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Pasteurellosis: An Outbreak Amongst Sheep.

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INTRODUCTION AND BRIEF SUMMARY OF LITERATURE.

CASES of pasteurellosis or infection with bipolar organisms in domestic animals and birds are frequently reported from different parts of South Africa; but, apart from the description of the disease in sheep given by Maybin (1931) and the account of Fowl Cholera by Henning and Coles (1933), the information furnished regarding the incidence of pasteurellosis in this country is unreliable and incomplete. According to some of the routine pathological specimens received at Onderstepoort, however, it would appear that pasteurellosis in sheep is far more widespread than is generally recognised. Maybin found the disease extremely prevalent in South-West Africa, where it affected goats as well as various breeds of sheep, the highest mortality occurring in Karakul sheep and their crosses; from the pathological specimens received at Onderstepoort it appears that in the Union Persian sheep are mostly affected, Merino sheep not having made any significant contribution.

In Europe haemorrhagic septicaemia of sheep was first described in 1889 (Galtier) and since then it has been recognised over wide areas of the continent. The relation, however, between European haemorrhagic septicaemia of sheep and the condition here described is not clear. Hutyra and Marek (1926) describe peracute, acute, subacute and chronic forms of the disease whereas in this outbreak these forms could not be differentiated—apart from one or two acute cases in the experimental animals only the chronic form with the development of pneumonia and pleuritis was seen.

Leyshon's (1932) description of " An Ovine Affection " coincides more closely with the condition recorded here. He observed cases of *pasteurella* infection in sheep where the animals lived from twelve hours to three or four days, and showed at autopsy pneumonia with numerous bipolar organisms present. His description agrees largely with Dungall's (1931) account of a contagious pneumonia of sheep occurring in Iceland between October and June; from some of these cases bipolar organisms pathogenic to sheep were isolated. Laikipia lung disease (Mettam, 1930) appears to be another form of this condition. Curasson and Didier (1932) record heavy mortality in sheep, especially amongst young animals, due to a *pasteurella* infection. It is interesting to note that these workers used a vaccine made from a formolised culture which was claimed to be effective in reducing mortality.

Numerous other workers including Miessner and Schern, Frohner und Zwick, Wiemann J., and others describe ovine pasteurella infections and allied conditions. A few years ago Schütze (1929) gave a comprehensive review of pasteurellosis.

History of the Present Outbreak.

In December 1931 the experimental farm of the University of Pretoria imported two Ryeland rams from England, and a year later five ewes of the same breed. The imported sheep were kept with a number of Merinos on the University farm. All were pastured under identical conditions in the same camps, fed from the same food and housed in the same buildings. During the winter of 1933 one of the rams (ram No. 1) became unthrifty and developed a short dry cough. It was treated for bronchitis and kept under observation. It improved slightly and served a few ewes, but in March, 1934, it suddenly developed acute tympanites associated with intense pulmonary distress from which it died within twenty-four hours. An autopsy revealed marked lesions of necrotic fibrinous pleuro-pneumonia, hydrothorax and hydropericardium, acute catarrhal gastro-entritis, tracheitis, pharyngitis and laryngitis, enlargement of the liver and spleen, and degenerative changes in the kidneys.

Cultures were made from the heart-blood and pneumonic areas. No growth resulted in the tubes inoculated with heart-blood, but several smooth whitish-grey, moist-looking, translucent colonies of different sizes appeared on the serum-agar slants inoculated with material from the lungs. A few single large colonies were picked, subcultured and studied. The organisms of the different colonies appeared to be much alike so that the growth obtained from only one colony was studied in detail. This culture was designated *pasteurella* 182 and is described below.

A week after the death of ram No. 1 the second ram (ram No. 2) also developed symptoms of acute pneumonia and died about fifteen hours after it was first noticed sick. An autopsy again revealed lesions of fibrinous pleuro-pneumonia, hydrothorax, hydropericardium, and inflammation of the pharynx, larynx and trachea. Both lungs were involved and there were fibrinous adhesions between the pulmonary and costal pleura. There was gastro-enteritis, degeneration of the kidneys, and enlargement of the spleen and liver. Cultures were again made from the heart blood and lung lesions and as in the case of ram No. 1 several greyish-white, moist, smooth, translucent colonies of different sizes developed on the media inoculated with the material from the lungs, but the tubes seeded with heart blood remained sterile. Morphologically the organisms from the different colonies were indistinguishable, and finally a culture obtained from one of the smaller colonies was kept and studied in detail, the others being discarded. This culture was called *pasteurella* 181 and is described below.

As a result of the death of both these animals the University was left without any male breeding stock and it was hoped that a purebred ram lamb (ram No. 3) the progeny of ram 2 now about five months old would soon be available for breeding purposes. As

the five Ryeland ewes and their progeny, including ram 3, did not thrive so well as the locally bred Merinos it was thought advisable to inoculate them with a vaccine made from culture 182, the vaccine used being a formolised saline emulsion of a twenty-four hours old serum-agar growth. All the sheep were inoculated once only. Soon afterwards all the ewes improved remarkably in condition, but ram 3, although showing a slight improvement, remained unthrifty. Early in August it suddenly became worse and developed acute pneumonia from which it died the following day. The most outstanding lesions found on autopsy were fibrinous pleuro-pneumonia, hydropericardium, hydrothorax, fatty degeneration of the liver and kidneys, gastro-enteritis and tumor splenis.

