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A Note on Strains of Tuberculosis from the Cape Kudu.

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PAINE AND MARTINAGLIA (1928), and some years later Thornburn and Thomas (1940), described the occurrence of tuberculosis in the Cape Kudu. A great deal of very interesting information about the disease is given in these articles and in the latter the interesting suggestion is made that the primary location of the lesions in the subparotid region may possibly be due to infection of the ears resulting from the animal scratching them with its hind feet, which may have been infected from the soil. An interesting feature of the lesions in the kudu is the tendency to the formation of a soft creamy pus, rather than the caseous type of material usually seen in bovine tuberculosis. A further interesting feature was the occurrence of actinophytic colonies resembling those seen in actinomycosis.

Pullinger (1941) in an article on the nature of swellings in the subparotid region in cattle, seen by him during large scale inspection of dairies supplying the Johannesburg area, describes the cases as being associated either with tuberculosis, actinomycosis or corynebacterium infections. He found these abscesses to be fairly common, but was unable to subscribe to the idea that injury to the ear formed the starting point for the tuberculosis cases. He suggested that infection through the conjunctiva might be a route by which the organisms reached the subparotid region.

The purpose of the present article is merely to record the isolation and typing of a number of strains of *Mycobacterium tuberculosis* obtained from specimens from kudu shot in the Albany district of the Cape Province between 1939 and 1941. They were forwarded by Mr. Thorburn, at that time Government Veterinary Officer at Grahamstown. A few transmission experiments carried out with these strains in cattle and goats are also recorded.

TYPING OF STRAINS.

Six strains of *M. tuberculosis* were isolated from material sent from the Albany district, one in 1939 and five during 1941, all from different animals. The lesions sent in for examination were in lymphatic glands in every case, and the pus in them was of a thick creamy type without any evidence of calcareous material. Acid fast bacteria were always found, usually in very small numbers, but in spite of an extensive search, no actinophytic granules were ever seen. All the strains were isolated by making cultures from guineapigs which had been inoculated with the tubercular material.

Of these six strains, five developed a moist type of growth on Dorset's egg medium and the growth was never profuse. The sixth one produced a rather dry crumbly type of growth and grew better than the others. On Griffith's litmus-glycerine-potato medium the growth of all the strains was very poor, which suggested the bovine type.

On 23.1.42, two of these strains, Kudu I and 3021, were used for typing the organism by inoculation of rabbits and guineapigs. The standard doses used were those recommended by Griffith, i.e., 10 mg. subcutaneously for rabbits and 1 mg. for guineapigs. The animals were all killed six weeks later. With both strains the guineapigs showed extensive generalized tuberculosis. All four rabbits showed extensive tuberculosis of the lungs, the organs being studded throughout with discrete nodules. The other organs showed very little, but in the kidneys in one case there were a few small caseous centres and in the other there were numerous small foci in the liver.

Judging from the lesions, a diagnosis of tuberculosis of the bovine type would be justified, but it was decided to inoculate guineapigs and rabbits with two of the other strains which were available.

Two rabbits and two guineapigs were inoculated with strains Kudu II and 4679, the rabbits were given 10 mg. each subcutaneously and the guineapigs 1 mg. When killed six weeks later the lesions were very similar to those seen in the previous inoculation. The guineapigs again showed very extensive generalized tuberculosis and the lesions in the rabbits were mainly confined to the lungs, which showed very pronounced lesions.

As a result of the inoculation experiments with these four strains of the tubercle bacillus from kudus, one would be justified in classing them as definitely of the *bovine* type.

Transmission Experiments.

A few experiments were carried out on the transmission of kudu tuberculosis to cattle and goats. The underlying idea was to imitate as far as possible the probable transmission method in the kudu, where the disease very probably starts from infection of scratches in the ears. The latter animals were chosen as being probably the domesticated animal species most closely related to the larger antelopes. Two goats, 59727 and 59768, were treated on 19.4.41 with pus from a lesion in a kudu. Goat 59768 received subcutaneously 1 c.c. of an emulsion of the pus in saline on the inside of the right ear. In goat 59727 the pus was mixed with sand and rubbed into a scarified patch on the inside of the right ear.

