

ISOLATION AND CHARACTERIZATION OF *Pasteurella multocida* FROM CAPRINE PNEUMONIC LUNGS

UGOCHUKWU, Emman Ikenna

Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. Email: emmaugochukwu@yahoo.com

Phone: +234 7039071914

ABSTRACT

By conventional microbiological methods, investigation was carried out in Nsukka and Enugu areas of Enugu State, Nigeria to determine the percentage frequency of occurrence of Pasteurella multocida from caprine pneumonic lungs in Nsukka and Enugu areas of Enugu State, Nigeria. In this study that spanned 12 years, a total of 350 pneumonic lung samples were collected from West African Dwarf goats, Sokoto Red goats and Fulani goats slaughtered in Nsukka and Enugu Municipal abattoirs in Enugu State. By cultural, biochemical and physiological attributes, four (4) of the isolates were characterized as Pasteurella multocida Both gross and histopathological lesions of the pneumonic lung specimens from which this aerobic bacterium was isolated were correlated with the organism. In spite of the low percentage frequency (1.14%) of isolation of Pasteurella multocida in this study, attention is drawn to the pathogenic potential of this organism for goats and other livestock in this part of Nigeria.

Keyword: Isolation, Characterization, *Pasteurella multocida*, Pneumonic, Caprine

INTRODUCTION

One of the major limiting factors in the successful rearing of goats and sheep in Nigeria is disease. This is rather disturbing since, goats contributes 40% of the meat consumed in Nigeria (Ademsun, 1986) and is said to adapt physiologically to harsh environmental conditions (Silanikove, 2000) of tropical forest belt of West Africa (Olapade and Onwuka, 2006).

Endoparasitism and respiratory diseases have been incriminated as being responsible for most of the morbidity and mortality in small ruminants (Ojo, 1971; Ojo, 1976; Ikede, 1977; Ikede, 1978; Okoh and Kaldras, 1980). Bacteriological studies on the occurrence and distribution of bacteria and mycoplasma in the pulmonary lesions of goats and sheep in Western Northern Nigeria have shown high frequency of occurrence of *Pasteurella hemolitica*, *multocida*, *soaphylococcus* species and *Mycoplasma arginini* (Ikede, 1977; Falade *et al.*, 1977; Ikede, 1978). The finding of Ikede (1977) in University of Ibadan farm confirmed that respiratory diseases were the major cause of death in farmed sheep and goats.

Not only was animal performance greatly affected in terms of poor growth, reduced productivity, debility and lowered resistance (Abubakar *et al.*, 1980) but also caprine pneumonia is globally a threat to the goat industry due to high mortality.

Worse still, concomitant secondary bacterial infection has been found to complicate a fatal viral disease of sheep and goats in West Africa sub region known as *Peste des Petit Ruminants*, (Ugochukwu, 1983).

In this investigation, the isolation, culture and identification of *P. multocida*, a potentially pathogenic aerobic bacterium and their possible role in the pathogenesis of caprine pneumonia are detailed.

MATERIALS AND METHODS

Animals: Animals used for this investigation were goats mainly of West African Dwarf breed, Sokoto Red and Fulani breeds slaughtered at Nsukka and Enugu Municipal abattoirs in Enugu State, Nigeria.

Sampling: Pneumonic lung samples were randomly collected from goats suffering from different clinical respiratory diseases. A total of 350 lungs were examined for pathological changes and all pneumonic lung specimens were aseptically collected in Bijou bottles with a pair of flamed thumb forceps and a pair of flamed scissors both dipped in methylated spirit. Each specimen was carefully numbered and labelled.

Microbial Studies: With a wire loop, an inoculum was obtained from the specimen bottle containing the pneumonic lung sample; a wet mount was made on a dry clean slide and examined under the microscope for the presence of aerobic bacterial organisms. Furthermore, the inoculum obtained from the pneumonic lung samples was streaked over blood and chocolate agar plates. The inoculated blood agar plates were incubated at 37°C for 24 hours in a glass jar using candle flame to reduce the degree of aerobiosis. The two objectives of this primary inoculation were, first to cultivate the organisms and secondarily to obtain discrete colonies. After 24 hours of aerobic incubation, the plates were brought out and the type and amount of growth of colonies were studied. The size, shape, colour and character of the colonies were recorded. Subsequently, the colonies were stained using Grain-stain technique and examined for microscopic morphology.

The following physiological, enzymatic and biochemical tests were conducted on each blood agar plates using standard bacteriological techniques. These tests include: Motility test, growth in nutrient

agar, growth in McConkey agar, growth under increased CO₂ tension, growth under anaerobic condition, catalase test, oxidase test, indole production test, hydrogen sulphide production, urease production, Nitrate reduction test, Oxidation-Fermentation (O – F) test and Sugar test in which glucose, lactose, sucrose or mannitol were incorporated separately.

RESULTS

Lung specimen from which *P. multocida* was isolated did not show significant histopathological changes except for the cellular infiltration involving mainly the neutrophils.