Cultures were made from the heart-blood, liver, spleen, lung lesions and pleuritic fluid. Numerous smooth translucent, shiny, greyish-white, moist colonies of varying sizes were observed on media inoculated with material from the lungs; the liver and pleuritic fluid also yielded growths. A number of the larger and some of the smaller colonies were picked, subcultured and studied. In morphology and staining characteristics the organisms from the large and small colonies were indistinguishable, but in virulence and bio-chemical reactions they showed noticeable differences. The organisms from the large colonies were apparently identical and a culture from only one (*pasteurella* 247) was kept for further study; great differences, however, were observed between the cultures obtained from two of the small colonies and between them and those obtained from the large colonies. The two small colonies studied yielded cultures 247a and 247c. The colonies found on the media seeded with liver were similar to the large colonies produced on the serum-agar by the lung material; the culture obtained from the pleuritic fluid became contaminated with *B. subtilis* and was discarded.

BACTERIOLOGY.

(1) Pasteurella 182.

Morphology and Staining Characteristics.

The organisms were generally small, ovoid and irregularly arranged in smears, sometimes pleomorphic, some being short bacillary (ovoid) in form while others were long and filamentous. They were non-motile. Bipolar staining was common and gave the bacilli a characteristic appearance, especially in very young cultures and in blood smears made from cases of septicaemia. In some cultures, however, the bipolar staining was not apparent. The organisms were Gram-negative.

Cultural Characteristics.

There was a moderate growth on most of the ordinary laboratory media. Freshly isolated cultures grew fairly well on nutrient agar, but after repeated subcultivation the growth became poorer and poorer. On serum-agar a much more abundant growth was obtained. Single colonies were raised, greyish-white, clear, translucent, moist, smooth, and spread peripherally during the course of a few days' growth. Fresh cultures could be washed off readily with saline,

but older ones became dull and viscous, and tended to adhere to the medium. Saline emulsions, even when made from fresh cultures, were generally flocculent. On solid media a maximum growth was usually obtained after two to three days incubation.

In broth a uniform turbidity was formed after twenty-four hours and a white, powdery, sometimes flocculent deposit collected at the bottom of the tube. The growth continued in broth for weeks so that in the course of a fortnight, or sooner, a thick flocculent deposit was found at the bottom, leaving the supernatant fluid almost clear. The deposit disintegrated with difficulty and it required a great deal of shaking before a uniform emulsion could be obtained. Sometimes a pellicle formed on the surface of the fluid. On blood agar there was no haemolysis and gelatin was not liquified.

Biochemical Reactions.

Acid without gas was formed in glucose, saccharose, mannite and maltose. Litmus milk was not altered, the methyl-red and Voges-Proskauer reactions were negative, while the methylene blue reductase and catalase tests were positive. Nitrates were reduced, ammonia and a small amount of hydrogen sulphide as well as indol were formed.

Virulence Tests.

(*a*) *For Guinea-pigs.*

Guinea-pigs 1 to 14 were inoculated as follows:—

Guinea-pigs.	Method.	Material.
1 and 2.....	Intraperitoneal	1/20 of a 24-hours old serum agar slant.
3 and 4.....	,,	1/200 of a 24-hours old serum agar slant.
5 and 6.....	,,	1/2000 of a 24-hours old serum agar slant.
7 and 8.....	,,	1/20000 of a 24-hours old serum agar slant.
9, 10, 11, 12.....	,,	1/200000 of a 24-hours old serum agar slant.
13 and 14.....	,,	0·05 c.c. of a 24-hours old broth culture.

Guinea-pigs 1, 2, 3, 4, 5, 6, 7, 13, and 14 were dead within twenty-four hours. Guinea-pigs 8 and 9 died after forty-eight hours, while 10, 11, and 12 survived for a week when they were reinoculated with the same dose of organisms as before. Within forty-eight hours all were dead. All the dead guinea-pigs showed lesions of severe fibrinous peritonitis, tumor splenis and enteritis, while some showed in addition hydrothorax and hydropericardium. *Pasteurella* in pure culture were obtained in media inoculated with heart-blood from guinea-pigs 5, 7, 8, 9, 10, 11, and 13. No cultures were made from the others.

Subsequently two more guinea-pigs were inoculated intraperitoneally with 1/200,000 of a twenty-four hours old serum-agar culture. Both died within forty-eight hours showing typical lesions of pasteurellosis and both yielded pure cultures of *pasteurella* in media inoculated with heart-blood.

(*b*) *For Rabbits.*

Rabbits 1 to 5 were inoculated as follows:—

Rabbits.	Method.	Material.
1.....	Intravenous.....	1 c.c. of a 24-hours old broth culture.
2.....	,, ...	2 c.c. of a 24-hours old broth culture.
3.....	,, ...	1/10 of a 24-hours old serum agar culture.
4.....	,, ...	1/100 of a 24-hours old serum agar culture.
5.....	,, ...	1/1000 of a 24-hours old serum agar culture.

Rabbit 3 died after a week, but *pasteurellae* could not be found in media inoculated with heart-blood and no outstanding lesions were revealed at autopsy. Two more rabbits were inoculated with 1/10 of a twenty-four hours old serum-agar culture; one of these died after four days, but again heart-blood cultures remained negative for *pasteurellae* and no lesions were observed. The other rabbits all remained healthy.

(*c*) *For Pigeons.*

Pigeons 1 and 2 were inoculated respectively with 1/10 and 1/100 of a twenty-four hours old serum-agar culture. Pigeon 1 died after forty-eight hours, but pigeon 2 survived. The inoculations were made intramuscularly.