No lesions developed during the next five months, so the inoculation was repeated on 8.9.41. The goats had not given tuberculin reactions at any time during the interval and one may presume the first inoculation failed to infect. Following the second inoculation, goat 59727 developed a nodular thickening with blister formation on the inside of the ear, followed by swelling of the subparotid gland on the right side. This gradually developed during the next three months, but subsequently began to decline in size again. Goat 59768 developed a permanent hard thickening of the right ear without any definite nodule formation. The right subparotid gland developed an appreciable enlargement during the three months following the inoculation, but the swelling was never as large as in the other goat. Both goats reacted positively to the intradermal test on 21.1.42.

Goat 59727 was killed on 27.2.42. At post mortem numerous hard caseous nodules were found in the skin at the scarification site in the ear. The right subparotid gland was enlarged and contained yellow caseous centres in which the material was slightly calcareous. Yellow calcareous centres were present in the right gland. There were numerous yellow calcareous lesions in the bronchial glands, and both lungs showed numerous small caseous nodules. Acid fast bacilli were found in the lesions.

Goat 59768 was killed on 9.4.42. It showed tubercular lesions in the conchus of the ear and in the subparotid, pharyngeal, prescapular, bronchial and mediastinal lymphatic glands. Both lungs showed cavernous tubercular lesions. Lesions of tuberculosis were present in the liver and spleen and in the periportal and mesenteric lymphatic glands. In both cases there was well marked generalization and it is probable that it would have become much more extensive if the animals had been allowed to live.

In cattle, three experiments were undertaken with kudu material. In the first one, two head of cattle, 7302 and 7505, were used. In one case, 7302, infected pus from a case of kudu tuberculosis was mixed with sand and rubbed into scarified patches on the inside of the left ear. In the other the material was inoculated under the skin of the inside of the ear. Both were negative to the tuberculin test before inoculation. Five months later 7302 showed a well-defined swelling in the left subparotid region but 7505 showed no changes. Both animals reacted positively to the tuberculin test, showing very marked reactions.

In a test carried out on 1.4.40, seven months after inoculation, good reactions were again obtained. The description of the reactions is as follows:—

	1/4/40.		3/4/40.		4/4/40.	
	Mamm.	*Avian.*	*Mamm.*	*Avian.*	*Mamm.*	*Avian.*
7302	8 mm.		50 mm.	20 mm.	About 50 mm.	23 mm.
7505	11 mm.		21 mm.	15 mm.	15 mm.	18 mm.

The swellings in the case of 7302 were very large, diffuse, painful and oedematous. A test with avian tuberculin was carried out at the same time and a definite positive reaction was obtained as well. One may consider it as being of the nature of a group reaction.

In 7302 the swelling under the ear had by this time become much larger and was 90 mm. in width, hard and painless. In 7505 no obvious swelling of the subparotid region could be seen. Figure 1 shows the reactions obtained in the case of 7302 with the two tuberculins. The mammalian is so large that it is difficult to see the margin, but the avian is well defined.

The two animals were kept under observation and on 1.2.41 7302 died suddenly, apparently from a severe enteritis. At post mortem the lesions of tuberculosis found were caseous abscessation of the left parotid gland, both bronchial and mediastinal glands and most of those on the left side of the neck. A caseous granulomatous area, about the size of a pigeon's egg, was found in the left lung. In the lesion in the affected glands faint stellate bodies like actinophytic granules with club-shaped rays were seen.

The other animal, 7505, was slaughtered on 6.7.40. Lesions of tuberculosis in the form of small caseous centres were found in the subparotid gland, which was enlarged. No other lesions were seen.

In both these cases, therefore, tuberculosis developed but no marked generalization had taken place.

This experiment was repeated with two more cattle on 8.9.41, numbers 7013 and 7285. Material was obtained from a case in a kudu and was mixed with sand and rubbed into a scarified place on the inside of the left ear of 7013. An emulsion of the pus was inoculated subcutaneously under the skin of the left ear in 7285.

