PRESENCE OF PASTEURELLA HAEMOLYTICA ANTIBODIES IN SERA OF APPARENTLY HEALTHY SUDANESE SHEEP

BY

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Introduction

Pasteurella haemolytica infection in sheep is economically important, it is characterized by pneumonia with frequent septicemic complications leading to arthritis, meningitis and death (Biberstein **et al**, 1960; Smith **et al**, 1972). Two biotypes of **P. haemolytica** were identified (Smith, 1961), within which 14 serotypes were determined (Biberstein & Thompson, 1966; **Pegram et al, 1979**). Serologically untypaple strains could also occur (Aarsleff **et al**, 1970). Type A strains are predominantly associated with enzootic pneumonoia and septicemia in young lambs, while type T strains can cause septicemia in both young and adult sheep (Biberstein & Thompson, 1966). However, most of **P. haemolytica** serotypes have been isolated from the nasopharynx of healthy sheep (Gilmour **et al,** 1974) and the onset of the disease in the carrier animal appears to be triggered by an outside "stressing" agent or factor (Biberstein **et al,** 1960).In the Sudan, six serotypes of **P. haemolytica** were isolated from the nasopharynx of healthy adult sheep (Shigidi,1976) and 30 % of a population of 400 sheep examined were reported as carriers. The present study was undertaken to show the prevalence of the disease in sheep, as indicated by presence of humoral antibodies against **P. haemolytica** serotypes, using an indirect haemagglutination assay. Sera of sheep from two different localities in the Sudan were tested in this investigation.

Materials & Methods

Materials:

1) 0.3 % neutral formalin in phosphate buffered saline (FPBS) at pH 7.0.

2) Ox red blood cells (RBC's)are freshly collected in Alsever's solution .They are washed three times in FPBS and made up to 5 % concentration.

3)**P. haemolytica** serotypes A_2, A_5, A_6, A_8, A_9 and T_3 **were kindly provided by DR. M.T.Shigidi of the Faculty of Veterinary Science, University of** Khartoum. They had been isolated from the nasopharynx of healthy Sudanese sheep in previous studies (Shigidi, 1976).Each serotype is grown overnight at 37° in nutrient broth and subcultured on blood agar to check for purity and to maintain the strain.

4) Collection of serum: Blood was collected from apparently healthy adult sheep by venous puncture. About 15 mls of blood were collected from each sheep in sterile containers. The blood was allowed to stand for I h at room temperature, followed by centrifugation and separation of serum.Serum samples were then transfered into sterile bijou bottles and stored at —18° until used.

Methods:

Indirect haemagglutination test was performed in microtiter plates (Nunclon,Denmark). A method modified from Biberstein & Thompson (1966) was used.

Broth culture of each **P. haemolytica** serotype was heated at 56° for 15 min to kill viable organisms. Ox RBC's were added to give a concentration of 0.5 % and incubated at 37° for 10 min to sensitize the RBC's. The culture was then washed 3 times in FPBS to remove excess antigen and made up to the original volume. Sera were diluted in FPBS in volumes of 0.05 ml, making serial doubling dilutions from $\frac{1}{4}$ to $\frac{1}{2048}$ in the microtiter plates. To one volume of diluted serum an equal volume of sensitized cells was added and allowed to stand at room temperature for 2 h. Agglutination indicates a positive reaction and is shown by an even mat of RBC's over the bottom of the well in the microtiter plate.

Control tests in which sensitized and unsensitized RBC's were added to a positive serum, as shown above, were run in parallel with every test.

Results

Indirect haemagglutination was performed on 109 and 77 sheep serum samples collected respectively from Sinnar and Khartoum areas of the Sudan.Each sample was screened against the 6 serotypes of **P.haemolytica** at dilutions of $\frac{1}{4}$ to $\frac{1}{2048}$. Samples showing agglutination at titres below $\frac{1}{32}$ were denoted as weakly positive, while those showing agglutination at titres of $\frac{1}{32}$ and above were considered strogly positive (technical pamphlets, Edinburgh, Scotland). Details of results are shown on Table 1 & 2

All 6 serotypes tested had shown positive agglutination with a number of sera collected from Khartoum and Sinnar areas. However, variations existed in both areas in the prevalence of serotypes

showing either weak or strong agglutination reactions. In Khartoum area the most prevalent serotypes showing agglutination at titres of $\frac{1}{32}$ and above as indicated by the percentage of strongly positive samples were A_8 (23.4 %), A_9 (16.9 %) and A_2 (15.6 %) compared to serotypes A_5 (28.4 %) and T_3 (15.6 %) in Sinnar area. Serum samples showing weak agglutination were generally more in number than those showing strong agglutination; mean percentages of 35.7 % and 34.5 % of samples obtained from Khartoum and Sinnar areas respectively were recorded as having weak agglutinating titres against **P. haemolytica** serotypes (see Tables 1 & 2). The most prevalent serotypes showing weak agglutination were A_6 (50.6 %), A_5 (45.4 %), A_9 (36.4 %) and T_3 (32.5 %) in Khartoum area and T_3 (57.8 %), A_5 (41.3 %) and A_8 (37.6 %) in Sinnar area. The incidence of strongly positive sera was higher in Khartoum samples than in Sinnar, mean percentages of 13.0 % and 10.7 % were recorded respectively in both areas. But it appears from the results that some serum samples from both areas showed weak agglutination reactions to more than one **P. haemolytica** serotype.