(*d*) *For Sheep.*

Sheep 37285 was inoculated intrapulmonarily with 10 c.c. of a twenty-four hours old broth culture of *pasteurella* 182 (15.3.34). On March 16th and 17th it was listless with an elevated temperature (107·5° F.) and accelerated respiration. On March 18th it died and an autopsy revealed a necrotic fibrinous pleuro-pneumonia with hydrothorax and hydropericardium. Culture media seeded with heart-blood yielded a pure culture of *pasteurella.*

Sheep 28078 was injected intrapulmonarily (19.3.34) with 2 c.c. of a twenty-four hours old broth culture. After twelve hours the animal became listless, its temperature shot up to 108° F. and its breathing became rapid and distressed. The temperature suddenly dropped to 106° F. on the next day and then to 104° F. on the day after that. On the fourth day the temperature was 102° F. and on the sixth day the animal died. The most outstanding lesions presented were hyperaemia and oedema of the lungs, and hydropericardium. The carcase was fairly decomposed at the time of autopsy and no cultures were made.

Sheep 38880 was inoculated intrapulmonarily (25.3.34) with a 2·5 c.c. of a twenty-four hours old broth culture. On the eighth day the temperature rose to 106° F. remained at that level for two days and then dropped to 103° F. on the tenth day a few hours before the animal died. Symptoms of listlessness and distressed breathing were also manifested during the febrile stage. An autopsy revealed lesions of fibrinous pleuritis, slight pericarditis, icterus and gastro-enteritis. *Pasteurellae* were obtained from the media seeded with heart-blood.

Sheep 37744 was inoculated intravenously (15.3.34) with 10 c.c. of a twenty-four hours old broth culture. The temperature shot up to 108° F. within twelve hours and the animal was found dead on the following day, showing lesions of pulmonary congestion and oedema, and epicardial haemorrhages. A pure culture of *pasteurella* was obtained from the heart-blood.

Sheep 32606 was inoculated intravenously with 2 c.c. of a twenty-four hours old broth culture. On the fourth day the temperature suddenly shot up to 107° F. while the animal became listless and showed rapid distressed breathing. During the following two days the temperature dropped, reaching 103·2° F. a few hours before the sheep died on the sixth day. Cultures made from the heart-blood yielded *pasteurellae* and the chief lesions observed were fibrinous pleuro-pneumonia, atelectasis of both lungs and enteritis.

Sheep 33993 was inoculated intravenously (22.3.34) with a saline emulsion of half a twenty-four hours old serum-agar culture. On the following day the temperature was 105·6° F. and the breathing distressed. After the first day the temperature rapidly dropped and was 103° F. a few hours before the animal was killed in extremis on the fourth day. The only pathological changes observed at autopsy were atelectasis of the right lung, enteritis and enlargement of the liver.

Sheep 36988 was inoculated intravenously with 1/10 of a twenty-four hours old serum-agar culture. On the following day its temperature had risen to 106·5° F. and its breathing was very rapid; on the second day the temperature had dropped to 102° F. and after that it fluctuated for several days between 102° F. and 105° F. Finally the temperature remained regular and the animal recovered.

Sheep 34333 was inoculated intravenously with 1/50 of a saline emulsion of a twenty-four hours old serum-agar culture. No obvious disturbance of health was noticed at any time.

The lungs and spleen of ram 2 were passed through a Latapie mincer and the emulsion obtained was utilised as follows:—

Sheep 27240 was inoculated intravenously (14.3.34) with 10 c.c. of the Latapie emulsion. On the second day the temperature was 105·6° F. and then fluctuated between 105·5° F. and 104° F. until the tenth day when it rose to 106·4° F. and on the twelfth it was 106·8° F. After this the temperature gradually dropped to 103° F. and the animal died on the twenty-fifth day. The most outsanding lesions recorded were purulent pleuro-pneumonia, (there were fractures of ribs on the right side with laceration of the corresponding lung) hydropericardium, fatty degeneration of the liver, and kidneys, and atelectasis of the right lung.

Sheep 38914 was injected intrapulmonarily (14.3.34) with a 10 c.c. of the Latapie emulsion. The temperature rose to 106·5° F. within twenty-four hours and then dropped to 102° F. on the second day; on the thirteenth day the temperature again rose to 106·8° F. and dropped on the following day as before. The sheep finally recovered.

On March 16th *sheep* 37698 was inoculated intravenously with 10 c.c. of a Berkefeld filtrate of the Latapie emulsion. On the second and fifth days the temperature suddenly rose to 105° F. and subsided on the following days. The animal showed no further reaction and remained healthy.

On March 16th *sheep* 37417 was inoculated into the right lung with 10 c.c. of a Berkefeld filtrate of the Latapie emulsion. Apart from a moderate rise in the temperature the sheep remained healthy.

Sheep 37831, 37818, 37798 were kept as controls in close contact with the above experimental animals. All remained healthy.

Immunising Properties.

An atempt was made to immunise laboratory animals with either formolised or live cultures of *pasteurella* 182. Guinea-pigs were inoculated with formolised saline emulsions of serum-agar cultures or with formolised broth cultures and rabbits were injected with live emulsions.

Eighteen guinea-pigs were inoculated subcutaneously at weekly intervals with progressively increasing doses of a formolised forty-eight hours old broth culture. The first dose was 0·5 c.c. and the final dose was 5 c.c. inoculated on two sides of the body. Six injections in all were given.

Six guinea-pigs were repeatedly inoculated with progressively increasing doses of a formolised saline emulsion of serum-agar cultures. The density of the emulsion corresponded to the opacity of the nephalometer tube (Burroughs and Wellcome) indicating a concentration of *B. coli* 3×10^9 per c.c. The dose varied from 0·5 c.c. to 2 c.c.

Rabbits were inoculated with increasing doses of live cultures; 1/1,000, 1/100, and 1/10 of a twenty-four hours old serum-agar slant being injected intravenously at weekly intervals, commencing with the smallest dose.

A week after the last inoculation the guinea-pigs were tested for immunity. They were inoculated intraperitoneally with various dilutions of saline emulsions made from twenty-four hours old serum-agar cultures. Those injected with amounts in excess of 1/200,000 of a culture all died, showing *pasteurellae* in the heart-blood, while about 50 per cent. of the guinea-pigs injected with 1/200,000 of a culture survived.

