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PORCINE MYCOBACTERIAL LYMPHADENITIS

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SUMMARY

In a survey of 368 tuberculous lesions in porcine lymph nodes collected from four abattoirs, nonchromogenic mycobacteria were isolated from 228 lesions (65%) and *M. tuberculosis* from 21 (5.7%). The lesions were indistinguishable from those caused by nonchromogenic mycobacteria.

Nonchromogenic, mainly *M. avium*-like mycobacteria were isolated from pig feed and the environment of pigs i.e. bedding and dust from piggeries where tuberculous lesions in the lymph nodes of pigs frequently occurred Seventy per cent of feed samples and 80% of dust and bedding samples yielded nonchromogenic *M. avium*-like mycobacteria.

Nonchromogenic Group III mycobacteria from pigs, feed, environment, man, cattle and fowls were compared with regard to morphology, physiology, biochemistrw and pathogenicity. The close resemblance of *M. intracellulare* to *M. avium* was demonstrated. Of the total of 85 environmental strains which were fully typed, none were *M. avium*, 34 were *M. intracellulare*, 45 belonged to the *M. terrae-M. gastri*-V subgroup saprophytes and 6 were non-classifiable.

It is concluded that nonchromogenic mycobacteria occurring freely in nature are responsible for a very high percentage of tuberculous lesions in the lymph nodes of pigs; this survey revealed a 94% incidence.

INTRODUCTION

The incidence of tuberculous lesions in lymph nodes of pigs showed a marked in crease in 1967 and 1968. Partial and total condemnation of pigs at abattoirs caused farmers considerable losses.

It is well known that different species of mycobacteria can cause tuberculous lymphadenitis in pigs. The bacilli enter through the tonsils and walls of the intestines, and are arrested in the regional lymph nodes. Generally only an incomplete primary complex forms. Lymphogenic spread occurs particularly with *M. bovis* and sometimes with *M. tuberculosis*, but *M. avium* is of low virulence to pigs. Neither macroscopic nor histological examination is of assistance in the differentation of the lesions produced by the various species of mycobacteria.

All three classical types of tubercle bacilli and atypical mycobacteria must be considered as possible sources of infection for man. In South Africa pulmonary tuberculosis in man is endemic and tuberculosis of cattle is frequently found in dairy cattle, while tuberculosis of domestic fowls occurs very rarely. Contact between pigs and fowls and between pigs and cattle occurs rarely, especially in modern piggeries (where most of the tuberculous lesions were found). On epizootiological grounds it is therefore unlikely that fowls or cattle can be incriminated as a source of tubercle bacilli for pigs. The atypical mycobacteria were differentiated by Runyon [1] into four groups:

Group I Photochromogens —
slow growers, of orange pigmentation when grown in the light but non-pigmented when grown in the dark.

Group II Scotochromogens —
slow growers, which are always pigmented whether grown in the dark or exposed to light.

Group III Non-chromogens —
slow growers, usually smooth colonies, non-pigmented even after exposure to light. Yellow pigmented variants are encountered but these are unaffected by light.

Group IV —
Rapidly growing mycobacteria, mature colonies at room temperature within one

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week, usually non-pigmented, but rarely chromogenic.

PREVIOUS SOUTH AFRICAN INVESTIGATIONS

In 1950 Fourie *et al*[2] described an outbreak of tuberculosis in pigs caused by the human strain. Robinson[3] typed 49 strains of mycobacteria isolated from pigs in 1954 and 30 were definitely *M. bovis*. In 1954-6 Robinson[4] studied the pathogenicity of *M. tuberculosis, M. bovis* and *M. avium* in pigs, and produced generalized lesions by dosing pigs with 1 or 3 mg of cultures of *M. bovis* and also with 1 mg intra-nasally, but it was not possible to limit these lesions to the lymph nodes of the head. Lesions caused by the human type were limited to lymph nodes after administering 10 mg per os, and no lesions resulted after 3 mg (2 pigs each) and 5 mg of *M. tuberculosis* culture *per os*.