These animals were both negative to the tuberculin test at the time they were put into the experiment. Both animals commenced to show a hard swelling in the left subparotid region about three months after inoculation. The swelling was more marked in 7013 but subsequently receded very considerably. In 7285 the swelling became gradually larger until after about 18 months, as it was losing condition and breathing with difficulty, the animal was killed.

At post mortem 7013, which was killed on 5.3.43, showed tubercular lesions in the external ear and in the subparotid, retropharyngeal and bronchial lymphatic glands. The lesions in the subparotid contained fluid creamy pus with caseous particles. There were several tubercular foci in both lungs in the vicinity of the hilus. No other lesions were seen.

Bovine 7285 was killed on 4.6.43 and showed advanced tubercular lesions. The subparotid, retropharyngeal, cervical, prescapular, bronchial mediastinal and periportal lymphatic glands showed extensive caseation and calcification. The pleura and peritoneum showed multiple granulomata (grapes) and both lungs showed multiple tubercular foci. Both animals were tested with tuberculin at intervals and always gave positive reactions. Number 7285 seemed to be highly sensitized, as up to the time of slaughter it always gave very large local reactions, at least 40 to 50 mm. or even larger in diameter, hot, painful, and oedematous.

A heifer, 7936, was inoculated subcutaneously on 8.9.41 with 2 c.c. of an emulsion of a three-weeks-old culture of a kudu strain on Dorset's egg. Three months later, although it had not shown any symptoms in the interval, it suddenly developed an acute dyspnoea and died within 24 hours. At post mortem lesions of miliary tuberculosis were found in every organ, but in particular the lungs, which were crowded with very small yellowish caseous nodules.

The experiments carried out with goats and cattle confirm the conclusion arrived at from the small animal experiments, that the tubercle bacillus from the kudu is of the bovine type.

During the time these experiments were being undertaken, two cattle were sent up from one of the farms where kudu tuberculosis occurred. These animals, 8628 and 8629, both showed very marked swellings in the subparotid region (see figures 2 and 3). The animals were not retained for experimental work but were killed.

Ox 8628 was killed on 14.6.40. The lesions were as follows: Advanced swelling and tubercular lymphadenitis in the right subparotid gland, less extensive lesions in the left. Lesions in the retropharyngeal and bronchial glands.

Ox 8629 was killed on 17.5.40. The lesions were: Swelling of the right subparotid gland with caseous tubercular lesions. Multiple tubercles in the lungs with tubercular bronchitis. Caseation and calcification of the bronchial, mediastinal and mesenteric lymphatic glands.

CONCLUSIONS.

Several strains of *M. tuberculosis* isolated from cases of tuberculosis in the kudu were shown to be bovine in type, as judged by their growth characteristics and the results of inoculation of laboratory animals, cattle and goats.

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Fig. 1.—Reactions to tuberculin in bovine 7302. Note swelling under the ear. The avian reaction A is behind the mammalian one M on the neck.

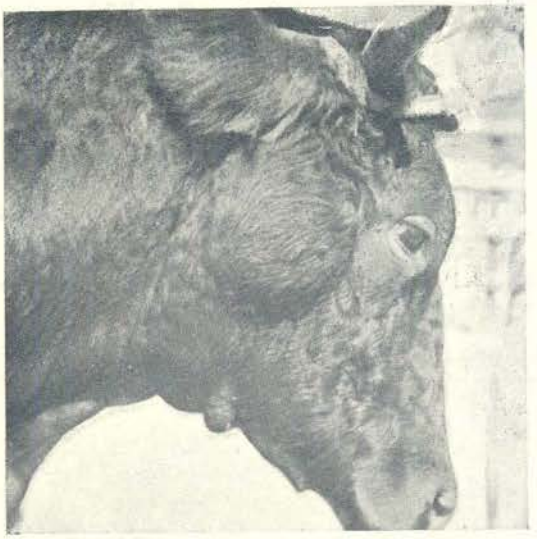

Fig. 2.—Ox 8629 showing subparotid swelling.

Fig. 3.—Ox 8628 showing subparotid swelling.