On account of the poor immunity produced in the guinea-pigs, it was decided to ascertain the agglutinin titre of the serum of some of the animals which had received the injections. Accordingly, four of them were bled to death and the serum separated. An agglutination test was made with the homologous organisms commencing with a dilution of 1/10. The test was entirely negative.

The same test was performed with four of the rabbits injected with the live emulsions, but apart from an incomplete agglutination in a dilution of 1 : 50 in one case the tests were negative.

The results of other workers, Schütze (1929), Cornelius (1929) also seem to indicate that the antigenic properties of pasteurellas are generally very feeble and that they are unreliable for the production of sera of a reasonable titre.

The use of precipitating sera, as described by Yusef (1935), for the recognition of pasteurellas has not been tried.

Results.

Pasteurella 182 has been found to be highly pathogenic for guinea-pigs and sheep, but rabbits and pigeons were extremely resistant to infection and withstood doses of virulent culture that proved to be lethal for sheep. The organism apparently has a predilection for pulmonary tissue and serous membranes because the lungs and pleurae were almost invariably affected extensively even when the infection was made by the intravenous route. Affected lungs passed through the Latapie mincer produced a definite reaction in sheep when the injection was made either intravenously or intrapulmonarily. The animal inoculated by the former route died from fibrinous pleuro-pneumonia, while the sheep that received the emulsion by the latter route recovered. Berkefield filtrates of the Latapie emulsions were harmless for sheep.

The immunity produced in guinea-pigs by repeated injections of formolised cultures was negligible, and in both guinea-pigs and rabbits inoculated with dead and live cultures respectively, appreciable amounts of agglutinin could not be demonstrated.

(2) Pasteurella 181.

In morphology, and staining characteristics this organism could not be differentiated from *pasteurella* 182. Cultures of *pasteurella* 181 on serum-agar retained the moist, translucent appearance longer than the other organism, they became less adherent to the underlying medium and did not develop the dullness so early. The primary freshly isolated colonies were smaller than the single colonies of *pasteurella* 182, but on subculturing the colonies became larger.

Biochemical Reactions.

Fermentation of sugars was found to be irregular; generally acid but no gas was formed in glucose, saccharose, maltose, mannite, and inosite. Indol was not formed, nitrates were not reduced and the methyl-red and Voges-Proskaues tests were negative; but ammonia and hydrogen sulphide were formed. The methylene blue reductase and catalase tests were both positive, but litmus milk remained unaltered.

Virulence Tests.

(*a*) *For Rabbits.*—Rabbit 1 was inoculated intravenously with 1 c.c. of a twenty-four hours old broth culture and rabbit 2 was inoculated with a 2 c.c. of the same culture.

(*b*) *For Guinea-pigs.*—Guinea-pigs 1 to 5 were inoculated as follows:—

Guinea-pigs.	Method.	Material.
1.....	Intraperitoneal.....	0·05 c.c. of a 24-hours old broth culture.
2.....	,, ...	0·1 c.c. of a 24-hours old broth culture.
3.....	,, ...	0·25 c.c. of a 24-hours old broth culture.
4.....	,, ...	0·5 c.c. of a 24-hours old broth culture.
5.....	,, ...	1 c.c. of a 24-hours old broth culture.

After forty-eight hours guinea-pigs 4 and 5 were dead, *pasteurellae* being recovered from cultures made from the heart-blood. Both the rabbits and the other three guinea-pigs remained healthy. The test was repeated on guinea-pigs with saline emulsions of serum-agar cultures, but death resulted only in those animals that received very large doses—1/5 and 1/10 of an agar slant.

Results.

Pasteurella 181 was only very slightly pathogenic for guinea-pigs, and rabbits remained unaffected even by employing comparatively large doses of virulent culture. It differed from *pasteurella* 182 culturally, biochemically and in virulence.

(3) Pasteurella 247.

In morphology, cultural and staining characteristics this organism was found to be identical with *pasteurella* 182.

Biochemical Reactions.

Like strain 182 this organism formed acid but no gas in glucose, saccharose, mannite and maltose. It did not change litmus milk or liquify gelatin. It gave negative methyl red and Voges-Proskauer tests, but positive methylene blue reductase and catalase reactions. It reduced nitrates, formed ammonia, a small amount of hydrogen sulphide, and indol.

Virulence.

In virulence also this organism closely resembled pasteurella 182.

Virulence Tests.

(*a*) *For Guinea-pigs.*

Guinea-pigs 1 to 6 were inoculated as follows:—

Guinea-pigs.	Method.	Material.
1.....	Intraperitoneal.....	0·5 c.c. of a 24-hours old broth culture.
2.....	,, ...	0·1 c.c. of a 24-hours old broth culture.
3.....	,, ...	1/20,000 of a 24-hours old serum agar slant.
4.....	,, ...	1/20,000 of a 24-hours old serum agar slant.
5.....	,, ...	1/200,000 of a 24-hours old serum agar slant.
6.....	,, ...	1/200,000 of a 24-hours old serum agar slant.

After forty-eight guinea-pigs 1, 2, 3, 4, 5, were dead, showing lesions of hydrothorax, hydropericardium and intense fibrinous peritonitis; *pasteurellae* were obtained in pure culture from the heart-blood of the dead animals. Guinea-pig 6 survived. Later four more guinea-pigs were inoculated each with 1/200,000 of a twenty-fuor hours old serum-agar slant; of these three died.

