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A comparative study of the serum antibody levels in sheep nasal carriers of *Pasteurella haemolytica*.

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SUMMARY

The population of *P. haemolytica* in the nasopharynx was examined in a group of 13 housed sheep, of mixed sex and breed, using the indirect haemagglutination test to identify the serotypes. The sheep were bled at fortnightly intervals over a 6-month period and the sera was tested for the presence of antibodies to serotypes A1, A2, A6, A7, A9, T3 and T10 using both the Indirect Fluorescent Antibody and Indirect Haemagglutination tests.

Five serotypes of *P. haemolytica* were isolated and comparisons of the 2 serological tests showed statistically that the F. A. test was more sensitive than the I. H. A. test, but possibly less specific.

INTRODUCTION

Pasteurella haemolytica is part of the normal bacterial flora of the nasal passages and tonsilar area of sheep, but is still of major world-wide importance as a pathogen of sheep and cattle (W.H.O. 1970). It is isolated in profusion from both species that have post mortem lesions indicative of an acute pneumonia, and in cases of septicaemia in sheep. The organism can be easily recognised using well documented biochemical tests. Although a clear difference exists on colonial morphology (Smith 1961) between the A types, predominantly colonised in the nasopharynx and the T type in the tonsil area (Gilmour et al, 1974), further differentiation must be done to identify the serotypes.

The role of the nasal carrier is far from clear, but a study of the different serotypes in the dynamics of the carrier state is essential for a clearer understanding of the problems involved in effective vaccine production, and other preventative measures. In such studies it would be useful to monitor the predominant serotypes in the nasopharynx and tonsil area by using simple serological tests. To date, no correlation has been demonstrated using the Indirect haemagglutination (I.H.A.) test but the use of an alternative sensitive test, possibly detecting different antibodies might prove more useful.

The Indirect Fluorescent Antibody test (I.F.A.) was chosen as an alternative which has been well tried in other fields and has on occasions proved itself more sensitive than other serological tests (Weir 1970), possibly detecting different antibodies.

This study examined nasal populations in a group of healthy housed experimental sheep, whilst examining the levels of circulating antibodies to 7 common serotypes of *P. haemolytica* as measured by the I.H.A. and I.F.A. tests and sought to correlate this with the serotypes isolated from the nasopharynx.

REVIEW OF THE LITERATURE

1. The Organism Pasteurella haemolytica

a. Morphology

The microscopic description of P. haemolytica as a non motile, gram negative pleomorphic short rod or coccobacillus with bipolar staining has been recognised for over 30 years. (Montgomery et al 1938, Dungal 1931).

Smith (1961) divided the organism into two biotypes, A and T, on the basis of colonial morphology. At 18-24 hours on sheep blood agar A type colonies had an even greyish colour with sometimes a darker centre. T type colonies were slightly larger measuring up to 2mm. in diameter, darker with brownish centres, the colour fading peripherally. Both colonies are of a smooth creamy texture and have equal degrees of haemolysis. Biberstein and Gills (1962) were unable to classify with certainty the two biotypes and consequently reverted to fermentation as a means of differentiation.

b. Biochemical properties

Biberstein et al (1960) required an organism to be:-

1. Haemolytic
2. Indole negative
3. Positive growth on MacConkey Agar
4. Negative reduction of litmus milk

before being identified as P. haemolytica. Smith (1961) tested the organism with 24 fermentable substances. Biberstein and Gills (1962) following the work of Smith (1961) took the fermentation of arabinose, mannose, lactose, trehalose, glycogen, starch, glycerol, salicin and the catalase test as the important

perameters. Their results agreed with Smith's findings, that all type T strains were positive fermenters of trehalose within 2 days, but negative with arabinose. Type A strains all gave positive reactions with arabinose, taking up to 7 days, but were negative with trehalose up to 10 days.
The other tests were found to be unreliable as a means of distinguishing between A and T types.

c. Antigenic relationship

Carter (1956) carried out indirect haemagglutination tests on 51 strains of *P. haemolytica* isolated from Canadian cattle that had died from shipping fever, and found them to be of one serological type. Biberstein et al (1960) modified the I.H.A. test and using 3 serotypes supplied by Carter, also found them to be homogenous. On testing many of their isolates from sheep, they found them to be heterogenous, being made up of 11 serotypes. They further concluded that the type specific substance is a freely diffusable surface material, absorbable on to the red blood cells, demonstrated by I.H.A. and that break down into types is correlated with certain ecological and physiological characteristics, such as host preference and association with disease.
Antiserum was produced in rabbits, by the method described by Edwards and Ewing (1955).
Biberstein and Gills (1962) using all known representative serotypes of *P. haemolytica* divided them into biotypes according to Smith (1961) and found on serotyping that 1,2, 5, 6, 7, 8, 9, 11 were type A whereas 3, 4, 10 were type T. They thought that

the two types might also vary in virulence.
Biberstein (1965) found that cross reactions do exist amongst cultures of P. haemolytica although they are of a low titre. The antigens responsible are distinct from the type specific antigens, as he demonstrated by reciprocal absorption experiments, and they do not as a rule interfere with the typing process. Muraschi et al (1965) found that P. haemolytica could be serotyped by gel diffusion, with no cross reactions, but it proved extravagent on sera.

2. Relationship of *P. haemolytica* with septicaemia and respiratory disease

In 1931 Dungal in Iceland described outbreaks of acute pneumonia in sheep. Death could occur in less than 12 hours, although most cases lived for 24-48 hours. Chronically infected sheep survived for up to 2 weeks. Pulmonary consolidation was evident at post mortem and a gram negative short rod, with bipolar staining was isolated, but under these conditions was non-haemolytic on blood agar. Dungal (1931) was able to produce pneumonic lesions, demonstrated at slaughter, by the intratracheal inoculation of broth culture. Montgomery et al (1938) later examined a representative sample from Dungal's outbreaks and found that it resembled a haemolytic pasteurella like organism. Montgomery et al (1938) also described cases of enzootic pneumonia in sheep in Wales, following severe changes in either management or the weather, but were unable to produce clinical disease by use of a 24 hour broth culture inoculated by the intratracheal and other routes. When they used a pathological lung suspension, clinical illness was produced but the thoracic lesions were atypical. Haemorrhagic septicaemia in ovines was described by three groups of workers in Europe, Woxholtt et al (1952), Stricker and Grunert (1956) and Stamp et al (1955). The later group of workers found that outbreaks occurred in the autumn with *P. haemolytica* being isolated from lung, spleen, heart blood, kidney etc. Intravenous inoculation of 7 - 9 month old lambs with 1ml of diseased lung or spleen suspension produced death and characteristic lesions in 12 hours. Those injected

intravenously with 2 ml of broth culture also died in 12 hours with typical post mortem and bacteriological pictures. Intra tracheal injections were not successful.

Biberstein and Kennedy (1959) found that in 6 flocks of Californian lambs in good condition, mortalities of up to 5% occurred when subjected to the stress of movement and shearing, thus confirming the findings of Stamp et al (1955).

Although P. haemolytica was thought to be the cause of the pathological lesions it was by then well recognised that unknown factors allowed the organism to multiply so dramatically. (Dungal (1931), Newson and Cross (1932), Anon (1949)).

In 1954-5 the importance of P. haemolytica in shipping fever in Canadian cattle was observed by Carter (1954) and Carter and McSherry (1955). Smith (1960) demonstrated the high degree of susceptibility of 3 week old lambs compared with the high level of resistance in adult sheep, to experimental injection with type A P. haemolytica, using small doses.

Smith (1960) working with type T, reproduced the characteristic clinical signs of respiratory distress, diarrhoea, and rapid prostration, together with the post mortem picture of congestion, haemorrhage and oedema, using large doses of broth culture or heat killed culture, given intravenously to 7 month old lambs and adults. They also found that bacteria were eliminated more rapidly from the peripheral circulation of the adult sheep compared with the lambs.

Florent and Godbile (1950) produced only a rhinitis following intranasal inoculation of sheep. Smith (1964) using type A *P. haemolytica* on adult sheep cultured intraperitoneally in an attempt to maintain virulence only managed to produce fatal pulmonary infection, resembling enzootic pneumonia, by the intrabronchial route of inoculation. This again indicates the involvement of other factors in outbreaks of enzootic pneumonia other than the presence of the organism.
Hore et al (1968) isolated Para influenza 3 (P.I.3) virus from the respiratory tract of lambs suffering from respiratory disease. No antibodies to the virus were found prior to clinical signs but were detected in those that were allowed to survive. Biberstein et al (1971) induced an experimental pneumonia with *P. haemolytica* when P.I.3 virus was inoculated 3 days previously. Earlier deaths, higher fatality rates, prolonged febrile periods in the survivors and more severe pathological changes, resulted from this combined infection. Virus inoculation alone gave only a slight illness. Shreeve et al (1972) concluded from their work that there was no evidence of P.I.3 being involved in 2 outbreaks of septicaemia, and 2 of pneumonia both involving sheep.