Discussion

The diffusible coat antigens of **P. haemolytica** show a high degree of specificity compared to the somatic antigens (Biberstein **et al**, 1960); they afford a means not only in classifying the strains of this rather heterogeneous species but also in the serodiagnosis of field cases (Biberstein & Thompson, 1966). They have been used in the present study for the detection of antibodies against **P. haemolytica** serotypes in sera of Sudanese sheep using the indirect haemagglutination test.

It is apparent from the results of this study that specific antibodies against **P. haemolytica** soluble surface antigens are present in sera of apparently normal adult sheep in Khartoum and Sinnar areas of the Sudan. The percentage of sheep having high level of agglutinating antibody titres against the different serotypes tested varies from 2.7 % to 28.4 % with a mean percentage of 13.0 % and 10.7 % in Khartoum and Sinnar areas respectively, while more than $\frac{1}{3}$ of the sheep examined contained low level of agglutinating serum antibodies against those serotypes. This high incidence of weak agglutination may be because these sheep had been infected and they recovered spontaneously from the disease or this weak haemagglutination tentatively indicates a great deal of cross reactivity with similar antigens (Biberstein, 1978). Alternatively these sheep were carriers, but circulating antibodies were reduced to minimum in them due to the production of specific local I_g A antibodies in the nasal epithelium which prevent colonization and enhance clearance of **P. haemolytica** (Walker, 1979). However, all sheep in this study, whether showing high or low antibody titres against **P. haemolytica** serotypes, were apparently healthy. This indicates that in the carrier animals other intrinsic or extrinsic factors beside the bacteria are responsible for the onset of the disease (Biberstein 1970). Moreover, humoral immune responses alone appear to be incapable of protection against the disease and perhaps cellular immunity is more effective in this aspect (Wells **et al** 1979).

The results may be of significance in the formulation of vaccines against ovine pasteurellosis as they give an indication of the serotypes most commonly found in disease conditions (Thompson *et al.* 1977) although positive titres for clinically sick animals were not presented. Only six serotypes of the 14 known serotypes of **P. haemolytica** have been isolated and identified in the Sudan (Shigidi, 1976) and all were included in this study. It is not unlikely that all the serotypes of **P. haemolytica** are present in this country as they have been recently isolated and identified in Kenya (Mwangota, 1975) and in Ethiopia (Pegram **et al,** 1979) and both countries have open borders with the Sudan. Work is progressing in our laboratory to establish and to confirm the presence of all the 14 serotypes using serodiagnostic methods.

Summary

109 and 77 sheep serum samples collected respectively from Sinnar and Khartoum areas of the Sudan were screened against serotypes A_2, A_5, A_6, A_8, A_9 and T_3, of **P. haemolytica** using indirect haemagglutination. Mean percentage of 13.0 % and 10.7 % of samples obtained from Khartoum and Sinnar areas respectively were recorded as having high agglutinating antibody titres against the different serotypes tested, while more than one third of the sheep population examined in both areas contained low level of agglutinating serum antibodies against those serotypes. The results may be of significance in the formulation of vaccines against ovine pasteurellosis as they may give an indication of the serotypes most commonly involved in disease conditions in the Sudan.

Acknowledgements

The authors would like to thank Dr. M.T.A.Shigidi of the Faculty of Veterinary Sciences, University of Khartoum for providing the six serotypes of *P. haemolytica*.

Table (I) :

Incidence in Khartoum: number of sheep showing the highest positive titres against**P. haemolytica** serotypes and percentages of strongly and weakly positive samples.

Serotype tested	No of sheep Examined	No of sheep showing positive titres 1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	weakly positive %	Strongly psitive %
A_2	77	8	6	3	4	6	2	—	—	—	—	22.0	15.6
A_5	77	19	12	4	3	2	1	—	—	—	—	45.4	7 8
A_6	77	15	13	11	4	1	1	—	—	—	—	50.6	7.8
A_8	77	6	11	4	7	7	3	1	—	—	—	27.3	23.4
A_9	77	16	8	4	5	7	—	1	—	—	—	36.4	16.9
T_3	77	15	7	3	2	2	1	—	—	—	—	32.5	6.5
Mean Percentage												35.7	13.0

Table (II) :

Incidence in Sinnar: Number of sheep showing the highest positive titres against **P. haemolytica serotypes and percentages of strongly** and weakly positive samples.

Serotype Tested	No. ofsheep Examined	No of sheep showing posivtive titres 1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	weakly positive %	Strongly positive %
A_2	109	12	12	5	3	4	1	—	—	—	—	26.6	7.3
A_5	109	12	22	11	14	11	2	3	1	—	—	41.3	28.4
A_6	109	4	9	4	2	1	1	—	—	—	—	15.6	3.7
A_8	109	20	14	7	3	2	2					37.6	6.4
A_9	109	16	8	7	2	—	1	—	—	—	—	28.4	2.7
T_3	109	16	28	19	8	7	—	2				57.8	15.6
Mean Percentage												34.5	10.7

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