

A serological survey of sheep sera for antibodies to *Pasteurella haemolytica* serotypes in the Sudan

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RÉSUMÉ

HUSSEIN (A. M.), ELSAWI MOHAMED (O.). — Enquête sérologique pour la recherche des anticorps dirigés contre différents sérotypes de *Pasteurella haemolytica* dans les sérums de moutons au Soudan. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (4) : 418-421.

Quatre cents échantillons de sérums de moutons collectés dans la région d'Omdurman et d'Elobeid au Soudan ont été analysés à la dilution de 1/100 pour la recherche des anticorps contre 14 sérotypes connus de *P. haemolytica*, au moyen du test d'hémagglutination passive (HAP).

Les résultats ont été positifs pour tous les sérotypes examinés et ont montré un plus grand nombre de biotypes A par rapport au biotype T.

Le plus fréquent était le type A₂ (17 p. 100), le plus rare le type A₁₁ (8,0 p. 100) ; la fréquence des autres sérotypes étant intermédiaire.

Les résultats confirment la présence des sérotypes de *P. haemolytica* dans les 2 régions concernées, ce qui nécessite la production d'un vaccin polyvalent pour protéger les animaux contre d'éventuels foyers de pasteurellose.

Mots-clés : Pasteurellose - *Pasteurella haemolytica* - Sérotypes - Mouton - Soudan.

SUMMARY

HUSSEIN (A. M.), ELSAWI MOHAMED (O.). — A serological survey of sheep sera for antibodies to *Pasteurella haemolytica* serotypes in the Sudan. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (4) : 418-421.

400 sheep serum samples, collected from Omdurman and Elobeid areas of the Sudan, were screened at 1/100 dilution for antibodies against 14 known serotypes of *P. haemolytica*, using the IHT. The results have shown positive sera to all serotypes tested, and an abundance of *P. haemolytica* biotype A over biotype T. The most prevalent serotype was A₂ (17.0 p. 100) and the least prevalent was A₁₁ (8.0 p. 100) while the other serotypes prevail in frequencies between A₂ and A₁₁. The results confirm the presence of *P. haemolytica* serotypes in those areas, which necessitates production of a multivalent vaccine to protect animals from future outbreaks of the disease.

Key words : Pasteurellose - *Pasteurella haemolytica* - Serotypes - Sheep - Sudan.

INTRODUCTION

The subdivision of *Pasteurella haemolytica*, the causative organism of ovine pasteurellosis, into several distinct serotypes was achieved by the agglutination reaction between bovine red blood cells sensitized with soluble coat antigens from *P. haemolytica* and antisera raised in rabbits against selected strains (4). Fifteen serotypes are now recognized and these can be divided into two biotypes, A and T (13, 8). Clinically, biotype A strains are predominantly

associated with enzootic pneumonia in sheep of all ages, and with septicaemia in young lambs while biotype T strains are associated with septicaemic pasteurellosis of older sheep (9). Serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13 and 14 belong to biotype A, and serotypes 3, 4, 10 and 15 to biotype T. Serologically untypable strains could also exist (1).

In the Sudan, infection caused by *P. haemolytica* has gained importance recently as the disease led to marked mortality in sheep kept in some confined farms. Only six serotypes of

P. haemolytica (A₂, A₅, A₆, A₈, A₉ and T₃) have been isolated and identified (15) and that was confirmed serologically in sheep from suspected areas (11). It is not unlikely that all the serotypes of *P. haemolytica* are present in the Sudan since they have been isolated and identified in Kenya (12), and in Ethiopia (13), and both countries have open borders with the Sudan. This communication reports the prevalence of *P. haemolytica* serotypes in some susceptible areas of the Sudan by screening sheep sera against all known serotypes using the indirect haemagglutination test (IHT).

MATERIALS AND METHODS

Materials

(1) 0.3 p. 100 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 p. 100 concentration.

(3) *P. haemolytica* serotypes A₁, A₂, A₅, A₆, A₇, A₈, A₉, A₁₁, A₁₂, A₁₃, A₁₄, and serotypes T₃, T₄, and T₁₀. Each serotype is grown overnight at 37° in nutrient broth and sub-cultured on blood agar to check for purity and to maintain the strain.

(4) Collection of serum : blood was collected from 400 adult sheep living in different localities in Omdurman and Elobeid areas of the Sudan. After separation from blood, the serum sample of each animal was transferred into a sterile bijoux bottle and stored at - 18° until tested.

Methods

(IHT) was performed in microtiter plates (Nunclon, Denmark). A method modified from BIBERSTEIN (5) 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 p. 100 and incubated at 37° for 10 min to sensitize the RBC's. The culture was then washed three times in FPBS to remove excess antigen and made up to the original volume. Sera were diluted 1 : 100 in FPBS and transferred in volumes of 0.025 ml into the microtiter plates. To one volume of

diluted serum an equal volume of sensitized cells was added and allowed to stand for 2 h at room temperature. Haemagglutination indicates a positive reaction.

Control tests in which sensitized and un-sensitized RBC's were added to a positive serum, as described above, were run in parallel with the test.