3. The Carrier State

P. haemolytica has been found to be a part of the normal bacterial flora of both the tonsil (Gilmour et al (1974)) and the nasopharyngeal region of sheep (Shreeve and Thompson 1970). In a study of American and British strains of *P. haemolytica* (Biberstein and Thompson (1966)) it was shown that A5, A8 and A9 were rare in sheep in the British Isles, but fairly common in the U.S.A. A2 was found to be a very frequent isolate in Britain and A_{11} was confined mostly to the normal nasopharynx, whilst A1 dominated in cattle.

Biotype T was relatively more abundant in septicaemia of lambs over 3 months of age and resistance to them takes longer to develop, compared with A types. It was thought that T types were more selectively pathogenic, as only 2.6% of the strains isolated from the nasopharynx were of that biotype. The incidence of *P. haemolytica* was low (10.7 - 31%) in healthy flocks, compared with the 12 - 69% level found in sheep exposed to pneumonia. The healthy flocks had the expected serotypes in the appropriate proportions whilst infected sheep had one or very few serotypes. There is a lower incidence of untypable strains in infected flocks. Attempts to infect sheep by massive repeated nasal inoculation resulted in failure of the strain to survive for more than a few days, with no lateral spread; other serotypes persisted in the nasopharynx throughout the experiment (Biberstein and Thompson 1966).

Shreeve and Thompson (1970) found that the number of serotypes isolated from the nasopharynx of young lambs, reared with the ewes, increased with age. They were able to isolate P. haemolytica 48 hours after birth. A2 was the commonest serotype isolated. Lambs were found to be more susceptible to implantation of P. haemolytica than were adult sheep. When lambs were removed from the ewe at birth, few typable strains were subsequently isolated, suggesting that prolonged and close contact with adult sheep aids the transfer of serotypes from the dam. No antibodies were found in lambs up to 3 - 4 weeks of age.

Gilmore et al (1974) found 65% of isolates from the tonsil, and only 6% from the nasopharynx of adult sheep were type T. They suggested that the predilection site found in adult sheep may allow the A types to enter the tissues by way of the lower respiratory tract, causing pneumonia, and the T biotypes are able to spread via the lymphatics to the blood stream, resulting in septicaemia.

Biberstein et al (1970) found that P. haemolytica could be isolated more frequently during the season when enzootic pneumonia occurred although the sheep remained healthy. Very little has been published concerning the level of humoral antibodies in sheep but Biberstein and Thompson (1966) found that in a group of 11 sheep, several individuals had antibodies to more than one serotype.

Shreeve and Thompson (1970) found that no antibodies to any serotype could be found in lambs at 3 - 4 weeks of age, although a broad spectrum of antibodies was found after this age. In ewes, antibodies to as many as 8 different serotypes were found at one time.

MATERIALS AND METHODS

1. Experimental Animals

Thirteen adult sheep of mixed sex and breed were selected from the experimental flock, which is housed in covered sheep pens at the Centre for Tropical Veterinary Medicine. New Zealand White Rabbits which were individually caged were used for the production of hyperimmune sera.

2. Methods of Sampling

Thirteen sheep were bled by jugular venepuncture into 10ml vacutainers (Becton-Dickinson, Rutherford, New Jersey) at fortnightly intervals. The tubes were left to stand at room temperature for 4 hours and then centrifuged (relative centrifugal force 700xg). The sera were pipetted off into bijouxs and stored at $-20^{\circ}C$. Swabs were taken from both left and right nostrils on the last 5 times that the sheep were bled. The swabs were moistened with brain heart infusion broth and then inserted into the nares until they came in contact with the nasopharyngeal wall and swabbed onto half a blood agar plate. On return to the laboratory they were plated out with a wire loop.

3. Identification of P. haemolytica

After 18 - 24 hours incubation at $37^{\circ}C$, the plates were examined and any organism which was haemolytic, and whose colony morphology resembled that which was described by Smith (1961), was removed and plated out onto Blood and MacConkey Agar (CM 7 Oxoid). Often

several colonies were taken from the initial plate.
All isolates were stained by Grams method (Cowan and Steel, 1975) and examined to see if they were gram negative coccobacilli or short rods. Tryptose water (CM 87 Oxoid) was inoculated and cultured at 37°C for 48 hours then a hanging drop motility and indole production test (Cowan and Steel, 1975) were carried out.
Indirect haemagglutination (I.H.A.) tests were only performed on organisms that demonstrated:-

1. B haemolysis on layered blood agar. (A medium on which it is easier to see haemolysis, as compared with when it is cultured on 6% blood agar).
2. Growth on MacConkey agar.
3. Non motile.
4. Indole negative.

Known serotypes of P. haemolytica were provided by Dr. N. Gilmour, Moredun Institute.

4. Indirect Haemagglutination Test (I.H.A.)

For the serotyping of P. haemolytica and the detection of humoral antibodies, strains of P. haemolytica were grown overnight at 37°C in brain heart infusion broth and subcultured onto blood agar to check for purity.
The broth cultures were placed in a water bath at 56°C for 15 minutes, to kill the organisms, and then cooled to 37°C before adding the 5% red blood cells (R.B.C.) to give a final concentration of 0.5%.

They were left at $37^{\circ}C$ for 10 minutes.

Each culture was then washed three times in formal buffered saline (F.B.S.) (relative centrifugal force 700xg) and then made up to the original volume. 0.025ml (Crookes Microtiter system, Flow Laboratories) of sensitised R.B.C. were added to an equal volume of diluted antisera in a round bottomed microtiter plate (Flow Laboratories) and left to stand at room temperature for 2 hours before reading.

When the antibody level in sheep sera was being measured, they were diluted with F.B.S. using Crookes Microtiter system (Flow Laboratories), starting at a dilution of 1/2, and an equal volume of R.B.C.'s previously sensitised with a known serotype, added to each well, to give a final dilution of 1/4.

Positive reactions were seen as an even layer of R.B.C.'s over the bottom of the well. Negative reactions produced a small button of R.B.C.'s in the centre of the well.

Four controls were used, namely, non-sensitised cells with serum, non-sensitised cells with F.B.S. sensitised cells with positive serum and sensitised cells with negative serum.

5. Indirect Fluorescent Antibody Test

Preparation of Antigen Coated Slides

Glass slides, (Chance, Propper Ltd., Smethwick, England), were cleaned in a defatting mixture and marked with two rows

of seven circles using a diamond marker. The various serotypes of P. haemolytica were grown overnight at 37°C on 6% sheep blood agar and then washed from the plates with phosphate buffered saline (P.B.S.) pH 7.3, diluted to Browns tube 2 with the same.

A drop of the suspension was placed on each circle on the slide using only one serotype per slide. The slides were air dried and fixed in cold (4°C) acetone (B.D.H.) for 15 minutes and then stored in a desicator at 4°C.

6. Indirect Fluorescent Antibody Test

The sera was diluted in two-fold serial dilutions in WHO perspex plates starting at a dilution of 1/4 with a transfer volume of 0.2ml.

One drop of sera at each dilution was placed on the circles up to a dilution of 1/128.

A drop of negative sera from a day old lamb that had received no colostrum and one drop of P.B.S. were placed on the remaining two circles and acted as negative controls.

A2 sheep sera with a titre of 1/64 was provided by Dr. N. Gilmour, Moredun Institute and was used as the positive control.

