Caprine Pneumonia III. Biochemical Characterization and Serological types of pasteurellae

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SUMMARY

TWO hundred isolates of *Pasteurella haemolytica* and 8 isolates of *P. multocida* were identified by biochemical and serological methods. Among *P. haemolytica* strains, 6 different serotypes were found while 25 isolates were untypable. The 8 isolates of *P. multocida* belonged to the same serotype.

INTRODUCTION

P. haemolytica has been isolated from pneumonic lung lesions in goats (Gourlay and Barber, 1960; Mugera and Kramer, 1967 and Pande, 1943). Recently Pegram (1974) isolated the organism from goats suffering from pneumonia and contagious caprine pleuropneumonia.

P. haemolytica was isolated from goats during the course of investigation into the aetiology of caprine pneumonia in Nigeria (Ojo, 1976a). All the isolates were made from pneumonic lung lesions in goats. Other bacteria isolated from pneumonic lung lesions in goats have been described (Ojo, 1976a, b). The present paper deals with the biochemical characteristics and serotypes of *Pasteurella* spp. isolated.

MATERIALS AND METHODS

Bacterial isolation

This has been described by Ojo (1976a). All the isolates were freeze-dried till tested. Strains of *P. multocida* were

obtained from the Federal Department of Veterinary Research, Vom. The strains were isolated from pneumonic lung lesions in goats by Johnson in 1957 (personal communication).

Biochemical characterization

For carbohydrate fermentation tests the Andrade peptone water (Oxoid) base medium was used. One per cent solution of the carbohydrates was added to the base medium. The pH was adjusted to 7.4. The following carbohydrate fermentations were carried out at 37°C for 14 days; glucose, lactose, arabinose, trehalose, salicin, xylose, sorbitol, dulcitol, maltose, mannose, starch, glycerol, manitol, fructose, raffinose, inulin, rhamnose and adonitol. Other tests carried out were catalase, oxidase, motility by hanging drop method, nitrate reduction—urease activity, decarboxylases, Hugh and Leifson fermentation test, ability to grow on MacConkey agar, indole formation, haemolysis on 5 percent human blood agar as well as 5% sheep blood agar—H₂S production using lead acetate paper, acid or alkaline production in litmus milk, ability to liquefy gelatin as well as methyl red (MR) and Voges-Proskauer (VP) reactions.

Capsule demonstration

The presence of capsule was demonstrated using the staining method of Jasmin (1945).

Mouse pathogenicity test

Eight weeks old White Swiss mice obtained from the Virus Research Laboratory, U.C.H., Ibadan and weighing between 16-18 gm were inoculated with 18 hour broth cultures of *Pasteurella* spp. The mice were housed in metal cages and provided with food and water *ad lib*. Two mice per isolate were used, one being inoculated intraperitoneally and the other subcutaneously with 0.5 ml of the broth culture. The mice were kept for up to 4 weeks. The mice which died were necropsied, peritoneal fluid and heart blood cultured on blood agar to ascertain the cause of death. *Pasteurella* sp thus isolated were serologically typed.

TABLE 1

Type strains of *P. haemolytica* and *P. multocida*

Strain	Serotype
H19	1
A15ER	2
863	3
57-1374	4
G13	5
A30S	6
B11	7
B5	8
B1	9
JF2	10
KC282	11
S209	12
3397	A
Insein	B
212	C
Kobe 6	D
PI235	E

Acridlavine test

The test was carried out for *P. multocida* strains only according to the method of Carter and Subronto (1973).

Production of antisera

The type strains of *P. haemolytica* were obtained from Professor E. L. Biberstein of the Department of Veterinary Microbiology, University of California. The type strains of *P. multocida* were also obtained from Professor G. R. Carter of Michigan State University and Professor J. E. Smith of the University of Surrey. The strains are shown in Table 1. The antisera were prepared in Californian breed of rabbits, weighing 2-3 kg. according to the method of Hooper and Sebesten (1974).

Serological typing

The indirect haemagglutination test was done according to the method of Biberstein and Thompson (1966).

RESULTS

Biochemical tests

The results of the tests are shown in Table 2. All the strains of *P. haemolytica* tested fermented glucose, sucrose, manitol with acid formation only. They were also catalase and oxidase-positive and haemolytic on both human and sheep blood agar. No strain fermented dulcitol, inulin, rhamnose and adonitol. All the strains were urease, indole, MR, VP, citrate utilization, gelatin liquefaction, arginine dihydrolase and lysine decarboxylase-negative. The 8 strains of *P. multocida* fermented sucrose, glucose, fructose, sorbitol, galactose, mannose and mannitol with acid formation only. No acid was produced in lactose, maltose raffinose, salicin, glycerol, rhamnose and

TABLE 2

The results of the cultural and biochemical characters of 200 strains of *P. haemolytica*

<i>Test</i>	<i>No. positive</i>	<i>No. tested</i>
Glucose	200	200
Sucrose	200	200
Mannitol	200	200
Catalase	200	200
Oxidase	200	200
haemolysis on blood agar	200	200
<i>Hush and Leifson</i> fermentation test	100	100
Growth on MacConkey agar	198	200
Fructose	180	200
galactose	174	200
raffinose	120	200
trehalose	105	200
nitrate reduction	96	100
arabinose	80	200
salicin	80	200
mannose	80	150
glycerol	70	200
sorbitol	70	150
maltose	70	200
xylose	65	200
lactose	50	200
litmus milk (Acid)	50	200
H ₂ S	25	200
Ornithine decarboxylase	25	100
Starch	3	200

inulin. They were all catalase and oxidase positive, H₂S positive and reduced nitrate to nitrite.