(*b*) *For Rabbits.*

Rabbit 1 was inoculated intravenously with 1/10, rabbit 2 with 1/100, and rabbit 3 with 1/1,000 of a twenty-four hours old serum-agar slant. After forty-eight hours rabbit 1 was dead, but no *pasteurellae* were found in media inoculated with heart-blood and no outstanding lesions were detected in the carcase. Two more rabbits were inoculated each with 1/10 of a twenty-fours hours old serum-agar slant and the result was the same as before. Rabbits 2 and 3 survived.

(*c*) *For Pigeons.*

Pigeon 1 was inoculated intramuscularly with 1/10 and pigeon 2 with 1/100 of a twenty-four hours old serum-agar slant. After forty-eight hours pigeon 1 was dead, but pigeon 2 survived.

(*d*) *For Sheep.*

Sheep 40531 was inoculated intravenously (8.10.34) with half the emulsion from a twenty-four hours old serum-agar slant of *pasteurella* 247. On the following day the temperature was very high (106·8° F.) and the animal showed distressed and rapid breathing. The symptoms persisted for five days when the animal died. An autopsy revealed intense fibrinous pleuritis, marked oedema and congestion of the lungs, hydrothorax, hydropericardium, fatty degeneration and bile pigmentation of the liver and enteritis. Cultures were made from the heart-blood, liver, pleuritic fluid and lungs, from all of which *pasteurellae* were obtained.

Sheep 40444 was inoculated intravenously (8.10.34) with 1/10 of a twenty-four hours old serum-agar slant. Within twenty-four hours the temperature rose to 107·7° F. but dropped to normal on the following day; on November 2nd and 9th the temperature again rose to 105° F. After this the temperature dropped and the animal recovered.

Sheep 40994 was injected intratracheally (17.10.34) with 1/10 of a serum-agar slant; the same dose was repeated after a week. The sheep remained apparently healthy.

Sheep 41034 was injected intratracheally (17.10.34) with one-half of a twenty-four hours old serum-agar slant, the same dose being repeated after a week. A certain amount of the first dose was expelled through the nose while the animal was being drenched. The day following the second injection the temperature rose to 106° F. and then gradually subsided for a week until it reached 102° F. After this the temperature rose periodically to 105° F. but the animal recovered.

Sheep 40488 was drenched (15.10.34) with 20 c.c. and sheep 40538 with 450 c.c. of a forty-eight hours old broth culture of *pasteurella* 247. Both animals remained apparently well.

Sheep 40414 was drenched (12.10.34) with about 100 c.c. of an emulsion made from the lungs of sheep 40531 (which died from an intravenous injection of *pasteurella* 247). On the tenth day its temperature rose to 107° F. and then suddely dropped to 102° F. on the following day; on the twenty-second day the temperature rose to 105·4° F. and again dropped to 102° F. within twenty-four hours; on the thirty-first day there was another rise to 105° F., after which the temperature gradually dropped to 101·8° F. and no further reaction occured.

Sheep 40777 was drenched on (12.10.34) with about 100 c.c. pleural fluid from sheep 40531 (which died from an intravenous injection of *pasteurella* 247). On the thirteenth day the temperature suddenly rose to 107° F. and then as suddenly dropped to 103° F. on the following day. After this the animal remained healthy.

Results.

Pasteurella 247 was shown to be pathogenic for guinea-pigs and sheep, both rabbits and pigeons being remarkably resistant to infection. In guinea-pigs infection was very readily set up by intraperitoneal inoculations, while in sheep death was produced by the intravenous route. Although a fatal infection did not result from an intratracheal injection of virulent material, sheep so injected showed a severe thermal reaction. When virulent cultures were given *per os* no reaction was observed, but when organ material from an experimentally infected sheep was given to two sheep by the same route a definite thermal reaction was set up in both cases.

The organism apparently has a predilection for pulmonary tissue and serous membranes as the most outstanding lesions developed in in the lungs and pleurae, even when virulent material was given intravenously.

(4) Pasteurella 247 (*a*).

In morphology and staining characteristics *pasteurella* 247 (*a*) was indistinguishable from *pasteurella* 247 but differed from it culturally, biochemically, and in virulence. In these respects it resembled *pasteurella* 181. Primary colonies of this organism on serum-agar were much smaller than those of *pasteurella* 247. After subculturing single colonies became much larger.

Biochemical Reactions.

Pasteurella 247 (*a*) formed acid but no gas in glucose, saccharose, mannite, maltose, and inosite. It did not change litmus milk, the methyl-red and Voges-Proskauer tests were negative; indol was not formed and nitrates were not reduced; ammonia and hydrogen sulphide were formed in small quantities; the methylene blue reductase and catalase reactions were positive.

Virulence Tests.

Guinea-pig 1 was inoculated with 0·1 c.c. of a twenty-four hours old broth culture, guinea-pig 2 was injected with 0·25 c.c. and guinea-pig 3 with 0·5 c.c. Guinea-pig 3 died after forty-eight hours, but Nos. 1 and 2 remained healthy. The test was repeated with three more guinea-pigs with similar results. *Pasteurellae* were recovered from the heart-blood of the dead animals.

These results indicated that *pasteurella* 247 (*a*) resembled *pasteurella* 181 not only in morphology, cultural and staining characteristics, but also in biochemical reactions and virulence. It cannot be regarded as identical with *pasteurella* 247 and 182.

(5) Pasteurella 247 (*c*).

In morphology and staining characteristics *pasteurella* 247 (*c*) was also very much like pasteurella 247, but differed from it culturally, biochemically and in virulence.

Although this organism also grew best on serum-agar it grew only moderately well in broth and poorly on nutrient agar; but the colonies were very much smaller than those of *pasteurella* 247 and a much weaker growth was obtained on laboratory media, including serum-agar.

Biochemical Reactions.