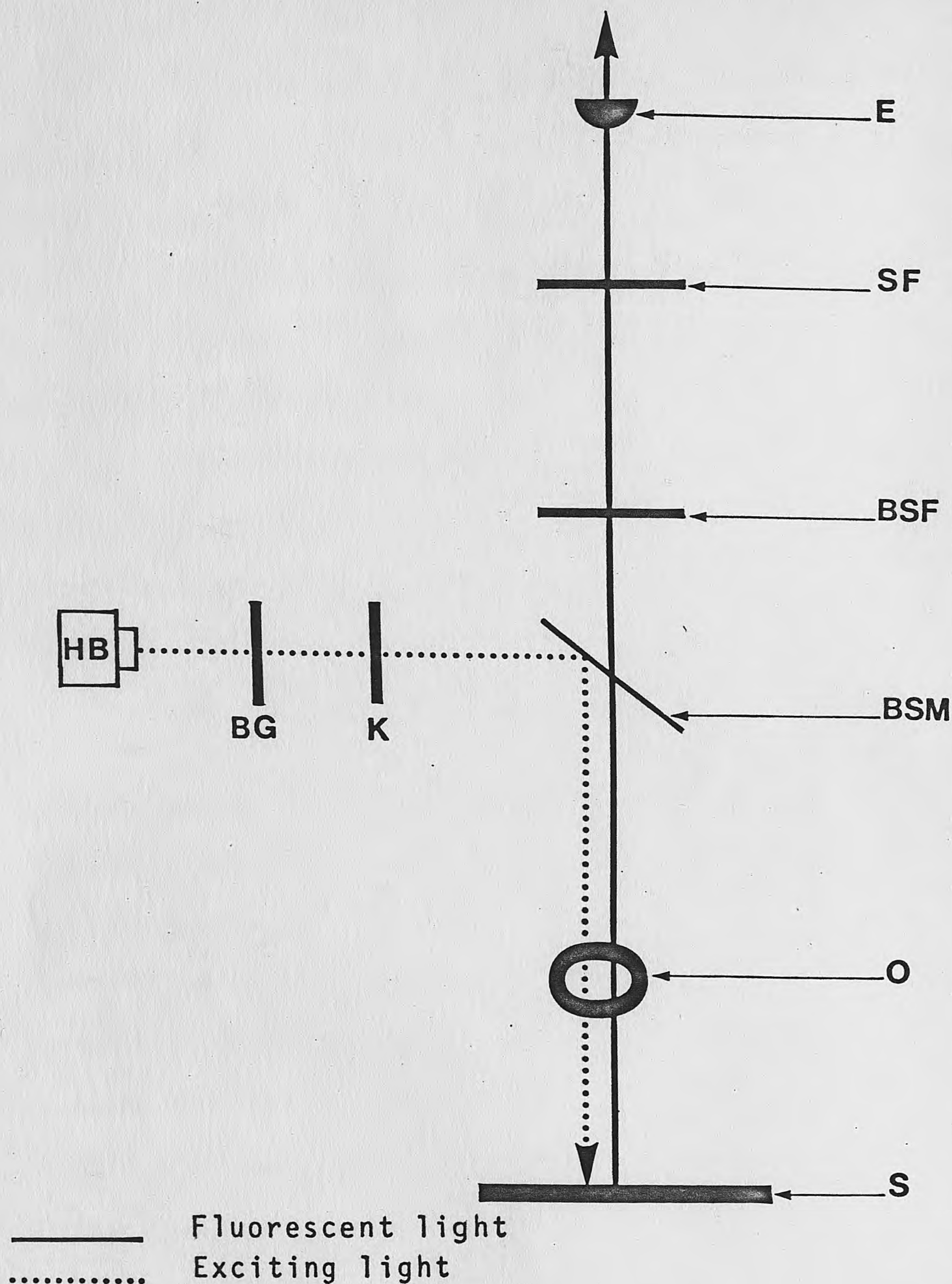
The slides were then placed in a humid chamber for 30 minutes, after which time they were washed in three changes of P.B.S. for 10 minutes.

Rabbit antisheep fluorescent antibody was then applied to the slides at a dilution of 1/15 and placed back in a humid chamber for 30 minutes, when it was again removed and washed in P.B.S. for 10 minutes with three changes and then blotted dry.

Slides were examined using incident light fluorescence (Fig. 1) with a Leitz (Wetzlar, West Germany) microscope, and Leitz non-fluorescent immersion oil.

The microscope was focused on individual organisms which showed varying degrees of fluorescence, xxx, xx and x, the end point was taken, as the change from xx to x fluorescence.

INCIDENT-LIGHT FLUORESCENCE - FIG. 1



HB = HBO 200 Mercury vapour lamp.
BG = 3mm. Blue glass 12 filter.
K = KP500 filter.
E = Eyepiece (x 6.3)
SF = Suppression filter (K510/530)
BSF = Built in suppression filter K515.)
BSM = Dichromic Beam splitting mirror TK510) Fluorescence vertical illuminator according to Ploem.
O = Objective (x54/0.95)
S = Specimen.

R E A G E N T S

7. Reagents for I.H.A.

Neutral formal buffered saline (F.B.S.).

Buffered tablet p.H. 7.0 (B.D.H. Chemicals Ltd., Poole).

One tablet per 100ml of distilled water.

Saline tablets (Code BR 53 Oxoid).

One tablet per 500ml of distilled water.

Formaldehyde solution 37 - 41% (B.D.H. Chemicals Ltd., Poole), shaken with excess magnesium carbonate and left to stand overnight.

3ml per litre of buffered saline.

Antisera to individual serotypes of P. haemolytica

Prepared in rabbits by the intravenous inoculation of 1/2, 1, 2, 3, 3, 3ml amounts of culture injected at 3-day intervals, previously prepared by growing overnight at 37°C on Blood Agar washed off and emulsified with F.B.S., incubated at 37°C for one hour and diluted to Brown's tube 2. The rabbits were bled 10 days after the last injection. (Edwards and Ewing, 1955). Sera was initially supplied by Dr. N. Gilmour, Moredun Institute, but later was produced in this laboratory by inoculating rabbits using the subcutaneous as opposed to the intravenous route with resultant low titres.

Sodium citrate

3.8 grammes of trisodium citrate (B.D.H. Chemicals Ltd., Poole) per 100ml of distilled water.

<u>Brain Heart Infusion Broth</u>

Code CM 225 Oxoid.

<u>Ox Red Blood Cells (R.B.C.)</u>

Ox blood was collected by jugular venepuncture into 10ml vacutainer tubes (Becton-Dickinson, Rutherford, New Jersey) into which 5ml of trisodium citrate solution had been injected. The R.B.C. were then washed 3 times in F.B.S. and made up to a final concentration of 5%.

<u>Reagents for Fluorescent Antibody Test (F.A.T.)</u>

Phosphate buffered saline (Dulbecco 'A') p.H. 7.3 (Oxoid Ltd., England).

One tablet per 100ml distilled water.

Rabbit antisheep immunoglobulin (fluorescent labelled) (Wellcome Reagents Ltd., Beckenham, England).

<u>Defatting Mixture</u>

50% Ethanol (B.D.H., Poole).

50% Diethyl Ether (B.D.H., Poole).

<u>Bacteriological Materials</u>

<u>Blood Agar</u>

5% Defibrinated sheep blood (Cruickshank et al 1975).

95% Columbia agar base (Code CM 331 Oxoid)

<u>Layered Blood Agar</u>

2mm layer of Nutrient agar (Code CM 3 R 2 Oxoid)

2mm layer of 6% blood agar.

<u>Nasal Swabs</u>

33cm pieces of 1.8ml diameter pliable aloy wire had

one end squeezed in a vice leaving a roughend surface.
The wire was then twisted in absorbant cotton wool (Vestric) to make swabs which were then wrapped in aluminium foil and autoclaved at 15 lbs per square inch for 20 minutes.

RESULTS

The results of the nasal swab cultures, with details of serotypes isolated on each occasion are set out on page 19a.

The results of two serological tests are set out on pages 20 - 32.

RESULTS - ISOLATES FROM SHEEP

Date	2.3.77	15.3.77	29.3.77	13.4.77	27.4.77
Sheep 1	A2 &T10		A2 &T10		A2
2	untypable	A2	A2	A2 &T10	A2
3					untypable & A2
4		A2			T10
5					A2 & T3
6	untypable			A2	
7	A2 & T3	A2 & T3			untypable
8		untypable	A2	A2 &T10	A2, T3 & T10
9	A9	A2 & untypable	A2	A2 &T10	A2
10	A9 & A2		A2		A2 & A7
11	A2				
12				A2	
13		A2 & T3	A2		

Typed by I.H.A. test using hyperimmune sera.

Blank spaces indicate that no *P. haemolytica* isolated. On occasions the plates were overgrown with other organisms, and could have prevented the serotypes from being isolated.

SHEEP 1

INDIRECT HAEMAGGLUTINATION TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/4	0	1/4	1/8	0	1/4	0
30.11.76	1/4	0	1/8	1/8	1/4	1/4	0
15.12.76	1/4	0	1/8	1/16	1/4	1/4	0
5. 1.77	1/4	0	1/4	1/8	0	1/4	0
18. 1.77	0	0	0	1/8	0	1/4	0
2. 2.77	1/4	0	1/4	1/8	0	1/4	0
16. 2.77	1/4	0	1/4	1/4	1/4	1/4	0
2. 3.77	1/8	0	1/4	1/8	0	1/4	0
16. 3.77	1/4	0	1/4	1/8	0	1/4	0
29. 3.77	0	0	1/4	1/4	0	1/4	0
13. 4.77	1/4	0	1/4	1/8	0	1/8	0
27. 4.77	1/4	0	1/4	1/8	0	1/4	0

SHEEP 1

FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/4	1/16	1/8	1/16	1/16	1/16	1/8
15.12.76	1/16	1/8	1/8	1/16	1/32	1/8	1/16
5. 1.77	1/4	1/4	1/16	1/32	1/8	1/16	1/16
18. 1.77	1/8	1/4	1/64	1/16	1/16	1/8	1/16
2. 2.77	1/4	0	1/8	1/16	1/16	1/8	1/16
16. 2.77	1/4	1/8	1/16	1/16	1/4	1/16	1/16
2. 3.77	1/16	1/32	1/16	1/32	1/16	1/32	1/16
16. 3.77	1/8	1/16	1/8	1/16	1/16	1/16	1/8
29. 3.77	1/16	1/32	1/16	1/16	1/16	1/8	1/16
13. 4.77	1/16	1/16	1/32	1/16	1/32	1/32	1/32
27. 4.77	1/16	1/8	1/16	1/16	1/32	1/16	1/16