Interpretation of results

(IHT) was performed on 400 sheep serum samples, and each sample was screened for antibodies against the 14 serotypes of *P. haemolytica*. Samples showing haemagglutination at titres of 1 : 100 were considered positive according to BIBERSTEIN (4). Positive samples for every serotype were expressed as a percentage of the total number tested.

RESULTS

All 14 serotypes tested had shown positive haemagglutination reactions with a number of sera (Table I). But however, variations existed in the prevalence of different strains. Generally, more sheep were carrying antibodies to serotypes belonging to A biotype ; samples showing positive haemagglutination reactions to A biotype constitute about 79.6 p. 100 of the total number of positive samples while those carrying antibodies to T biotype were only 20.4 p. 100. The variations in the prevalence of the different serotypes range from 17.0 p. 100 to 8.0 p. 100. Serotypes A₁, A₂, A₆ and A₁₂ were the most prevalent while serotypes A₅, A₇, A₁₁ and T₁₀ were the least prevalent. It also appears from the results that some serum samples had shown positive reactions to more than one *P. haemolytica* serotype. Only serotype T₁₅ was not included in this study.

DISCUSSION

The results indicate that all *P. haemolytica* serotypes tested were present in the Sudan. The serological typing of *P. haemolytica* is concerned entirely with soluble surface antigens, which are supposed to be highly specific

TABLE N°1 - Prevalence of the different serotypes of *P. haemolytica* in Omdurman and Elobeid areas of the Sudan

<i>P. haemolytica</i> serotype	N° tested	N° positive	N° negative	Prevalence (percentage positive) p.100
A ₁	400	58	342	14,5
A ₂	400	68	332	17,0
A ₅	400	35	365	8,7
A ₆	400	62	338	15,5
A ₇	400	40	360	10,0
A ₈	400	54	346	13,5
A ₉	400	44	356	11,0
A ₁₁	400	32	368	8,0
A ₁₂	400	56	344	14,0
A ₁₃	400	44	356	11,0
A ₁₄	400	51	349	12,7
T ₃	400	51	349	12,7
T ₄	400	46	354	11,5
T ₁₀	400	42	358	10,5

(4). The (IHT) procedure, in one of its several modifications, is usually employed in serotyping the strains (5). Occasionally, some strains were found to give low-titre cross-reactions with the typing antisera of other serotypes (2). Such cross-reactivity between strains is more obvious when the indirect enzyme-linked immunosorbent assay (ELISA) is used (7). ELISA is more sensitive than IHT and it consequently demonstrated common antigenic relationships between strains which were not apparent by IHT (6). This may set typing of *P. haemolytica* serotypes by IHT questionable. But this fact may remain invalid as cross-protection between *P. haemolytica* serotypes did not occur (10) and toxicity of the serotype is neutralized more effectively by a serotype specific antiserum rather than a heterogeneous antiserum (14).

The test employed in this report is according to the standards of BIBERSTEIN (4) in which haemagglutination at a serum dilution of

1 : 100 is considered positive (5). At this dilution, non-specific agglutination reactions are minimized. In other similar standards, positive sera were usually screened for *P. haemolytica* serotypes at a dilution of 1 : 50 (3), which is lower than the dilution used in this study. The highest prevalent serotype in this study is A₂ (17 p. 100) and the least prevalent is A₁₁ (8.0 p. 100) while the other serotypes prevail in frequencies between 17.0 p. 100 and 8.0 p. 100. The serum samples were collected from sheep living in different localities within one area and from other nomadic sheep known to be moving continuously from one place to another. The results therefore reflected the occurrence of a wide range of serotypes. The results have also shown an abundance of biotype A over biotype T, this could be interpreted that sheep pneumonia and lamb septicaemia are encountered in those areas more often than the classical septicaemic pasteurellosis of adult sheep (5). But con-

cerning the epidemiology of the disease the result has confirmed the occurrence of 14 serotypes of *P. haemolytica* and a multivalent vaccine is needed to protect sheep and lambs from future outbreaks of the disease.

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RESUMEN

HUSSEIN (A. M.), ELSAWI MOHAMED (O.). — Encuesta serologica para la búsqueda de anticuerpos contra diferentes serotipos de *Pasteurella haemolytica* en los sueros de carneros en Sudan. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (4) : 418-421.

Se analizaron con la dilución 1/100 400 muestras de suero de carneros recogidas en la región de Ondurman y de Elobeid en Sudan para la búsqueda de anticuerpos contra 14 serotipos conocidos de *P. haemolytica* por medio de la prueba de hemaglutinación pasiva (HAP).

Los resultados fueron positivos en todos los serotipos

examinados y mostraron un número de biotipos A mayor que el de biotipo T. Era el tipo A₂ (17 p. 100) el más frecuente y el tipo A₁₁ (8 p. 100) el más escaso : siendo intermedia la frecuencia de los demás serotipos.

Los resultados confirman la presencia de serotipos de *P. haemolytica* en ambas regiones, lo que necesita la producción de una vacuna polivalente para proteger los animales de focos posibles de pasteurelisis.

Palabras claves : Pasteurelisis - *Pasteurella haemolytica* - Serotipos - Carneros - Sudan.

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