No change occured in litmus milk, indol was not formed, the Voges-Proskaues and methyl-red tests were negative, nitrates were very slightly reduced, and ammonia was formed in very small quantities, the methylene blue reductase test was weakly positive and the catalase test was negative. Sugars were not fermented at all. The growth on sugars and on the various media used for biochemical tests was very poor, a fact which possibly explained the absence or mildness of reactions.

Virulence Tests.

Guinea-pig 1 was injected intraperitoneally with 0·5 c.c. of a twenty-four hours old broth culture and guinea-pig 2 with 1·0 c.c. of the same culture. Both animals remained healthy.

These results show that a third and entirely different type of *pasteurella* was obtained from the cultures made from the lungs of ram 3. It differed from strains 247, 247(a), 182, and181, and it was the least pathogenic of all the strains isolated and studied.

Clinical Observations.

These are confined to the three natural cases seen in the Ryeland rams and the cases of pasteurellosis produced in sheep by the injection of strains 182 and 247 of the organism. Of the three natural cases seen one was noticed to be sick for only twenty-four hours before death occured. The other two showed symptoms of pneumonia for periods of two to three weeks before succumbing. Individual experimental sheep varied clinically to a large extent apparently

depending upon the amount of material and the method used. For example, sheep 37744 received 10 c.c. of a broth culture on the afternoon of 15.3.34 and died on the next day, the disease assuming a septicaemic form. On the other hand, sheep 27240 received 10 c.c. of a lung emulsion from one of the early cases on 14.3.34 and died from pleuro-pneumonia on 9.4.35 nearly a month after the injection.

In the animals that died from the septicaemic or acute form of the disease symptoms observed consisted of severe respiratory distress with accelerated breathing and pulse rates and marked hyperthermia. Within twenty-four hours the animals were dead. A number of the experimental sheep, however, as well as two of the natural cases showed considerable similarity in clinical symptoms. The high febrile reaction occuring a few days after the injection was characteristic and it was always associated with accelerated respiration and pulse rate; the breathing in addition becoming distressed. After reaching its highest point usually between 107° and 108° F. the temperature always dropped to about 104° F. shortly before death.

Sheep 32606 which received 2 c.c. of a broth culture intravenously on 19.3.34 may be taken as well representative of the usual case seen. The temperature remained between 102° and 104° F. up till the morning of 22.3.34, when it commenced to rise and by the afternoon of the 23rd it had reached 107° F. Then it dropped almost as suddenly as it had risen and was 104° F. on the afternoon of 24.3.34 shortly before the animal's death on the 25th. On the 23rd the animal remained down with a greatly accelerated pulse and respiration rates, and had a slight bilateral muco-purulent nasal discharge. In the animals which recovered an intermittent fever was maintained for periods of up to three or four weeks. The temperature fluctuated between 103° F. and 108° F. eventually reaching a normal level. During the period of fever the pulse and respiration rates were increased but dropped again as the fever decreased.

Whilst it would be unwise to make a diagnosis of pasteurellosis upon clinical grounds alone the symptoms seen can be usefully correlated with *post-mortem* appearances and laboratory examinations.

PATHOLOGICAL ANATOMICAL CHANCES.

These are confined to the *post-mortem* appearances seen in sheep; both in the natural cases from the University farm and in the cases artificially produced with strains 247 and 182 of the organism and ending fatally. They may be roughly classed with those seen in sheep that died from the septicaemic or acute form i.e. within one or two days and those seen in animals which were sick for a week or more.

Changes in the animals which died from the acute or septicaemic form were limited to severe hyperaemia and often oedema of the lungs with enlargement of the spleen. The latter change was never very severe, the largest spleen seen measuring $10 \times 7{\cdot}5 \times 2$ cms. Hydrothorax and hydropericard were usually seen, and in some instances there was a hyperaemia of the mucous membrane of the

gastro-intestinal tract. Slight fatty degeneration of the liver and kidneys was noticed in these cases. No emaciation was seen except in the case of the third Ryeland ram which was losing condition for some months before it developed acute pneumonia and died suddenly.

In the animals which died after a more lengthy course of the disease, much more significant and severe lesions were noted at autopsy. They usually showed some degree of emaciation; those that had been ill for some time were often very poor in condition. In one sheep (27240) two ribs on the right side were fractured but this was apparently the result of a traumatic injury. The changes which may be classed as occurring almost invariably were those in the respiratory tract. There was frequently a muco-purulent discharge from the nostrils and in many cases a severe hyperaemia of the pharynx, larynx and trachea. The lungs and pleural membranes were the seat of severe damage in almost every animal, either fibrinous pleuro-pneumonia or purulent broncho-pneumonia being present. Usually the lung was found partially deflated and heavier than normal. It was firm to the touch and of a mottled bluish-red or light red colour. The pleural membranes, both costal and pulmonary were frequently covered with a fibrinous yellow deposit up to 2 cms. in thickness and the lung was often attached by fibrinous material to the costal wall over varying areas. A large amount of turbid yellow or red fluid was usually present in the thoracic and, to a lesser extent, the pericardial cavity.

On sectioning the lung, which cut like a solid organ such as liver, the cut surface varied in colour from a light pink to a dark red, often with lighter coloured yellow areas. A reddish turbid fluid could be expressed and was present in the bronchioli. Frequently light coloured areas of soft necrotic tissue were seen. The whole of the lung tissue was never hepatised—the parts usually affected being the anterior and inferior parts of both lungs—and the junction between the normal and healthy tissue was always clearly demarcated. In some of the cases parts of the lungs were atelectatic, being completely collapsed and dark red in colour.

Changes in the other organs were limited to fatty degeneration of varying severity in the kidneys and liver and occasionally a slight enlargement of the spleen. The gastro-intestinal tract sometimes showed hyperaemia in the mucosa.