NT = Not tested
0 = less than 1/4
Serotypes isolated - A2 and T10

SHEEP 2

INDIRECT HAEMAGGLUTINATION TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/4	0	1/4	1/16	1/4	1/8	0
30.11.76	1/4	0	1/4	1/16	0	1/8	0
15.12.76	1/4	0	1/8	1/16	1/4	1/4	0
5. 1.77	1/4	0	1/8	1/32	1/4	1/4	0
18. 1.77	1/4	0	1/8	1/16	1/4	1/4	0
2. 2.77	1/4	0	1/8	1/32	1/4	1/4	0
16. 2.77	1/8	1/4	1/4	1/8	1/8	1/4	0
2. 3.77	1/8	1/4	1/4	1/4	1/4	1/4	0
16. 3.77	1/8	1/4	1/4	1/8	0	1/4	0
29. 3.77	1/4	0	1/8	1/8	1/4	1/8	0
13. 4.77	1/8	0	1/4	1/8	1/4	1/8	0
27. 4.77	1/4	0	0		0	1/4	0

SHEEP 2

FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/8	1/32	1/8	1/16	1/16	1/16	1/8
15.12.76	1/8	1/16	1/16	1/32	1/32	1/32	1/16
5. 1.77	1/8	0	1/32	1/32	1/16	1/16	1/32
18. 1.77	1/8	1/4	1/32	1/16	1/8	1/8	1/8
2. 2.77	0	0	1/8	1/16	1/16	1/8	1/16
16. 2.77	1/4	0	1/16	1/16	1/32	1/8	1/16
2. 3.77	1/8	1/32	1/16	1/32	1/32	1/16	1/16
16. 3.77	1/4	1/8	1/8	1/4	1/16	1/8	1/8
29. 3.77	1/16	1/16	1/16	1/32	1/8	1/16	1/8
13. 4.77	1/8	1/32	1/16	1/32	1/16	1/32	1/32
27. 4.77	1/16	1/4	1/4	1/32	1/16	1/8	1/8

NT = Not tested
0 = Less than 1/4
Serotypes isolated - A2, T10 and untypable.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 3

date \ serotype	A1	A2	A6	A7	A9	T3	T10
16.11.76	0	0	1/4	1/8	0	0	0
30.11.76	0	0	1/4	1/4	0	1/4	0
15.12.76	0	0	1/4	1/8	0	0	0
5. 1.77	0	0	1/4	1/8	0	1/4	0
18. 1.77	0	0	1/4	1/8	0	1/4	0
2. 2.77	0	0	1/4	1/16	0	1/4	0
16. 2.77	1/4	0	1/4	1/4	0	1/4	0
2. 3.77	0	0	0	1/4	0	1/4	0
16. 3.77	0	0	1/4	0	0	1/4	0
29. 3.77	0	0	1/8	1/8	0	0	0
13. 4.77	0	0	1/4	1/4	0	1/4	0
27. 4.77	0	0	0	1/8	0	0	0

SHEEP 3 FLUORESCENT ANTIBODY TITRES

date \ serotype	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/8	1/32	1/8	1/16	1/16	1/16	1/8
15.12.76	1/8	1/8	1/16	1/8	1/8	1/16	1/16
5. 1.77	1/16	0	1/8	1/8	1/16	1/16	1/32
18. 1.77	1/8	1/16	1/16	1/16	1/8	1/32	1/16
2. 2.77	1/4	0	1/4	1/4	1/8	1/16	1/64
16. 2.77	1/4	1/4	1/16	1/4	1/8	1/16	1/32
2. 3.77	1/8	1/32	1/16	1/8	1/16	1/32	1/16
16. 3.77	1/16	1/16	1/4	1/8	1/8	1/8	1/8
29. 3.77	1/32	1/8	1/16	1/16	1/8	1/16	1/4
13. 4.77	1/16	1/32	1/16	1/16	1/16	1/32	1/16
27. 4.77	1/8	1/4	1/8	1/32	1/16	1/16	1/32

NT = Not tested
0 = less than 1/4
Serotypes isolated - A2 and untypable.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 4

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	1/4	1/16	1/4	1/8	0
30.11.76	1/4	0	1/4	1/32	0	1/16	0
15.12.76	1/4	0	1/4	1/32	1/4	1/16	0
5. 1.77	1/8	0	1/4	1/32	1/4	1/16	0
18. 1.77	1/4	0	1/4	1/32	1/4	1/8	0
2. 2.77	1/8	0	1/4	1/32	0	1/8	0
16. 2.77	1/32	0	1/4	1/32	1/4	1/16	0
2. 3.77	1/8	1/4	0	1/16	1/4	1/16	0
16. 3.77	1/8	0	1/4	1/16	0	1/16	0
29. 3.77	1/16	0	1/4	1/32	1/4	1/16	0
13. 4.77	1/8	0	1/4	1/16	0	1/8	0
27. 4.77	1/4	0	0	1/16	1/4	1/8	0

FLUORESCENT ANTIBODY TITRES

SHEEP 4

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/8	1/16	1/8	1/16	1/16	1/16	1/8
15.12.76	1/8	1/8	1/32	1/16	1/16	1/32	1/16
5. 1.77	1/16	1/64	1/32	1/32	1/64	1/16	1/64
18. 1.77	1/16	0	1/32	1/16	1/16	1/3	1/8
2. 2.77	1/4	0	1/8	1/8	1/8	1/4	1/16
16. 2.77	1/8	1/8	1/16	1/4	1/8	1/16	1/16
2. 3.77	1/8	1/16	1/16	1/16	1/32	1/32	1/16
16. 3.77	1/16	1/16	1/16	1/8	1/8	1/16	1/4
29. 3.77	1/32	1/16	1/16	1/16	1/8	1/8	1/8
13. 4.77	1/16	1/32	1/16	1/16	1/16	1/16	1/8
27. 4.77	1/8	1/16	1/8	1/16	1/32	1/16	1/32

NT = Not tested
0 = Less than 1/4
Serotypes isolated - A2 and T10

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 5;

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	1/4	1/8	1/4	1/8	0
30.11.76	1/4	0	1/4	1/8	1/4	1/16	0
15.12.76	1/8	0	1/8	1/16	1/8	1/4	0
5. 1.77	1/4	0	1/4	1/16	1/8	1/4	0
18. 1.77	1/4	0	1/4	1/8	1/8	1/4	0
2. 2.77	1/4	0	1/4	1/8	1/4	1/8	0
16. 2.77	1/4	0	1/4	1/8	1/8	1/8	0
2. 3.77	1/8	0	1/4	1/4	1/8	1/8	0
16. 3.77	1/4	0	1/8	1/8	1/4	1/8	0
29. 3.77	1/8	0	1/8	1/8	1/8	1/4	0
13. 4.77	1/16	0	1/4	1/8	1/8	1/8	0
27. 4.77	1/8	0	1/4	1/8	1/4	1/8	0

SHEEP 5 FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/32	1/32	1/8	1/16	1/32	1/16	1/8
15.12.76	1/8	1/8	1/32	1/16	1/16	1/32	1/16
5. 1.77	1/32	0	1/16	1/32	1/16	1/16	1/16
18. 1.77	1/8	1/16	1/16	1/16	1/8	1/16	1/8
2. 2.77	1/8	1/4	1/8	1/8	1/4	1/16	1/32
16. 2.77	NT	NT	NT	NT	NT	NT	NT
2. 3.77	1/16	1/32	1/16	1/16	1/16	1/16	1/32
16. 3.77	1/16	1/32	1/8	1/8	1/32	1/8	1/8
29. 3.77	1/16	1/16	1/32	1/32	1/16	1/8	1/8
13. 4.77	1/8	1/16	1/32	1/32	1/32	1/16	1/16
27. 4.77	1/16	1/16	1/16	1/32	1/64	1/32	1/32

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2 and T3.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 6

date \ serotype	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/4	0	1/4	1/4	0	1/4	0
30.11.76	0	0	0	1/4	0	1/4	0
15.12.76	0	0	0	1/8	0	1/4	0
5. 1.77	0	0	1/4	1/4	0	1/4	1/4
18.1.77	0	0	1/8	1/4	0	1/4	0
2. 2.77	0	0	1/4	1/8	0	1/4	0
16. 2.77	1/4	0	1/4	1/4	0	0	0
2. 3.77	1/4	0	0	1/4	0	0	0
16. 3.77	1/4	0	0	1/4	0	0	0
29. 3.77	0	0	0	1/8	0	0	0
13. 4.77	1/4	0	1/4	1/4	0	1/4	0
27. 4.77	1/4	0	1/4	1/4	0	1/8	0

FLUORESCENT ANTIBODY TITRES

SHEEP 6

date \ serotype	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/4	1/16	1/8	1/16	1/16	1/8	1/8
15.12.76	1/16	1/16	1/16	1/16	1/32	1/16	1/16
5. 1.77	1/4	1/16	1/16	1/16	1/32	1/16	1/16
18. 1.77	1/8	1/16	1/16	1/32	1/32	1/16	1/16
2. 2.77	1/4	1/16	1/8	1/8	1/16	1/16	1/8
16. 2.77	1/8	0	1/32	1/16	1/16	1/8	1/16
2. 3.77	1/8	1/16	1/8	1/16	1/32	1/32	1/16
16. 3.77	1/8	1/8	1/16	1/32	1/64	1/16	1/16
29. 3.77	1/8	1/16	1/16	1/32	1/16	1/32	1/16
13. 4.77	1/4	1/16	1/8	1/16	1/32	1/16	1/16
27. 4.77	1/16	1/16	1/8	1/8	1/16	1/16	1/8

