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Study on the Isolation and Pathogenicity of *Pasteurella multocida* Type A in Calves in Saudi Arabia

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Abstract: A total number of 400 nasopharyngeal and nasal swabs, collected with symptom suggestive of respiratory diseases, were examined. *Pasteurella multocida* were isolated in a percentage 2.5 from the total samples. Antigenic typing revealed that 50% of the isolates belong to capsular type A, 25% to type C and 25% to type E. Calves experimentally infected with *P. multocida* type A demonstrated typical clinical signs of haemorrhagic septicaemia, including fever, anorexia, submandebular and brisket oedema, congested mucous membrane, nasal discharge, moist rales in lungs, increase in respiratory rate, increase in pulse rate, tachycardia and recumbency. The severity of infection and mortality rate was more pronounced in calves infected with 10 ml of virulent *P. multocida* ($6x10^8$ c.f.u. ml^{-1}) type A. Different organs were demonstrated to be predilection sites for *P. multocida*; these include lung, heart, trachea, spleen, liver, pharynx and oedematous fluid in the neck and nasal cavity. Infected calves which escaped death and completely recovered showed the presence of *P.multocida* in the nasal cavity during the whole period of the experiment.

Key words: *Pasteurella multocida*, incidence, antigenic types, pathogenicity, calves, Saudi Arabia

INTRODUCTION

Haemorrhagic septicaemia (HS), caused by specific serotypes of *Pasteurella multocida*, is one of the most serious acute, fatal diseases principally affecting cattle and buffaloes in Asia and Africa. Different serotypes of *P. multocida* have been identified these are A, B, C, D and E[1]. In Saudi Arabia *P. multocida* infection and the associated economical losses have not been adequately assessed. However only two detailed studies on haemorrhagic septicaemia have been reported by Hafez *et al.*[2] and AL-Dughaym[3]. Although routine vaccinations are carried out in the Kingdom, there seems to be no available base-line information's about the various prevalent serotypes of *P. multocida*.

This research was aimed to isolate *P. multocida* from suspected cases of haemorrhagic septicaemia in calves, serotyping of the isolates and to confirm experimentally the virulence and the pathogenicity of the predominant isolates in calves.

MATERIALS AND METHODS

Sample: Nasopharyngyeal samples were collected by sterile cotton swabs from 74 calves with pneumonic lesions at AL-Ahsa abattoir. Nasal swabs were collected from 326 calves presented to Veterinary Teaching Hospital with clinical signs suggestive of respiratory tract involvement. The nasopharyngyeal and nasal swabs were used for isolation and identification of *P. multocida*.

Isolation and identification: Blood agar (Oxoid), MacConkey's agar (Oxoid), Nutrient broth (Oxoid), Brain-heart infusion (BHI) broth (Biolife) and Brain-heart infusion (BHI) agar (Liofil chem) were used for isolation of *P. multocida*. The organism was identified by using API 20 E strip (France). Hyperimmune sera were prepared in the rabbit by using reference strains of *P. multocida* type A, B, C, D and E (received from the Public Health Laboratory Service, London). The isolated strains were antigenically typed by using the rapid slide agglutination (RSA) and agar gel immunodiffusion (AGID) techniques.

Experimental infection: The microorganism used for experimental infection was a virulent strain *P. multocida* type A. isolated during the present study.

Nine conventionally reared Friesian non-vaccinated calves aged about 2 to 3 months, ranging from 60 to 70 kg body weight were used. They were fed maintenance ration consisting of grass and alfalfa hay and a commercial grain

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mixture. Calves were acclimatized for at least two weeks before experimentation. They were kept in isolation houses at the premises of the Veterinary Teaching Hospital (VTH). All calves were negative to the presence of *P. multocida*. Calves were divided into three groups (n=3). Group (1) comprised the calves which were inoculated intratracheally (I/T) with 10 ml of *P. multocida* type A cultures containing $6x10^8$ c.f.u. $/ml^{-1}$. Group (2) consisted of the calves which were inoculated I/T with 5 ml of *P. multocida* type A cultures containing $6x10^8$ c.f.u. ml^{-1}. Group (3) were kept as non- infected control.

RESULTS AND DISCUSSION

In the present investigation the isolation percentage of *P. multocida* was 4.1 and 2.15% from the abattoir and Veterinary Teaching Hospital respectively. The over all isolation percentage of *P. multocida* from the total calves examined was 2.5% (10 isolates of 400 samples) (Table 1). The various characteristic of the *P. multocida* isolated during the present investigation are in an accord with the findings of Shigidi and Mustafa[4] and Francis and Carter[5]. Hafez *et al.*[2] reported the isolation of 17.5% *P. multocida* from calves in the Eastern region of Saudi Arabia. Variation in the isolation percentage have been reported by a different authors (3 by Huq and Grumbles[6]; 19.5 by Atsumi *et al.*[7] and 7.6% by Hossam[8]). Researchers who collected samples from cattle with frank clinical signs of haemorrhagic septicaemia or directly from lungs with obvious pathological lesions reported high incidence of *P. multocida*, among these are Kielstein and Schimmel[18] who reported an isolation rate of 50.4% of 115 calves with pneumonia in ten herds and Hossam[8] reported an incidence of 25.2% in samples collected from lung lesions.