HISTO-PATHOLOGICAL CHANGES.

In every case that terminated fatally specimens of internal organs were collected and fixed in formalin; sections were cut by either the freezing or the paraffin embedding method. Organ smears were also made and stained with Giesma. The stains used in the sections were Haematoxylineosin, van Giesen, Sudan III, and Giemsa.

The sheep that died from the acute form of the disease showed very few histological changes: the blood content of the spleen was increased and the lymphoid follicles enlarged. Bipolar organism were demonstrated without difficulty in spleen smears from these cases.

In the lungs hyperaemia and oedema was observed. The alveoli were filled with clear serous material and the capillaries were distended with blood.

In animals that lived a week or more after the infection characteristic changes developed in the respiratory organs. In the lungs a constant feature was the severe hyperaemia present. Every capillary blood vessel was packed with blood cells. In almost every case areas of consolidation were present. The alveoli in these areas were filled with a mass of fibrinous material, neutrophiles and red blood corpuscles. The fibrin was in the form of a network of very fine strands in which the infiltrating cells and red corpuscles were enmeshed. Large phagocytic cells (macrophages) were also present. The fibrin, rather difficult to identify with the Haematoxylin-eosin stain was very clearly shown up by Wiegert's method (Fuchsin, Methylviolet and Lugol's iodine). In these cases of fibrinous pneumonia in sections cut by the paraffin method and stained with Giemsa numerous bacteria were present amongst which bipolar staining organisms could be clearly identified. Necrotic areas were seen in which all cell structure had disappeared and which showed up as light pink staining (Haematoxylin-eosin) homogeneous structureless foci surrounded by a zone of neutrophiles.

Cases were seen in which as well as a fibrinous pneumonia, areas of purulent broncho-pneumonia were also present. Here the alveoli contained no fibrin but were blocked with a mass of neutrophiles and large phagocytic cells. In some of the sheep this type of pneumonia only was present, and no bipolar organisms were seen, although numerous cocci and short bacillary organisms could be demonstrated.

In some sheep the pneumonic changes were accompanied by a fibrinous pleuritis. In sections the pleural membrane of both the lung and the costal wall was roughened and thickened and covered with a dense layer of fibrin which again appeared on a network of fibrils enclosing here and there red blood corpuscles and a few neutrophiles. In the sections stained with Giemsa bipolar organisms, as well as other bactera, could be demonstrated fairly easily. In smears made from the consolidated parts of the lungs numerous bacteria were present including frequent bipolars.

The histological changes in the lung can then be summarised as including acute and sub-acute fibrinous and purulent broncho--pneumonia, frequently with areas of necrosis and fibrinous pleuritis.

In other organs such as liver and spleen a hyperaemia was occasionally noted, whilst in the liver and kidneys fatty degeneration to a greater or lesser extent was usually present.

DISCUSSION.

A virulent form of pasteurellosis in Ryeland sheep at the experimental farm of the University of Pretoria was investigated. Of the three natural cases studied, two had been suffering from a chronic pulmonary infection for several weeks before they died while the third (ram 3) was not noticed sick for more than twenty-four hours before death. Ryeland ewes kept under identical conditions

and in close contact with the rams did not suffer in the same way. It is true that at one time these ewes were unthrifty and that they improved in condition after they had been inoculated with a formolised emulsion of *pasteurella* 182, but there is no proof that they suffered from pasteurellosis or that the improvement could be attributed to the inoculation; ram 3 which received the same treatment finally succumbed to a pulmonary disease from which an organism (*pasteurella* 247) identical with *pasteurella* 182 was obtained.

Primary cultures made from the lungs of ram 1 produced a growth containing several colonies of different sizes; one of the largest of these was picked and subcultured for a detailed study. It yielded culture 182 which was composed of small ovoid (short bacillary), bipolar-staining, Gram-negative organisms corresponding to the description of *pasteurellae* given by Topley and Wilson (1929). This organism proved to be highly pathogenic for guinea-pigs and sheep, but apparently non-pathogenic for rabbits and pigeons; it was found to have a predilection for pulmonary tissue and serous membranes; when a live culture was injected intravenously into sheep pathological changes developed in the lungs, pleurae and pericardium in preference to other parts of the body. In the three natural cases studied the lungs, pleurae and pericardium were affected most extensively. Moreover, the lesions presented by the experimentally produced cases closely resembled those observed in the natural cases.

Repeated inoculations of formolised cultures of *pasteurella* 182 did not appreciably increase the resistance of guinea-pigs to infection with virulent live cultures and the serum of the "immunised" guinea-pigs was devoid of any agglutinin content. Likewise no agglutinins could be demonstrated in the serum of rabbits inoculated with live cultures.

On serum-agar media seeded with material from the diseased lungs of the second ram several colonies of different sizes developed; one of the smaller colonies was picked, subcultured and studied. The organisms of this culture (*pasteurella* 181) were also ovoid, bipolar-staining and Gram-negative; but although morphologically indistinguishable from *pasteurella* 182, it differed from it culturally, in virulence and in biochemical reactions, the most striking of which was its inability to form indol and to reduce nitrates. For guinea-pigs it was barely pathogenic, very large doses being necessary for the production disease.

On account of the similarity of the pathological changes presented by these two rams and the apparent similarity of the primary growths on serum-agar seeded with pulmonary material from rams 1 and 2 it is suggested that each of the primary cultures contained at least two different types of colonies, one of them yielding virulent organisms corresponding to *pasteurella* 182 while the other gave rise to non-virulent bacteria like *pasteurella* 181. It is further suggested that the small colonies of the primary growths contained the non-virulent organisms while the large ones yielded virulent cultures. The bacteriology of the cultures obtained by seeding pulmonary material from ram 3 on serum-agar slants supports this view.