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2 and untypable.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 7

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/4	0	1/4	1/4	0	1/4	0
30.11.76	1/8	0	1/4	1/4	0	0	0
15.12.76	1/4	0	1/4	1/16	0	1/4	0
5. 1.77	1/4	0	1/4	1/4	0	1/4	0
18. 1.77	1/4	0	1/4	1/4	0	1/4	0
2. 2.77	1/4	0	1/4	1/8	0	1/4	0
16. 2.77	1/8	0	1/4	1/4	0	1/4	0
2. 3.77	1/8	1/4	0	1/4	0	1/4	0
16. 3.77	1/8	0	1/4	1/4	0	1/4	0
29. 3.77	1/8	0	1/4	1/8	0	1/4	0
13. 4.77	1/8	0	0	1/4	0	1/4	0
27. 4.77	1/4	0	0	1/4	0	1/4	0

SHEEP 7 FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/16	1/32	1/16	1/16	1/16	1/8	1/16
15.12.76	1/16	1/16	1/64	1/16	1/32	1/16	1/16
5. 1.77	1/8	1/16	1/64	1/16	1/16	1/32	1/64
18. 1.77	1/16	1/4	1/16	1/16	1/8	1/32	1/16
2. 2.77	1/8	0	1/16	1/16	1/16	1/32	1/32
16. 2.77	1/8	0	1/16	1/16	1/16	1/16	1/32
2. 3.77	1/16	1/16	1/32	1/16	1/32	1/32	1/16
16. 3.77	1/32	1/32	1/32	1/32	1/16	1/16	1/8
29. 3.77	1/32	1/16	1/32	1/16	1/16	1/32	1/16
13. 4.77	1/64	1/32	1/16	1/32	1/64	1/16	1/32
27. 4.77	1/16	1/8	1/8	1/8	1/32	1/16	1/32

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2, T3 and untypable.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 8

date \ serotype	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	1/16	1/16	0	0	1/4
30.11.76	1/8	0	1/16	1/32	0	1/4	1/4
15.12.76	1/8	0	1/16	1/16	0	1/4	1/4
5. 1.77	1/16	0	1/16	1/16	0	1/8	0
18. 1.77	1/16	0	1/8	1/16	0	1/4	0
2. 2.77	1/8	0	1/8	1/16	0	1/4	0
16. 2.77	1/16	0	1/16	1/8	1/4	0	1/4
2. 3.77	1/4	1/4	1/8	1/16	1/4	0	1/4
16. 3.77	1/16	1/4	1/8	1/8	0	0	0
29. 3.77	1/16	1/4	1/16	1/8	1/4	0	0
13. 4.77	1/4	0	1/4	1/8	0	1/4	0
27. 4.77	1/4	0	1/4	1/8	1/4	0	0

FLUORESCENT ANTIBODY TITRES

SHEEP 8

date \ serotype	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/16	1/16	1/16	1/16	1/16	1/8	1/8
15.12.76	1/16	1/32	1/32	1/32	1/16	1/32	1/32
5. 1.77	1/32	0	1/16	1/32	1/32	1/32	1/64
18. 1.77	1/16	1/8	1/16	1/32	1/64	1/16	1/16
2. 2.77	1/4	1/4	1/8	1/16	1/16	1/32	1/64
16. 2.77	1/4	1/4	1/8	1/8	1/8	1/16	1/32
2. 3.77	1/16	1/16	1/16	1/32	1/32	1/16	1/32
16. 3.77	1/32	1/16	1/8	1/32	1/16	1/8	1/8
29. 3.77	1/32	1/16	1/16	1/16	1/16	1/8	1/16
13. 4.77	NT	NT	NT	NT	NT	NT	NT
27. 4.77	1/32	1/16	1/16	1/16	1/32	1/16	1/32

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2, T3, T10 and untypable.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 9

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/4	0	1/8	1/8	0	0	0
30.11.76	1/4	0	1/4	1/8	0	1/4	0
15.12.76	1/4	0	1/4	1/16	1/4	1/4	0
5. 1.77	1/4	0	1/4	1/16	1/4	0	0
18. 1.77	1/4	0	1/4	1/16	0	1/4	0
2. 2.77	1/4	0	1/4	1/16	1/4	1/4	0
16. 2.77	1/8	0	1/4	1/16	1/4	1/4	0
2. 3.77	1/4	0	0	1/8	1/4	0	0
16. 3.77	1/8	0	1/4	1/16	0	1/4	0
29. 3.77	1/8	0	1/4	1/16	1/4	1/8	0
13. 4.77	1/4	0	1/4	1/16	1/4	0	0
27. 4.77	1/8	0	0	1/16	0	1/4	0

SHEEP 9

FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/32	1/32	1/16	1/32	1/32	1/16	1/16
15.12.76	1/32	1/16	1/128	1/16	1/64	1/16	1/16
5. 1.77	1/64	0	1/64	1/32	1/64	1/16	1/32
18. 1.77	1/32	1/16	1/64	1/32	1/32	1/32	1/8
2. 2.77	1/4	0	1/16	1/8	1/16	0	1/16
16. 2.77	1/8	1/16	1/16	1/16	1/32	1/8	1/16
2. 3.77	1/16	1/16	1/16	1/32	1/64	1/16	1/16
16. 3.77	1/16	1/16	1/16	1/32	1/32	1/8	1/16
29. 3.77	1/32	1/16	1/16	1/64	1/32	1/4	1/8
13. 4.77	1/16	1/32	1/32	1/32	1/32	1/32	1/32
27. 4.77	1/16	1/16	1/4	1/32	1/32	1/16	1/32

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2, A9, T10 and untypable.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 10

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	1/4	1/4	0	1/4	0
30.11.76	1/8	0	1/4	1/4	1/8	0	0
15.12.76	1/8	0	1/4	1/4	1/8	1/4	0
5. 1.77	1/4	0	1/4	1/4	1/4	1/4	0
18. 1.77	1/4	0	1/4	1/16	1/8	1/4	0
2. 2.77	1/8	0	1/4	1/4	1/8	1/4	0
16. 2.77	1/16	0	1/4	1/4	1/8	0	0
2. 3.77	1/8	0	0	0	1/8	0	0
16. 3.77	1/8	0	1/4	1/4	1/8	0	0
29. 3.77	1/8	0	1/8	1/4	1/8	0	0
13. 4.77	1/8	0	1/4	1/4	1/8	1/4	0
27. 4.77	1/4	0	0	1/4	1/4	0	0

SHEEP 10 FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/16	1/32	1/16	1/16	1/16	1/8	1/16
15.12.76	1/32	1/32	1/64	1/32	1/32	1/16	1/16
5. 1.77	1/64	1/4	1/32	1/16	1/32	1/16	1/32
18. 1.77	1/16	1/4	1/64	1/16	1/32	1/16	1/8
2. 2.77	1/8	1/8		1/8	1/32	1/8	1/8
16. 2.77	1/4	1/4	1/32	1/8	1/16	1/16	1/32
2. 3.77	1/8	1/16	1/16	1/8	1/32	1/16	1/32
16. 3.77	0	1/16	1/16	1/16	1/16	1/8	1/8
29. 3.77	1/16	1/8	1/16	1/16	1/16	1/4	1/8
13. 4.77	1/8	1/32	1/16	1/32	1/16	1/16	1/8
27. 4.77	1/16	1/16	1/8	1/16	1/32	1/16	1/32

NT = Not tested
0 = Less than 1/4.
Serotypes isolated. - A2, A7 and A9.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 11

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	1/4	0	0	1/4	0
30.11.76	1/4	0	1/4	1/4	0	1/4	0
15.12.76	1/4	0	0	1/16	1/4	1/4	0
5. 1.77	1/4	0	1/4	1/8	1/4	1/4	0
18. 1.77	1/4	0	1/4	1/16	1/4	1/4	0
2. 2.77	1/4	0	1/4	1/16	0	1/4	0
16. 2.77	1/16	0	0	1/8	0	1/4	0
2. 3.77	1/4	0	0	1/4	1/4	1/4	0
16. 3.77	1/8	0	1/4	1/4	0	1/4	0
29. 3.77	1/4	0	0	1/4	0	1/4	0
13. 4.77	1/8	0	1/4	1/4	0	1/4	0
27. 4.77	1/4	0	1/4	1/8	1/4	0	0

SHEEP 11

FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/16	1/16	1/8	1/16	1/16	1/16	1/16
15.12.76	1/16	1/16	1/64	1/32	1/32	1/32	1/8
5. 1.77	1/32	0	1/64	1/32	1/32	1/32	1/64
18. 1.77	1/64	1/4	1/64	1/16	1/16	1/32	1/8
2. 2.77	1/4	1/4	1/8	1/16	1/16	1/16	1/64
16. 2.77	1/4	0	1/32	1/8	1/32	1/16	1/16
2. 3.77	1/8	1/16	1/32	1/16	1/32	1/32	1/16
16. 3.77	1/4	1/32	1/8	1/32	1/16	1/16	1/16
29. 3.77	1/16	1/16	1/32	1/16	1/16	1/8	1/8
13. 4.77	1/32	1/8	1/16	1/16	1/64	1/32	1/32
27. 4.77	1/16	1/16	1/8	1/32	1/16	1/16	1/64