More recently identification of mycobacteria isolated from pigs by the Tuberculosis Section of the Veterinary Research Institute showed that the majority of lymph node lesions were caused by mycobacteria other than *M. tuberculosis* or *M. bovis*. Kleeberg[5] examined 55 specimens in 1957/58 and found mycobacteria in 82%. Further identification revealed that 50% of the strains were mycobacteria of the avian group, 20% were *M. tuberculosis* and 30% *M. bovis*. In 1960 Van Rensburg *et al*[6] undertook a special survey at the Estcourt bacon factory, and detected tuberculous lesions in 7.66% of 13 000 pig carcasses. No attempts at isolation of the organisms were made, the significance of the study being that these figures were seven times as high as abattoir figures for the country as a whole at that time. During 1961-65 Worthington[7] isolated 54 strains and found 35 to be of the avian group, nine *M. bovis* and ten *M. tuberculosis*. At the same time, Stottmeier *et al*[8] and Kleeberg[9] made a study of atypical mycobacteria isolated from man and cattle respectively. Four strains of the *M. avium*-like *M. intracellulare* were found in man, and nine such strains plus one strain of *M. avium* in cattle.

When wild birds were blamed as a source of carriers of the *M. avium*-like mycobacteria, Worthington[7] examined 35 such birds caught in and around piggeries where the tuberculosis problem had arisen. No mycobacteria could be isolated from their faeces but the livers of eight birds harboured a few mycobacteria. Only one strain of *M. intracellulare* was isolated, the others being saprophytes.

In 1963/64 Worthington and Kleeberg[10] compared 14 *M. avium*-like strains isolated from pigs with nine strains isolated from tuberculous fowls. Using the nitrate, niacin, urease and catalase tests, cultural characteristics, drug sensitivity pattern, specific sensitin reactions and pathogenicity tests in guinea pigs, rabbits, fowls and pigeons, they identified three porcine strains and all fowl strains as true *M. avium* and the other 11 porcine strains as *M. intracellulare*. There was no difference in their ability to ferment eight different organic salts. All fowl and porcine strains grew at 42° and 44°C. There was no difference in colonial morphology, and in relation of large to small colonies (about 1:250) in both types. All strains were resistant to isoniazid, PAS and streptomycin. Tuberculins were prepared from two true *M. avium* and two typical porcine strains, and three guinea pigs each were sensitized with 12 different porcine strains and a number of different avian strains. Specificity differences and sensitivity profiles proved that there were no antigenic differences between the porcine strains *(M. intracellulare)* and the avian strains *(M. avium)* as judged by sensitin specificity methods. *M. avium* strains from pigs were recognisable by their high virulence in fowls causing death within 8 weeks after intravenous (i.v.) injection of 0.01 mg of bacterial weight.

Ten pigs were infected i.v. with 0.5 mg wet bacterial weight of 10 different strains, one *M. tuberculosis* isolate from a baboon serving as control, three *M. avium* strains from fowls and six strains from pigs. The baboon strain caused miliary tuberculosis of the lungs. The *M. avium* strains and three of the porcine strains caused mainly hepatic and splenic lesions, but these could have been overlooked as only a *tumour splenis* and a slightly enlarged pale liver was seen, and in two cases also small tubercles. Histologically there was a diffuse infiltration of epithelioid and giant cells, and many acid-fast organisms were visible. The other three porcine strains caused no lesions and only one could be re-isolated.