Serological typing

Six serotypes of *P. haemolytica* were found including 25 strains which were

untypable. The serotypes are shown in Table 3. About 42 percent of the strains were serotype 2. The strains of *P. multocida* were not typable by the indirect haemagglutination test. Heavy flocculent precipitate was produced within 5 min in 1:1,000 aqueous solution of acriflavine, hence they were considered type D (Carter and Subronto, 1973).

DISCUSSION

Reports on *Pasteurella* spp. of goats are very scarce. Much of the works reported have been on few outbreaks of pneumonia in goats (Mugera and Kramer, 1967; Gourlay and Barber 1960, and Pegram, 1974). Except for the report of Pegram (1974) no serological typing was

TABLE 3

Serological types of *P. haemolytica* isolated from goats

H. A. type	No.	Incidence of predominant serotype (percent)
1	—	0
2	85	42.5
3	—	0
4	—	0
5	—	0
6	40	20
7	20	10
8	13	6.5
9	—	0
10	7	3.5
11	10	5
12	—	0
Untypable	25	12.5

Capsule demonstration

Seventy five out of 100 strains of *P. haemolytica* examined for capsule were found to possess capsule. Capsule could not be demonstrated for the 8 strains of *P. multocida*.

Mouse pathogenicity test

None of the strains of *P. multocida* was pathogenic to mice. Of the 100 strains of *P. haemolytica* tested, 65 were pathogenic to mice by the intraperitoneal route only. All the mice inoculated by the subcutaneous route were still alive after 4 weeks. About 60% of the mice which died from the intraperitoneal infection did so within 24 hr while the others died between 24 and 72 hr. The organisms were isolated from the peritoneum, heart blood and spleen of the dead mice.

done by the earlier workers. Perhaps the reason for the few reports is that goats are found predominantly in the developing countries where the economic importance of the animal is not fully realised.

Newson and Cross (1932) showed that there was diversity of cultural reactions among *P. haemolytica*, hence Biberstein Gills and Knight (1960) considered the following criteria in order of diminishing importance for the species.

- Haemolysis on beef blood agar particularly thinly poured blood
- Absence of indole production which was uniformly characteristic
- Growth on MacConkey's agar.
- Failure to reduce methylene blue milk. Temporary reduction at the bottom of the tube may occasionally occur on the 2nd or 3rd day of inoculation.

- (e) Fermentation of glucose and sucrose and variable other carbohydrates, with the production of moderate acidity.

These criteria equally apply to the strains under test except that litmus milk instead of methylene blue milk was used. The diversity of cultural reactions among *P. haemolytica* which Newson and Cross (1932) observed was quite evident among the strains tested in this study.

Cameron and Smit (1970) found serotypes 1, 4, 6 and 7 to be the most prevalent among the 55 isolates of *P. haemolytica* from sheep in South Africa. The survey of serotypes of *P. haemolytica* isolated from calves by Wray and Thompson (1971) showed that serotypes 1 and 2 constituted 59 per cent and 20% respectively of the isolates from cases of clinical pneumonia. Isolates from nasal swabs of healthy calves were mainly of serotypes 1 and 2, being 50% and 37% respectively. Shreeve, Biberstein and Thompson (1972) examined diseased flocks of sheep in Scotland and found that there was variation in the serotypes from flock to flock. Serotype 2 was predominant among 9 out of 11 flocks while serotype 7 was predominant among 4 flocks. The 3 isolates from goat reported by Pegram (1974) were of serotypes 2.

The present study shows that the prevalent serotypes are types 2 and 6. All the isolates were from cases of pneumonia. The question arises whether serotype 2 has a greater potential pathogenicity than any of the other serotypes. This is to be investigated. The serotype of *P. multocida* reported in this study is the same as that reported by Carter and Bain (1960). The biochemical reactions of the isolates are also similar to those recorded by other workers (Mugera and Kramer, 1967; Gourlay and Barber, 1960; Biberstein, Shreeve and Thompson, 1970;

Shreeve, Ivanov and Thompson, 1970).

Although 65 percent of *P. haemolytica* isolates tested were pathogenic to mice by the intraperitoneal route, none of them killed mice when inoculated subcutaneously. This may indicate that the isolates are not all that virulent. The virulence might have been reduced by the subculture of the organisms, though most of them were only subcultured 3 or 4 times before they were freeze-dried.

ACKNOWLEDGEMENTS

My sincere thanks to Professors E. L. Biberstein, G. R. Carter and J. E. Smith for the supply of type strains of *Pasteurella* spp. I also thank Mr. M. O. Oladele for the secretariat works. The work is supported by the University of Ibadan Senate Research Grant Paper No. 678.

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