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 12

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	1/4	1/16	0	1/4	0
30.11.76	1/8	0	1/8	1/16	0	1/4	0
15.12.76	1/4	0	1/4	1/16	0	1/4	0
5. 1.77	1/4	0	1/8	1/16	0	1/4	0
18. 1.77	1/8	0	1/4	1/16	0	1/4	0
2. 2.77	1/4	0	1/4	1/32	0	1/4	0
16. 2.77	1/8	0	1/4	1/16	0	1/8	0
2. 3.77	1/8	0	1/4	1/16	1/4	1/8	0
16. 3.77	1/8	0	1/4	1/16	0	1/8	0
29. 3.77	0	0	0	1/16	0	1/4	0
13. 4.77	1/8	0	1/4	1/16	1/4	1/4	0
27. 4.77	1/8	0	1/4	1/16	0	1/8	0

SHEEP 12 FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/8	1/16	0	1/16	1/8	1/4	1/4
15.12.76	1/16	1/16	1/16	1/8	1/32	1/16	1/16
5. 1.77	1/8	1/16	1/32	1/8	1/32	1/16	1/32
18. 1.77	1/8	1/8	1/8	1/16	1/16	1/8	1/8
2. 2.77	1/4	0	1/8	1/8	1/8	1/16	1/8
16. 2.77	1/8	1/4	1/8	1/8	1/16	1/16	1/8
2. 3.77	1/16	1/32	1/8	1/32	1/32	1/8	1/16
16. 3.77	1/32	1/16	1/16	1/8	1/16	1/4	1/8
29. 3.77	1/16	1/16	1/8	1/16	1/16	1/8	1/8
13. 4.77	1/16	1/32	1/8	1/16	1/32	1/16	1/16
27. 4.77	1/16	1/16	1/16	1/32	1/16	1/32	1/16

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2.

INDIRECT HAEMAGGLUTINATION TITRES

SHEEP 13

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	1/8	0	0	1/8	1/4	1/4	0
30.11.76	1/8	0	1/4	1/8	0	1/4	0
15.12.76	1/8	0	1/4	1/16	0	1/4	0
5. 1.77	1/8	0	1/4	1/8	1/4	1/4	1/4
18. 1.77	1/8	0	0	1/4	1/4	1/4	0
2. 2.77	1/8	0	1/4	1/16	1/4	1/4	0
16. 2.77	1/8	0	1/4	1/4	1/4	1/4	0
2. 3.77	1/8	0	0	1/4	1/4	1/4	0
16. 3.77	1/16	0	1/4	1/4	1/4	1/8	0
29. 3.77	1/16	0	1/8	1/8	1/4	1/16	0
13. 4.77	NT	NT	NT	NT	NT	NT	NT
27. 4.77	NT	NT	NT	NT	NT	NT	NT

SHEEP 13 FLUORESCENT ANTIBODY TITRES

serotype / date	A1	A2	A6	A7	A9	T3	T10
16.11.76	NT	NT	NT	NT	NT	NT	NT
30.11.76	1/8	1/16	1/8	1/32	1/32	1/16	1/8
15.12.76	1/32	1/16	1/16	1/16	1/64	1/16	1/16
5. 1.77	1/16	1/8	1/32	1/16	1/32	1/16	1/32
18. 1.77	1/8	1/8	1/32	1/8	1/32	1/16	1/16
2. 2.77	1/4	0	1/16	1/8	1/8	1/16	1/8
16. 2.77	1/4	0	1/8	1/8	1/8	1/8	1/16
2. 3.77	1/4	1/16	1/16	1/32	1/32	1/16	1/16
16. 3.77	1/16	1/16	1/16	1/8	1/32	1/8	1/8
29. 3.77	1/16	1/16	1/32	1/32	1/32	1/8	1/4
13. 4.77	1/8	1/16	1/16	1/8	1/64	1/8	1/16
27. 4.77	1/16	1/16	1/16	1/16	1/8	1/32	1/16

NT = Not tested.
0 = Less than 1/4.
Serotypes isolated - A2 and T3.

HYPERIMMUNE SERA PRODUCTION

The New Zealand White rabbits were bled following subcutaneous inoculation and the antibody levels were measured using the I.H.A. test. All sera collected on the 10th day gave a titre of 1/8.

ENVIRONMENT

The temperature in the buildings in which the sheep were housed was recorded daily but no dramatic changes occurred. They varied between -1°C and $+11^{\circ}$C.

The ambient humidity varied between 50% and 60%.

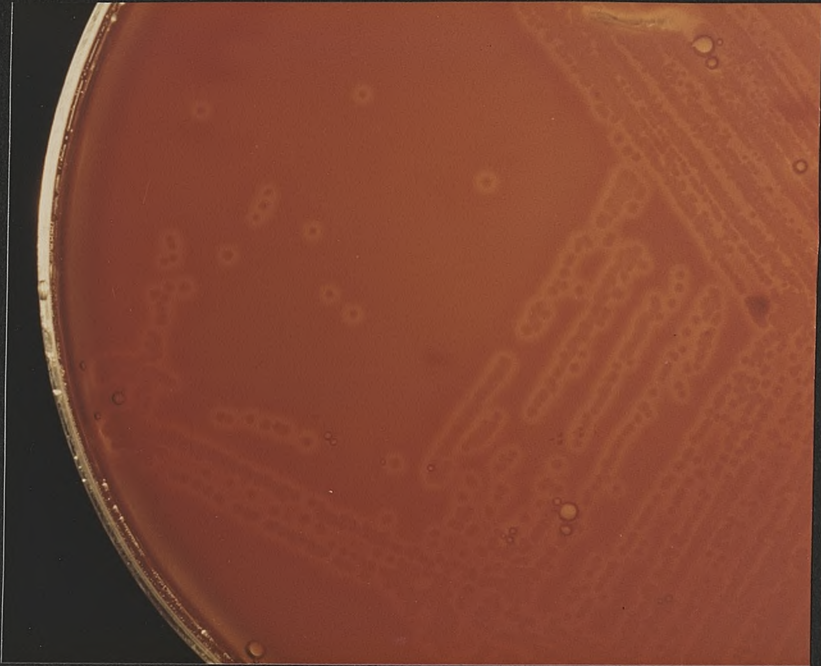


PLATE 1

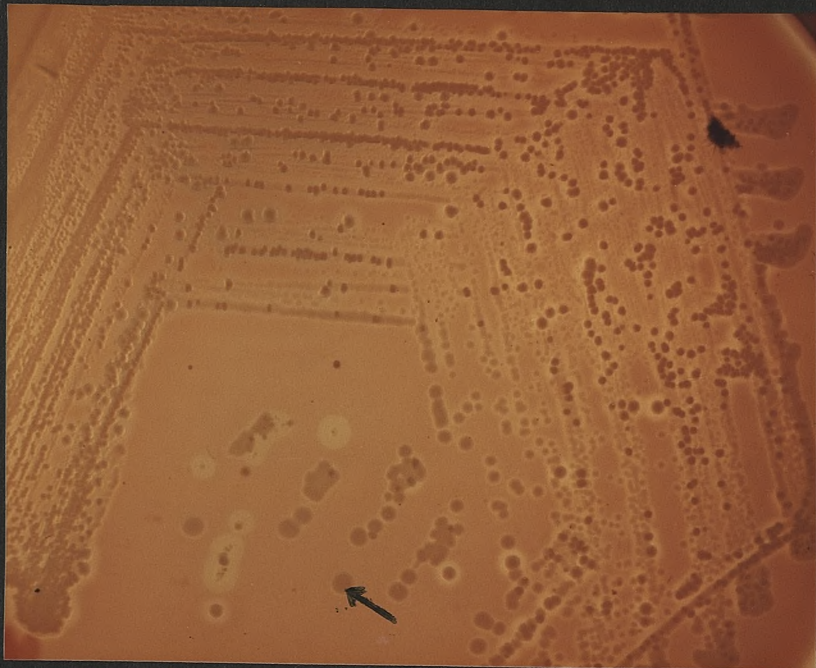


PLATE 2

PLATE 1.

Typical fresh A type isolate of P. haemolytica showing a wide zone of haemolysis on layered blood agar. Compare with T types seen on Plate 4.

PLATE 2.

Typical nasal swab on layered blood agar, with A1 serotypes arrowed.

