of 2001-2004 and the authors diagnosed this disease in the hospital of the faculty of veterinary medicine in Shahid Chamran university in Ahvaz in May 2005, therefore this high seroprevalence may be due to these outbreaks. On the other hand, in Khouzestan province, vaccination against *P. multocida* is applied only when the outbreak of haemorrhagic septicemia occurs. At such situation, if vaccination be accomplished, all populations of cattle or all herds will not be covered. Therefore, the majority of titers we found may be due to natural infections. On the other hand, a IHA titer is indicative of natural exposure and confers solid immunity, whereas the IHA response to vaccination is poor (De Alwis *et al.*, 1982). In fact, the sensitivity of IHA test is low to reveal the vaccination induced immunity. In a group of vaccinated animals, immune to direct challenge by *P. multocida*, only 27% showed IHA antibody (De Alwis *et al.*, 1982; De Alwis *et al.*, 1990). Antibodies against serogroups B, E and A were demonstrated by IHA, respectively in 93%, 98% and 98% of calves tested in USA (Swada *et al.*, 1985). In the observation of De Alwis and Smandasa (1982), the incidence of naturally acquired immunity was related to level of incidence of haemorrhagic septicemia in the region and ranged form 0.47% in the low incidence areas to 7.2% in the moderate incidence areas and 36.1% in the high incidence area.

Among 6 cattle we recognized as carriers of *P. multocida*, 5 animals were positive serologically and 2, 2, and one of them had titres 1: 128, 1: 64, and 1: 32, respectively. Healthy cattle could be carrier of *P. multocida* in its nasopharynx. It may then temporarily contaminate the environment by its saliva. Other animals which are physiologically and immunologically susceptible may become infected from the temporarily contaminated environment. An animal that carriers the virulent strain of *P. multocida* in its nasopharynx may have acquired a natural active immunity. When the resistance of a herd is lowered, susceptible animals may become infected with a virulent strain present in a resistant host. It is also possible that cattle may develop a natural immunity by means of a subclinical infection with a strain of *P. multocida* (Wijavanta *et al.*, 1968). On the other hand, the carrier animal of *P. multocida* exists in active or latent states. During the latent state, the organism persists in the tonsils whilst in the active state it also appears in the nasopharynx which is followed by a prolonged latent carrier state (Mohan *et al.*, 1968). In seropositive animals of which the organism could not be isolated from nasopharynx, the organism might persist in other organs like tonsil and the animals were latent carriers.

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مطالعه فراوانی حاملین پاستور لامولتوسیدا و ارتباط آن با وضعیت ایمنی آنها در گاوهای کشتار شده در کشتارگاه اهواز

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چکیده

به منظور بررسی میزان فراوانی حاملان پاستور لامولتوسیدا و ارتباط آن با وضعیت ایمنی آنها، این مطالعه بر روی ۲۵۰ راس گاو کشتار شده در کشتارگاه اهواز در استان خوزستان صورت گرفت. جهت انجام این مطالعه بلافاصله بعد از کشتار ۱۰ میلی لیتر خون و نمونه از نازوفارنگس با سواب استریل اخذ گردید. سوآبها در آگار حاوی ۵ درصد خون گوسفند کشت داده شدند. محیطهای کشت به مدت ۲۴ ساعت در انکوباتور ۳۷ درجه سانتیگراد نگهداری و بعد از این مدت، کلنی های مشکوک به پاستور لامولتوسیدا خالص گردیده و سپس با استفاده از روش های بیوشیمیایی تعیین هویت گردیدند. جدایه های پاستولامولتوسیدا ۱ تعیین تیپ شده و بیماری زایی آنها نیز در موش مورد ارزیابی قرار گرفت. نمونه های سرم از نظر وجود آنتی بادی ضد پاستولامولتوسیدا ۱ با استفاده از روش آگلوتیناسیون غیر مستقیم (IHA) آزمایش شدند و نمونه هایی که در رقت های مساوی یا بالاتر از ۱:۱۶ دارای آنتی بادی ضد پاستور لامولتوسیدا بودند مثبت در نظر گرفته شدند. از مجموع ۲۵۰ راس گاو ۶ راس در نازوفارنگس حامل باکتری فوق بودند. ارتباطی بین سن یا جنس با آلودگی وجود نداشت. کلیه جدایه های پاستور لامولتوسیدا متعلق به تیپ B بوده و باعث تلف شدن موش ها در عرض ۲۴ ساعت بعد از تزریق باکتری گردیدند. در IHA، میزان ۸۴/۸ درصد نمونه ها واحد پادتن ضد پاستور لامولتوسیدا ۱ بودند. تعداد ۵ راس از ۶ راس گاو حامل پاستور لا از نظر سرمی مثبت بودند.

واژه های کلیدی: پاستور لامولتوسیدا، حاملان، گاو، اهواز، ایران.

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