The authors then attempted to transmit the disease *per os*. Five mg of six different porcine strains were given daily for 4—6 days and suspensions were squirted into the back of the throat. Small amounts of the suspensions were probably inhaled. No visible lesions were produced in three pigs, le-

sions in bronchial lymph nodes were produced in two and in mesenteric lymph nodes in two pigs, but one of those strains transpired to be *M. avium.*

A further experiment was carried out on six pigs; three being given 15 mg of an avirulent strain daily for 4 days and three the same amount of a virulent strain. The pigs were slaughtered after two months. The avirulent strain caused no visible lesions and cultures were negative. One of the other three pigs was also negative and two showed numerous minute dull yellow foci in the mesenteric lymph nodes which would probably have been overlooked at routine meat inspection. It was therefore possible to cause tuberculous lesions in pigs by feeding *M. intracellulare* strains isolated from pigs. It was noticed that, regardless of the host, strains of *M. avium* and *M. intracellulare* caused disease mainly in the liver and spleen when infected i.v., whereas mammalian strains caused miliary tuberculosis of the lungs in pigs and rabbits.

OVERSEAS LITERATURE

Countries which have eradicated bovine tuberculosis and are successfully controlling human tuberculosis are still encountering many tuberculous lesions in pigs. More recent studies, which include identification of the mycobacteria, show the majority of these lesions to be caused by *M. avium* or the avian-Battey sub-group.

When bovine tuberculosis was still prevalent in West Germany in 1956 Schulze [11] isolated 44 strains of tubercle bacilli from 54 pigs and found *M. bovis* in 38 (86%), and *M. avium* in 6 samples (14%). Fluorescence microscopy was positive in 48%, and light microscopy in 18% of lesions.

In 1962 Schliesser [12] investigated 70 strains which were isolated from mesenteric lymph nodes of 100 pigs; he found three *M. bovis* strains, 64 *M. avium* or *M. avium*-like strains, two *M. fortuitum* and one *M. smegmatis.* He considered those strains which are culturally and biochemically identical to *M. avium* as belonging to the species *M. avium,* regardless of possessing low or no virulence for fowls.

In 1963 Nassal *et al.* [13] bacteriologically examined 552 suspicious lesions and 92 obviously tuberculous lesions in the lymph nodes of pigs. From suspicious lesions they cultured 333 strains, of which 93.7% were *M. avium,* 6.6% were *M. bovis,* and 1.5% were *M. tuberculosis.* From the obvious tuberculous lesions they isolated 60 strains; 88.3% were *M. avium,* 6.7% were *M. bovis,* and3.3% were *M. tuberculosis.* They regarded other bacteria like corynebacteria, *Escherichia coli* and cocci as secondary invaders. Histological confirmation was possible in 95%. In 1965 Nassal [14] reported on an investigation of 103 slaughter pigs with isolated lymphadenitis. Mycobacteria were found in 76% of 79 lymph nodes; 92% were *M. avium.* In four cases he isolated *M. avium* from the muscles of such pigs.

Janetschke [15] in East Germany reported on a survey of 14 588 pigs of which 7% showed tuberculous lesions in lymph nodes; 97.3% of these were the result of peroral infections and 2.7% were found in bronchial lymph nodes. No bacteriological work was done.

In 1964 Kauker and Zettl [16] studied a large piggery where lymph node lesions occurred frequently and isolated mycobacteria of the avian group from the sawdust used as bedding. They produced lesions in the lymph nodes of five young pigs caused by feeding the sawdust. They maintained that *M. avium* infection in pigs caused clinical symptoms with fever which could lead to generalization and death, but the avian-Battey type mycobacteria could not cause generalization.

Hellmann [17] isolated mycobacteria from 27 pigs, 22 being *M. avium,* four *M. bovis* and one *M. tuberculosis.* He found that lesions caused by *M. avium* could not be identified easily by their pathology or by microscopy. He found *M. avium* in ten and mammalian mycobacteria in five of 15 imported pigs. The majority of his *M. avium* strains grew at 22°C.

In 1967 Seeger *et al.* [18] investigated 333 pigs with lesions in the mesenteric lymph nodes and 100 pigs without lesions. From the former he isolated mycobacteria from 216 animals (64.7%), and from the 100 lymph nodes showing no lesions he isolated 3 atypical Group II mycobacteria. His typing revealed 93% *M. avium,* 2.7% *M. bovis* and 4.2% atypicals; of the latter two strains were Group III.