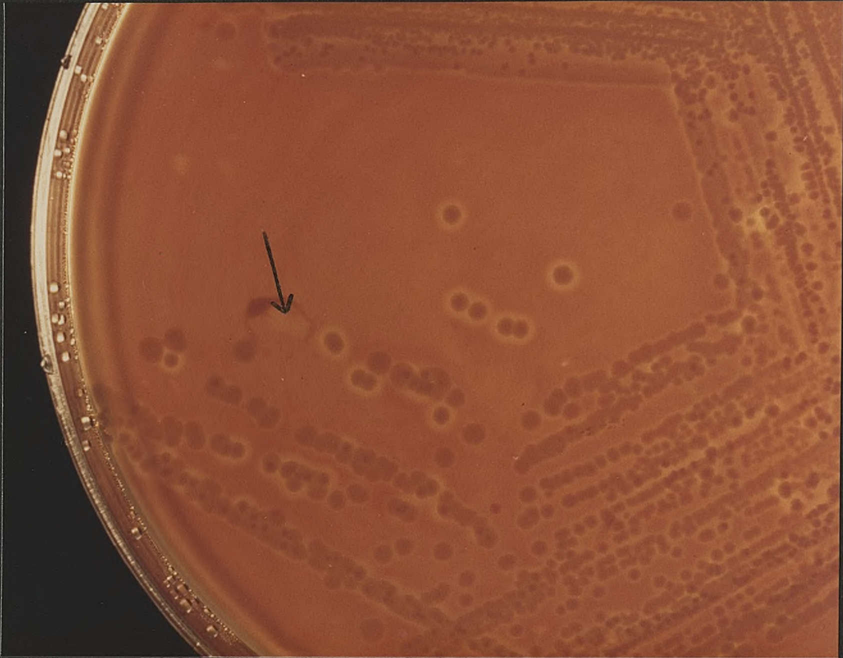


PLATE 3

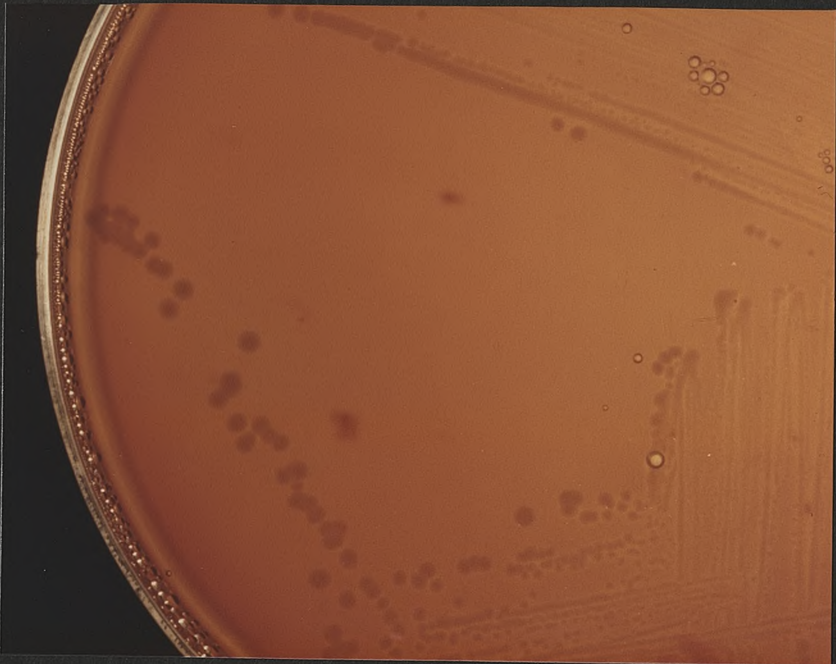


PLATE 4

PLATE 3.

Slightly haemolytic Al serotype on layered blood agar with other organisms which have a wide zone of haemolysis. Arrow shows two colonies which have been moved to demonstrate clearing of the media beneath the colonies.

PLATE 4.

Slightly haemolytic Al serotype on blood agar demonstrating the difficulty in seeing Beta haemolysis. Compare with Al serotype on layered blood agar (PLATE 1.).

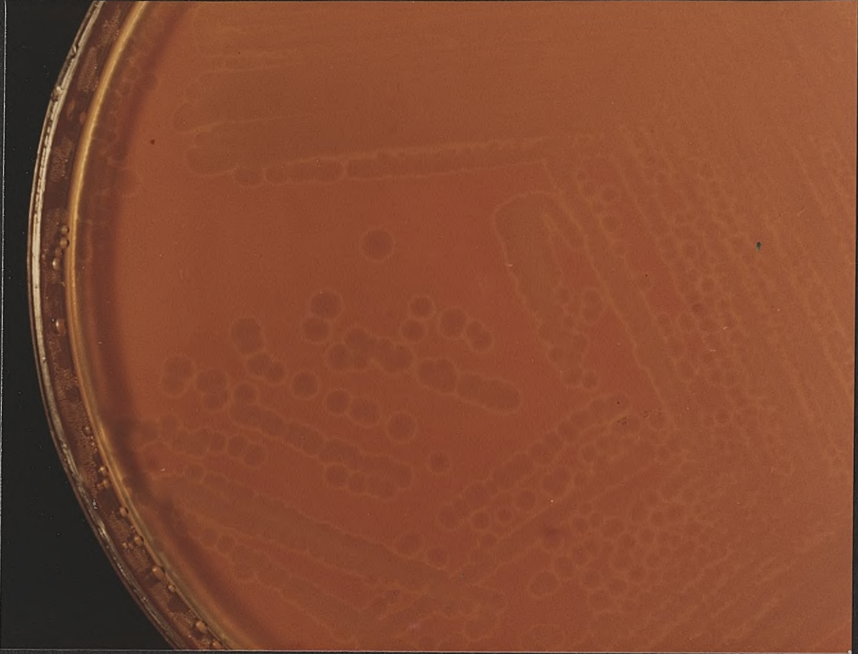


PLATE 5

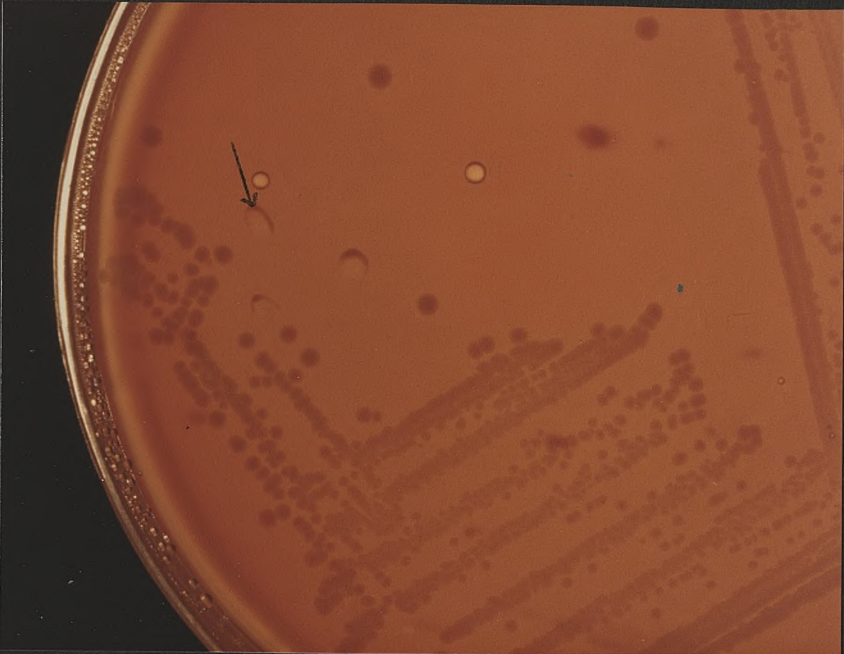


PLATE 6

PLATE 5.

T10 serotype on layered blood agar showing the larger colonies.
Compare with A1 serotype (PLATE 1.)

PLATE 6.

Slightly haemolytic T4 serotype on blood agar requiring colonies
to be moved to see the Beta haemolysis.

t values (Fig. 2)

Sheep							
1	3.43C	5.93D	4.30C	6.53D	8.20D	8.86D	17.89D
2	0.93	3.30C	6.71D	2.47B	9.59D	7.02D	11.50D
3	7.93D	4.71D	5.16D	2.17	15.59D	11.84D	9.02D
4	1.60	5.40D	9.64D	-1.49	8.48D	1.30	8.20D
5	3.08B	6.38D	9.0D	4.71C	3.75C	4.33C	10.47D
6	5.61D	9.46D	7.42D	6.90D	17.89D	7.47D	17.32D
7	5.59D	5.06D	10.70D	4.02C	13.79D	15.59D	13.97D
8	1.56	5.48D	1.5	2.69B	9.00D	10.85D	8.23 D
9	4.87D	6.42D	7.60D	3.19C	17.10D	3.54C	4.63D
10	1.34	7.2D	5.31D	6.90D	7.29D	9.9D	10.22D
11	2.59B	5.04D	5.84D	4.66D	12.33D	11.84D	9.25D
12	3.32C	7.25D	3.36C	-1.30	15.73D	3.46C	9.93D
13	0.00	4.99C	6.28D	2.0	6.83D	3.58C	10.09D

$P < 0.05 \quad > 0.01 = B$

$P < 0.01 \quad > 0.001 = C$

$P < 0.001 \quad = D$

A comparison of F.A.T. and I.H.A. tests was carried out by calculating the t values (Fig. 2 page 40).

Linear correlation r values were calculated for each test (Fig. 3 page 41).