That the diseased tissues of naturally infected animals can set up a condition in sheep which is like the naturally occuring pasteurellosis is borne out by the effect of Latapie emulsions of the affected tissues of ram 2 on experimental sheep. Moreover, the fact that a severe fibrinous pleuro-pneumonia was produced in one animal suggests that the lungs contained a pathogenic organism as well as the slightly pathogenic pasteurella 181.

When ram 3 died from a condition in which the symptoms and pathological changes were apparently identical with those recorded in ram 1 and 2 another opportunity was presented for studying the bacteriology and pathology of the disease.

Serum-agar media were seeded with material from the lungs, liver and pleural cavities. The tubes inoculated with pulmonary material yielded several moist, greyish-white, smooth, translucent colonies of different sizes scattered over the surface of the media. A number of the different colonies, small and large, were picked and subcultured. The organisms obtained from the large colonies were found to be alike and a culture obtained from only one, *pasteurella* 247, was kept; the colonies that appeared on the media inoculated with liver were of the large variety and resembled *pasteurella* 247. The tubes seeded with pleuritic fluid also yielded a growth but as it was contaminated with *B. subtilis* it was discarded.

Culture 247 was found to be identical with strain 182, not only in morphology, cultural and staining characteristics, but also in virulence and biochemical reactions. Like *pasteurella* 182 it formed indol and reduced nitrates, and was also highly pathogenic for guinea-pigs and sheep, but not for rabbits and pigeons. An intravenous inoculation of sheep with this organism produced lesions which were not only similar to those presented by natural cases (rams 1, 2, and 3) but also to those observed in sheep infected experimentally with *pasteurella* 182.

An attempt was also made to set up an infection either by intratracheal injections or by dosing of live cultures. As a result of the intratracheal injections a marked thermal reaction was set up, but the oral administration, even of large quantities (450 c.c. broth culture) of live cultures had no apparent effect on the sheep.

Several of the small colonies scattered over the serum-agar media seeded with lung material from ram 3 were picked. The cultures (247a and 247c) obtained from two of these were finally kept and studied. The organisms of the one were found to differ from those of the other, and the bacilli of both cultures differed from those obtained from the large colonies (*pasteurella* 247).

Pasteurella 247a was noticed to resemble *pasteurella* 181 not only in morphology, cultural and staining characteristics, but also in virulence and biochemical reactions. Like 181 it did not produce indol or reduce nitrates and it fermented the same sugars; it was also found to be very slightly pathogenic for guinea-pigs, comparatively large doses being necessary to cause death in the inoculated animals.

Pasteurella 247c yielded a much poorer growth than either 247a or 181. It differed from both of these culturally, in biochemical reaction and in virulence. For guinea-pigs it was non-pathogenic, even in very large doses.

That virulent and avirulent forms of *pasteurellae* can be recovered from the same organ of an animal suffering from pasteurellosis has been proved.

SUMMARY AND CONCLUSIONS.

From the three cases of pasteurellosis in sheep studied, five different strains of *pasteurellae* were obtained, 182 from ram 1, 181 from ram 2, and 247, 247a, and 247c from ram 3.

Of these strains 182 and 247 were highly pathogenic for both sheep and guinea-pigs, and almost non-pathogenic for rabbits and pigeons. Both showed the same biochemical reactions and both produced similar pathological changes in experimental animals inoculated with live cultures. They resembled each other also in morphology, staining and cultural characteristics. *Pasteurellae* 182 and 247 can therefore be regarded either as identical or so closely related that they cannot be differentiated by the methods employed. Both these organs have a predilection for pulmonary tissue and serous membranes and both produced lesions in experimental animals that could not be differentiated from those found in natural cases studied. These two organisms are considered to have been the cause of the mortality among the Ryeland sheep at the experimental farm of the University of Pretoria. An identical disease in experimental sheep was produced by the injection of organ emulsions and cultures made from the original cases from the University farm. Berkeveld filtrates of organ emulsions from natural cases did not produce the disease.

So far no success has yet been attained with immunisation tests in laboratory animals and no properly controlled immunisation experiments have been carried out with sheep.

The pathogenesis of the disease under natural conditions is still obscure.

Pasteurellae 181 and 247a cannot be distinguished from each other by the tests employed; both are very slightly pathogenic for guinea-pigs and both have the same biochemical reactions, and they agree in morphology, cultural and staining characteristics. Both have originated from small colonies picked from primary cultures of pulmonary material.

Pasteurella 247c does not resemble either of the two groups of organisms mentioned above. It is entirely non-pathogenic for laboratory animals.

These results indicate—

(1) that the small colonies picked from the primary growths on media seeded with material from affected lungs yielded cultures which were either non-pathogenic (247c) or only very slightly pathogenic (247a, 181);

(2) that the large colonies obtained from similar growths gave rise to highly pathogenic cultures (182 and 247);

(3) that when several colonies were picked from the same primary growth, highly pathogenic, slightly pathogenic and non-pathogenic cultures may be obtained e.g. cultures 247, 247a, and 247c);

(4) that if only one colony is picked from the primary growth either a highly pathogenic culture (182) or one which is barely pathogenic (181) may result.

It is possible that the non-pathogenic and slightly pathogenic *pasteurellae* occur as saprophytes in the respiratory passages of sheep in certain areas and that they invade the lungs only when the way has been paved for them by the entrance of pathogenic *pasteurellae* of the type 182 and 247. These latter enter the tissues and set up disease under conditions which have not yet been determined.

In making a bacteriological study of a case of pasteurellosis, therefore, it is recommended that several colonies of different sizes be picked from the primary growth, and that the pathogenicity of each one be studied separately. Only in this way may the presence of pathogenic *pasteurellae* be determined.

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