Bergmann and Götze [19] studied 188 tuberculous lymph node lesions and cultured 130 lesions. One hundred and twelve strains were isolated and typing showed 104 to be *M. avium,* seven *M. bovis* and one *M. tuberculosis.*

Stoll and Siam [20] examined 225 head and mesenteric lymph nodes bacteriologically; 80.8% were positive on microscopy and 71.1%

positive on culture. Of their 160 strains 74.3% were *M. avium* and another 8.7% were *M. avium* mixed with atypicals, six (3.75%) were *M. bovis*, and three (1.8%) were *M. tuberculosis*. Another 18 atypical strains were found. They admit, however, that they did not test the pathogenicity of *M. avium* when contact between pigs and fowls had been established. In two cases with no contact with fowls the strains were non-pathogenic in fowls but biochemically resembled *M. avium*.

In another German study of 9 514 pigs in 1961 by Westphal *et al.*[21] 73 were found to have lesions in the lymph nodes. Fifty-eight of these were confirmed to be tuberculous by microscopy or histology. Thirty-three strains of mycobacteria were isolated and all but one were found to be *M. avium*. They believe that atypicals and corynebacteria could not be blamed for these lesions.

In Austria, Baumann and his co-workers described avian-Battey type mycobacteria in 1955[22, 23, 24] and 1956[25]. They first maintained that infection with these mycobacteria, called *M. suis*, could be distinguished histologically from infection with mammalian mycobacteria. They later found the pathogenicity of *M. suis* in six different mammalian species to be very low. They claimed that their strains were distinct from the other known types of mycobacteria. These were later named *M. avium* var. *suis*, but were probably *M. intracellulare*.

Baumann[26] later tried to infect pigs *per os* and caused reactions to avian tuberculin but in only one piglet with a previous erysipelas infection was caseation of a lymph node and pneumonia produced.

Elian *et al.*[27] in 1953 studied 3 143 pigs slaughtered in Cairo. Tuberculous lesions were seen in the submaxillary lymph nodes of 57 and in the lungs of five. Mycobacteria were recovered from the 57 lymph nodes, 60% being *M. tuberculosis*, 24% *M. bovis* and 16% *M. avium*.

In Australia, Kovacs[28] in 1962 examined lymph node material from 117 pigs and found 28% positive for acid-fast bacteria indistinguishable from the nonchromogenic Battey type. Tammemagi and Simmons[29] in 1968 reported tuberculous lymph node lesions in 65% of 77 pigs at the Brisbane abattoir. Of the 1 768 pigs from one piggery 67% had visible lymph node lesions, especially of the mesenteric nodes. Three Battey type mycobacteria were isolated.

In Michigan Mallman *et al.*[30] in 1962 isolated approximately 100 strains of Group III mycobacteria from swine with and without gross lymph node lesions. These isolates produced disease in pigs after intradermal inoculation. In a survey of swine lesions from a Detroit abattoir 85% of the mycobacterial isolates were *M. avium* and the rest were nonchromogenic mycobacteria.

Scammon *et al.*[31] in 1963 isolated 43 strains of *M. avium*-like mycobacteria from lymph nodes of pigs from the Los Angeles abattoirs. These closely resembled true *M. avium* and Battey organisms except for the degree of virulence. Mallmann *et al.*[32] in 1964 reported on 177 strains of Group III mycobacteria of which 93 were from pigs, 39 from skin lesions of cattle, 28 from cattle lymph nodes and 17 from soil and feed.

In Scotland, Roberts and Hamilton[33] in 1968 examined tuberculous submaxillary lymph nodes of pigs, and found 42% of 50 cases positive on culture. They were presumed to be *M. avium*.