From physiological and biochemical tests, four out of a total of 153 aerobic bacteria isolates were characterized to be *P. multocida*. No haemolytic species was recovered out of the 350 pneumonic lung examined bacteriologically, only (1.14% were positive for *P. multocida* (Figure 1). Physiological and biochemical characteristics of *P. multocida* isolated from caprine pneumonic lungs are shown on Table 1.

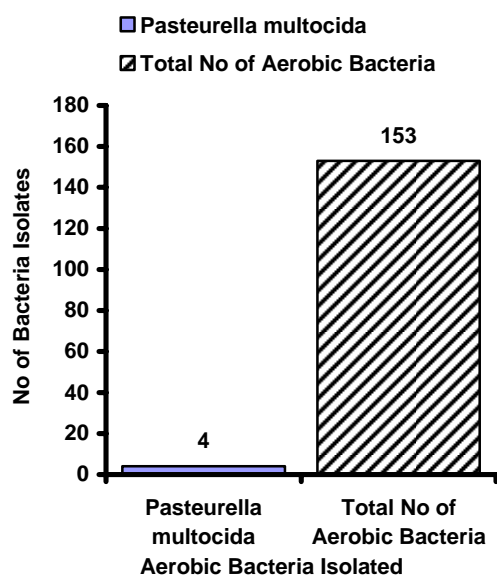


Figure 1: Prevalence of *Pasteurella multocida* from caprine pneumonic lungs

DISCUSSION

Previous investigations in Northern and Western parts of Nigeria led to successful isolation of *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Proteus species*, *Pseudomonas aeruginosa*, *Streptococcus species*, *Corynebacterium pyogenes* and *P. haemolytica* from goats (Ojo, 1976; Ikede, 1977).

In this investigation centred on the isolation of *Pasteurella* from caprine pneumonic lungs, the four species were biochemically and physiologically characterized to be *P. multocida*. Incidentally, *P. haemolytica* was not isolated in this study in this part of the country. The failure to isolate *P. haemolytica*

in this bacteriologic investigation cannot be effectively explained until further investigation to determine the prevalence of this species in this part of the country has been fully explored.

P. multocida is a potential pathogenic bacteria organism, which has been incriminated, in both human and animal infections where it causes often times severe respiratory abnormalities that can terminate in death (Dritz *et al.*, 1996).

The successful isolation of *P. multocida* in this investigation is interesting not only because of its traditional role as a disease-causing aerobic bacteria but also because of its toxigenicity (Hall *et al.* 1987; Cheville and Rimler, 1989) making it a highly invasive, pathogenic and virulent microorganisms.

Its ability to initiate infection in different anatomic structures of the body in man as well as in animals has been documented (Dritz *et al.*, 1996).

Its toxin has been reported to have deleterious effects on organs systems and immuno-responsiveness (Hall *et al.*, 1987; Cheville and Rimler, 1989).

Although the percentage isolation was relatively low (1.15%), the possible role of *P. multocida* in the aetiology and pathogenesis of caprine pneumonia should not be underestimated. Although, it may be found occasionally as a normal inhabitant of the respiratory system, experimental evidence has shown that under certain conditions associated with debilitation, nutrition and climatic factors, this organism may singly or in concert with other organisms flare up to cause severe infections with high morbidity and mortality. The neutrophilic infiltration in pneumonic lung samples from which *P. multocida* was isolated is not characteristic but rather a common finding in bacterial infection.

The isolation of *P. multocida* from caprine pneumonic lungs, in this investigation, is more disturbing in the light of the prevalence of a highly fatal viral disease of goats in this parts of West Africa sub region, since recent works have implicated concomitant secondary bacterial infection in the severity, pathogenesis and pathology of *Pestes Des Petit Ruminants* (PPR) (Ugochukwu, 1983; Ugochukwu, 1985, Ugochukwu and Agwu, 1991).

However, further scientific work is needed to clearly elucidate the precise role of *Pasteurella multocida* and indeed other species of *Pasteurella* in the pathology of caprine pneumonia in this unique geo-climatic region of the world.

Moreover, more research is needed on the risk factors due to cross infection to human population considering the fact that goats are kept in close proximity to human population in cultural setting of this part of Nigeria under bacteriological investigation.

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Table 1: Detailed physiological and biochemical properties of Isolates of *Pasteurella*

Isolate Number	Hemolysis (sheep blood)	Motility	Growth in MacConkey agar	Growth in nutrient agar	Growth under increase CO ₂	Growth under anaerobic condition	Catalase	Oxidase	Indole production	H ₂ S production	Urease production	Nitrate reduction	O-F	Acid in sugars			
														Glucose	Lactose	Maltose	Sucrose
1	-	-	-	-	+	-	+	+	+	-	-	+	F	+	-	+	+
2	-	-	-	+	+	-	+	+	+	-	-	+	F	+	+	+	+
3	-	-	-	-	+	-	+	+	+	-	-	+	F	+	-	+	+
4	-	-	-	-	+	-	+	+	+	-	-	+	F	+	+	+	+

Key: + indicates positive reaction; - indicates negative reaction; F indicates that carbohydrate break-down is fermentative

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