Linear Correlation r values (Fig. 3)

I.H.A.	A1	A2	A6	A7	A9	T3	T10
Sheep 1	-0.02	NC	-0.34	-0.37	-0.43	-0.39	NC
2	0.47	0.26	-0.37	-0.62	-0.12	0.06	NC
3	0.05	NC	-0.17	-0.09	NC	0.02	NC
4	0.54	0.14	-0.45	-0.41	-0.09	-0.23	NC
5	0.37	NC	0.12	-0.36	0.13	-0.08	NC
6	0.43	NC	-0.02	-0.03	NC	-0.11	-0.19
7	0.38	0.14	-0.63	-0.16	NC	0.41	NC
8	-0.26	0.42	-0.71	-0.81	0.56	-0.38	-0.57
9	0.63*	NC	-0.62	0.48	-0.02	0.21	NC
10	-0.05	NC	-0.17	-0.16	0.31	-0.42	NC
11	0.32	NC	-0.10	-0.04	-0.01	NC	NC
12	-0.02	NC	-0.53	-0.03	0.39	0.52	NC
13	0.68*	NC	0.39	-0.43	0.54	-0.17	NC

F.A.T.	A1	A2	A6	A7	A9	T3	T10
Sheep 1	0.55	0.36	0.27	-0.30	0.24	0.36	0.39
2	0.25	0.04	-0.35	0.05	-0.13	-0.17	-0.06
3	0.33	0.04	-0.01	0.33	0.11	0.05	-0.06
4	0.33	0.04	-0.30	0.22	-0.11	-0.21	-0.12
5	-0.2	0.29	0.24	-0.04	0.40	-0.20	0.26
6	0.22	0.22	-0.20	-0.36	0.01	0.38	0.09
7	0.52	0.04	-0.41	0.06	0.43	0.34	0.02
8	0.26	0.13	-0.34	-0.21	0.03	-0.32	0.01
9	-0.33	0.21	-0.61	0.33	-0.42	0.002	0.21
10	-0.44	0.41	-0.36	-0.02	-0.22	-0.07	-0.09
11	-0.14	0.25	-0.33	-0.01	0.09	-0.32	0.50
12	0.49	0.23	0.23	0.44	0.16	0.30	0.20
13	-0.05	0.18	0.14	-0.15	-0.22	-0.19	-0.17

NC = No calculations.

DISCUSSION

The technique described for the isolation of P. haemolytica from the nasopharynx was successful to the extent that the expected pattern of isolates occurred, with 5 different serotypes being isolated, and in several sheep isolates were made on all 5 occasions. In contrast, several sheep yielded only 1 or 2 isolates and occasionally the plates were overgrown with other organisms.

Further isolates could possibly have been obtained by placing swabs in broth, after swabbing (Shreeve & Thompson, 1970), and subsequently plating out a drop of the broth, by using growth inhibiting substances in the blood agar, (Morris 1958), or by the use of nasal washings instead of nasal swabs. This latter method could result in the nasal population failing to return to normal, before the next sampling date. In these experiments initial swabbing was carried out with dry swabs but later a change to swabs moistened in tryptose phosphate broth was made because of the poor isolation rate with dry swabs. Drying of the organism would not be the problem as swabs were applied to the blood agar immediately after collection, but it is possible that the broth dilutes the nasal antibodies (Smith et al 1975), and so improves the isolation rate.

The techniques used in this work may have favoured the isolation of the A2 serotype and not the less frequently isolated ones.

A comparison of isolation rates of different serotypes of *P. haemolytica* with various swabbing techniques would be of interest.

Attempts to reduce the amount of contamination were made by inserting a plastic tube, 5 cms. long and 1.3 cms. diameter, into the nares and then introducing the swab. This upset the sheep and made sampling more difficult and was therefore discontinued.

When organisms resembling *P. haemolytica* were found on a plate several colonies were removed, even if they appeared to be of one biotype, in case more than one serotype was present. All colonies regardless of morphological simularity should ideally have been removed to ensure no serotype was missed but this was not always practicable and a representative sample was taken instead.

The importance of colony morphology and B. haemolysis cannot be over-stressed (PLATE 3.). On occasions individual serotypes can vary in the degree of haemolysis visible and consequently layered blood agar was found to be of assistance (PLATE 3 & 4.). However, it was still necessary to move colonies to check for clearing of the blood agar. Isolates could have been missed (PLATE 6.) if the media was not consistant from week to week.

Problems did arise, and if there was any doubt, further plates were prepared. At the beginning of the work Columbia Base Agar, after autoclaving, was left in a water bath at 55°C overnight. When the plates were poured the following morning although appearing normal, on incubation they became dark and would not readily support the growth of P. haemolytica. No explanation for this phenomena could be found. If the Columbia Base was not given time to cool, then chocolate agar was the end product.

The non-haemolytic strain of P. haemolytica isolated by Dungal (1931) was found to be slightly haemolytic by Montgomery et al (1938) emphasising the importance of a standardised media. Non-haemolytic colonies from nasopharyngeal swabs in this work, were ignored.

When defibrinated blood from the experimental flock was used in the blood agar, no changes in colony morphology or haemolysis with the standard serotypes was noticed. This contrasts with Carter's (1975) view that antibodies in the media may inhibit haemolysis. Comparisons of the growth characteristics of different serotypes of P. haemolytica, on blood agar containing antibodies to the various serotypes, would be of interest.

As strains of P. haemolytica appear to vary in degrees of haemolysis, and possibly therefore in other characteristics, the same strain should be used throughout an experiment. To maintain such strains it was necessary to sub-culture at least once a week and even blood agar slopes stored at 4°C could only maintain the organism for periods of about 4 weeks. Montgomery et al (1938) and Dungal (1931) also found problems with the survival of P. haemolytica.

The I.H.A. test, although yielding reproducible results (repeated tests gave titres within one dilution of that previously recorded), failed to detect antibodies to both A2 and T10; although these 2 serotypes were commonly isolated. I.H.A. antibody levels to serotype A7 were comparatively high in almost all the sheep except for sheep No. 10 from which A7 was isolated. These figures may indicate that when the serotype is isolated from the nasopharynx, the I.H.A. antibody levels are low, but when high, they prevent the serotype being established.

Gilmour et al (personal communication) found that when using gluteraldehyde treated ox red blood cells (Shrai et al 1975) the detectable level of antibodies by the I.H.A. test, were not reproduceable unlike those found using fresh Ox red blood cells. Gluteraldehyde treated cells were more adhesive and consequently more difficult to resuspend, following centrifuging.

Fresh Ox red blood cells were used throughout this series of I.H.A. tests.

It is of interest that the growth media and p.H. of the F.B.S. appeared critical. Nutrient broth No. 2 and brain heart infusion broth both proved satisfactory but tryptose phosphate broth was not so.

Linear correlation r values (Fig. 2) indicate that the I.H.A. antibody levels were random and did not show any deviation from the mean that was of statistical significance. However, the r values were significant in sheep 13 and 9 to serotype A1 which was never isolated, but the fluctuations in antibody levels over the 6 months were probable in the 5% that would occur by chance.

Values of r calculated for the F.A.T. titres (Fig. 2) show there is no statistical rise or fall over the 6 months period for any of the serotypes. Antibodies were detectable against all serotypes unlike the findings of the I.H.A. test. t values (Fig. 3) calculated show that the titres obtained by the F.A.T. test were significantly higher than those obtained by the I.H.A. test. This might indicate that the F.A.T. test shows greater sensitivity, but of course it may be detecting different antibodies. However, the F.A.T. test is unlikely to be as consistent as the I.H.A. test, due to the problem of selecting an end point which is a subjective decision varying from day to day. Moreover, the fluorescent

preparations could give different readings depending on the quality of fluorescent microscope used; in this case a very sophisticated one. The reading of the slides requires considerable care because when looking at a tightly gathered mass of organisms, they can appear positive, yet when focused on an individual P. haemolytica bacillus, it might be found negative.

Attempts to photograph the varying degrees of fluorescence failed, as the organism faded quickly, and the required exposure time was too long. A possible failing with the indirect F.A.T. test, is, that when P. haemolytica is grown on different medias, the composition of the extracellular substances alters (Wetzel and Collier, 1974). This could affect the brightness of the fluorescence. It is of interest that prepared antigen slides should be stored in a desicator at 4°C to prevent moisture damage.

If more facilities had been available at the time, marked changes in the temperature and humidity could have been made, possibly resulting in changes in the nasal population which in turn may have affected the F.A. and I.H.A. titres.

As far as serotyping is concerned, this could have been carried out using the quicker F.A.T., as a direct comparison with the normally used I.H.A. test.

In view of the poor correlation between nasal population and circulating antibodies, it would be of interest to study nasal antibodies eluted from nasal swabs (Smith et al 1975). Using the same serological tests, one might then determine whether there is a closer relationship with nasal population than was found studying the circulating antibodies.

CONCLUSION

With the tests used in this experiment, the humoral serological response is of no value in determining the serotypes of *P. haemolytica* present in the nasopharynx. There is a distinct statistical difference in the 2 serological tests, with the F.A.T. appearing to be more sensitive. A study may follow of sheep exposed to varying conditions of temperature and humidity to investigate the possible resultant change in nasal population and antibody levels.

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