From Finland, Vasenius[34] in 1965 reported the examination of 1 254 porcine lymph node lesions. *M. tuberculosis* and *M. bovis* were found in 0.6%, atypical mycobacteria in 9% and *C. equi* in 17%. Microscopically 27% of the lesions and 39% of the cultures were positive, 6.7% being *M. tuberculosis* and *M. bovis*.

In Czechoslavakia Hejlicek[35] in 1966 and Popluhár *et al.*[36] examined lymph node lesions ond organs from 160 pigs and isolated 65 strains i.e. 41%, 46 being *M. avium*, 15 were *M. bovis* and four were mixed infections of *M. avium*, *M. bovis* and saprophytic mycobacteria. In Prague Jirina[37] in 1966 examined 61 216 pigs and found 363 with tuberculous lymph node lesions. *M. avium* was isolated from 10 cases.

In France, Lafont and Lafont[38] in 1962, in a study of 159 porcine lymph nodes, isolated 54 strains of *M. bovis*, five of *M. tuberculosis*, 18 of *M. avium* and 35 of atypical mycobacteria.

MATERIALS

A total of 368 lesions resembling tuberculosis in lymph nodes of pigs slaughtered at the abattoirs of Estcourt, Durban, Heidelberg and Vereeniging were examined. The tuberculous nature of the lesions could be confirmed in all cases by taking into account their size, colour and consistency and the presence of caseation or calcification. Lymph

node material was chosen at random at the abattoirs and put into sterile glass bottles. In the case of material from Durban, Estcourt and Vereeniging a detergent (Desogen) was added to prevent contamination and decomposition during transport. All samples including fresh pathological material from Heidelberg were received in good condition. They were in transit for 12—96 hours and were refrigerated on arrival until used. Most specimens were accompanied by full particulars.

The pigs originated from places as far apart as Port Elizabeth, Paulpietersburg, Durban and Rustenburg.

Twenty-eight samples of pig feed consisting of the ready mixed feeds or their components, were collected on six farms where tuberculous lesions had become a problem. All specimens originated from the Transvaal, mostly around Pretoria. Seventeen feed samples were taken at the Veterinary Research Institute, Onderstepoort, which purchases most of its feed. An additional seven samples of cattle feed were taken from two farms where sensitization of cattle to avian tuberculin had occurred for a number of years. Eight samples of wood shavings intended as bedding in pig sties were collected, as well as some dust inside piggeries, soil given as a feed supplement, dry grass, lawn cuttings, and lucerne either freshly cut or dried and baled or milled. These samples were collected in plastic bags.

The nonchromogenic mycobacteria isolated were compared with the following strains from the Onderstepoort collection:—

24 strains from pigs, 16 from cattle, (lymph nodes and lung tissue) 10 from domestic fowls and 13 from sputum of man with pulmonary mycobacteriosis or tuberculosis.

The comparative study was limited to mycobacteria of the nonchromogenic, slow-growing Group III of Runyon [1]. These strains had been maintained on egg medium at -20° C.

METHODS

A. Isolation of Mycobacteria

Aseptic techniques were followed throughout the study, and all cultures were incubated for 8 weeks at 37°C in the dark unless otherwise specified. The following isolation methods were used to recover mycobacteria from the different materials:—

Decontamination and Culturing of Porcine Lesions: The lymph nodes were trimmed of the surrounding tissue and fat, dipped into methylated spirit and flamed to kill the contaminating surface bacteria. A portion or all of the lesions was then removed and ground in a mortar or tissue grinder. Decontamination was usually effected by using 2% HCl, but for samples which were not so well preserved 3% HCl was used. The material was left in the acid for 15 minutes, and after centrifugation for 10 minutes the sediment was poured off, 5 ml distilled water added and the contents shaken vigorously. After recentrifugation for 10 minutes the sediment was used for inoculation.

A loopful of decontaminated material served as the inoculum for the following media — two Löwenstein-Jensen slants (L.J.), two Herrold's medium slants with mycobactin (H.M.), and one Kirchner-Hermann medium (K.H.). The Löwenstein-Jensen medium was that of the International Union Against Tuberculosis, and the Kirchner-Hermann and the Herrold's media were prepared according to the Laboratory Manual of Tuberculosis Methods [39].