The eight isolates of *P. multocida* identified during the present study were found to belong to type A (50%), type C (25%) and type E (25%). No isolates was found to belong to type B or type D. (Table 1). The association of *P. multocida* type A as a predominant isolates with bovine pneumonia have been shown by Atsumi *et al.*[7], Purdy *et al.*[9] and Hossam[8]. The isolation of type E form bovine pneumonia were also reported by Shigidi and Mustafa[4], Hossam[8]. During this study 2 isolates were found to belong to Type C. Bain[10] suggested that type C should be dropped because it is not an important pathogenic type.

Control calves were apparently healthy during the whole period of the experiment. The severe clinical signs observed in the infected two groups of calves included anorexia, fever, submandibular and brisket oedema, congested mucous membranes, serous nasal discharge,

Table 1: Isolation percentage and antigenic typing of *P. multocida* isolated from naturally infected calves

Location	Total number of calves	Number of isolated	%
Abattoir	74	3	4.1
Calves with respiratory infection	326	7	2.15
Total	400	10	2.5
Total number examined	Type A	Type C	Type E
* 8	4 (50%)	2 (25%)	2 (25%)

* Two strain were untyped due to technical error
NT/ Tests against type B and D antigens were negative

moist rales, increase in respiratory rate and increase in pulse rate, terminally there was tachycardia and recumbency. One calf from the three calves inoculated with 5 ml died in the 3rd day and two calves from the three calves infected with 10 ml died at the 4th and 6th day (Table 3). These symptoms of haemorrhagic septicaemia are generally in accord with those reported by Radostits *et al.*[11] and Dowling *et al.*[12]. Dullness, respiratory distress and high rectal temperature were prominent signs in the second day; the clinical signs reached its peak in the 5th days. These findings confirm those of Dowling *et al.*[12] who studied the pathogencity of *P. multocida* serotype A in calves. Survived animals showed gradual recovery, this was also observed by DE Alwis[13], Dowling *et al.*[12], Gourlay *et al.*[14] and Shoo[19].

During the present investigation mortality rate was one out of three calves inoculated with 5 ml of the inoculum and two out of three calves that inoculated with 10 ml (Table 2) the difference in the rate of death between the two groups could be explained by the difference in the inoculum size[12,14], while the difference in the susceptibility of calves in both group could be attributed to an interaction between a number of factors such as burden of infections, individual defense mechanism and immunity level. DE Alwis[13] attributed the phenomenon that some animals succumb to clinical disease while others develop the disease to what is described as an "arrested infection" leading to naturally acquired immunity and to the different pattern of morbidity and mortality among infected animals.

In this investigation *P. multocida* was recovered from different organs of dead or scarified infected calves (Table 3). The nasal cavity was also positive to the presence of the organism in most of the infected calves.

The isolation of the *P. multocida* from the nasopharynx and other internal organs in natural or experimental infections was reported by Gourlay *et al.*[14], DE Alwis *et al.*[15].

P. multocida was recovered from the nasal cavities of infected calves which apparently recovered from the infection (Table 3). Such recovered calves may act as

Table 2: Clinical state, recovery and death (%) in calves experimentally infected intratracheally with 5 and 10 ml of *P. multocida* ($6x10^8$ ($6x10^8$ c.f.u. ml^{-1}) type A

Calves	Total No. of calves	* Severe clinical signs	* Mild clinical signs	Recovery	Death
Control non - infected calves	3	_	_	3	--
Calves infected with 5ml of virulent *P. multocida*	3	1	2	2	1 (33.3%)
Calves infected with 10ml of virulent *P. multocida*	3	3	_	1	2 (66.7%)

*Severe clinical signs: anorexia, fever, congested mucous membranes nasal discharge, moist rales, submandibular and brisket oedema and terminally tachycardia and recumbency. *Mild clinical signs: mild signs of moist rales, slight serous nasal discharge and reduced appetite.

Table 3: Isolation *P. multocida* of type A from the nasal cavity and other organs of the calves infected intratracheally with 5 and 10 ml of *P. multocida* ($6x10^8$ c.f.u ml^{-1})

		Isolation of the organism from different organs						
Calves	Isolation period from nasal cavity (days)	Neck (oedema)	Trachea	Heart	Lung	Pharynx	Spleen	Liver
Infected with 5 ml (Died at 3rd day)	Not isolated	+ve	+ve	+ve	+ve	-ve	+ve	-ve
Infected with 10 ml (Died at 4th day)	Not isolated	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Infected with 10 ml (Severely ill, scarified at day 6)	2nd – 6th day	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Infected with 5 ml (recovered, scarified at day 14)	2nd – 14th day	-ve	+ve	-ve	+ve	-ve	-ve	-ve
Infected with 5 ml (recovered)	2nd–*14th day	*NT	NT	NT	NT	NT	NT	NT
Calve Infected with 10ml (recovered)	2nd–*14th day	NT	NT	NT	NT	NT	NT	NT

• 14th : End of the experiment • NT : Not tested

carrier animals. This carrier state, as indicated by DE Alwis *et al.*[16], is important in the spread of haemorrhagic septicaemia, especially when carrier animals move from endemic to non-endemic areas. The transience of this nasopharyngeal carrier state was also demonstrated in haemorrhagic septicaemia exposed cattle and buffaloes in the felid[17] as well as among experimentally exposed buffaloes[15].

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