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

N.A. Al-Humam, A.M. Al-Dughaym, G.E. Mohammed, F. M. Houssawi and A.A. Gameel
Department of Clinical Studies, Department of Microbiology and Parasitology, Department of Pathology, College of Veterinary Medicine and Animal University, King Faisal University, P.O. Box 1757, Al-Salama 13982, Saudi Arabia

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, submandibular and brisket oedema, congested mucous membrane, nasal discharge, moist rales in lungs, increased 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 (6x10^6 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, 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 as 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.[3] and AL-Dughaym.[4] Although routine vaccinations are carried out in the Kingdom, there seems to be no available base-line information 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: Nasopharyngeal samples were collected by sterile cotton swabs from 74 calves with pneumatic lesions at AL-Hlsa abattoir. Nasal swabs were collected from 326 calves presented to Veterinary Teaching Hospital with clinical signs suggestive of respiratory tract involvement. The nasopharyngeal 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 (Bioline) 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
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 6x10⁶ 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 6x10⁶ 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 accord with the findings of Shigidi and Mustafiœ and Francis and Carter. Haefez et al. 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 different authors (3 by Huq and Grumbles, 19.5 by Atsumi et al. and 7.6% by Hossam). Researches 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 who reported an isolation rate of 50.4% of 115 calves with pneumonia in ten herds and Hossam 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., Purdy et al. and Hossam. The isolation of type E form bovine pneumonia were also reported by Shigidi and Mustafiœ, Hossam. During this study 2 isolates were found to belong to Type C. Bain 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, moist mules, increase in respiratory rate and increase in pulse rate, terminal 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. and Dowling et al. 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. who studied the pathogenicity of *P. multocida* serotype A in calves. Survived animals showed gradual recovery, this was also observed by DE Alwis, Dowling et al., Gourlay et al. and Shouk

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, 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 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., DE Alwis et al.

*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 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 |
| * g | 4 (50%) | 2 (25%) | 2 (25%) |

* Two strain were untyped due to technical error
* NT Tests against type B and D antigens were negative
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 field27 as well as among experimentally exposed buffaloes83.

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REFERENCES