Decontamination of Plant Material, Soil, Dust and Wood Shavings:

(a) Five grams of plant material was placed in a homogenizer and about 20 ml of 0.01% Tween 30 in distilled water was added. The material was homogenized using a Buehler homogenizer for 1 minute at 15 000 rpm. The mycobacteria adhering to the material were brought into suspension by the surface-wetting action of Tween and the very vigorous agitation. This is due to Tween being absorbed on the lipid portion of the mycobacteria surface by means of its long aliphatic molecular chain. This ethylene oxide chain forms a hydrophilic layer around the bacterium, thus keeping it in a uniform state of suspension in the aqueous Tween solution [40].

The supernatant was initially decanted directly into a centrifuge tube. Later, however, it was filtered through a very porous filter paper disc in order to remove the bulky floating plant debris which is troublesome due to swelling when in contact with NaOH. Thereafter the paper-filtered suspension was filtered through a fine grade absorbent cottonwool plug in order to remove fungal hyphal elements and spores. As plant material and soil are heavily contaminated with all

other groups of micro-organisms, the problem was to select decontamination conditions and culture media so that the mycobacteria were selectively isolated. Mycobacteria grow very slowly, even on a good medium, while fungal and bacterial contaminants grow very much faster.

After five minutes of cotton wool filtration the suspension was centrifuged, the sediment divided into three parts and each treated as follows:—

(i) with 3.75% HCl for 25—30 minutes (ii) with 4% NaOH for 25—30 minutes (iii) with pancreatin-Desogen for 3 or 5 hours.

The method using pancreatin-Desogen (p.D.) was as modified by Stottmeier and Kleeberg [41]. The procedure employs pancreatin in a 0.25% concentration and the surface active quarternary ammonium compound Desogen in a 1.5% concentration in phosphate buffer pH 8.0, and the material is exposed for 5 hours at room temperature.

Cultures were prepared by transferring two drops of the mixture onto the medium which was left horizontal for one day. This allowed of equal distribution and fixation of the bacteria onto the surface of the egg medium.

(b) In the second procedure, according to Beerwerth [42], 5 g of plant material was placed in a homogenizer treated with about 50 ml 4% NaOH and homogenized for one minute. After 10 minutes the supenatant for 15 minutes. The supernatant was decanted and centrifuged at 3 000 rpm. and the sediment treated with a 20-fold amount of 5% oxalic acid, mixing well. After 15 minutes the tube was recentrifuged for 15 minutes and the supernatant discarded. The sediment was used as the inoculum.

Because of the possible toxicity of oxalic acid to mycobacteria, Beerwerth's method was modified in that the sediment was washed with 5 ml distilled water and collected by repeated centrifugation.

The four types of material obtained by the various procedures were each inoculated in triplicate on the two types of solid media using a total of 24 media bottles per plant sample.

All the mycobacterial colonies were described with regard to their morphology, growth rate, pigmentation, size and number. Smears were made from each representative colony type, stained by the Ziehl-Neelsen method and examined for acid fastness and bacterial morphology.

Biological Tests: Material from each porcine lesion was injected into one albino guinea pig weighing about 500 g and bred in the tuberculosis free Onderstepoort colony. The inoculum (1ml) was injected subcutaneously in the right groin. The animals were fed on balanced dry rations, green lucerne and water *ad lib* and were sacrificed and autopsied six weeks later.

Gross pathology was evaluated by Mitchison's index (1:100) [43]. Tuberculous and suspicious lesions were removed for culturing, decontaminated with 2% HCl and handled as previously described.

Microscopy: From the cut surface of each lesion a smear was prepared, stained according to Ziehl-Neelsen and examined for a minimum of five minutes for acid-fast bacilli.

B. Identification of Mycobacteria

Colonial Morphology: A mature colony of known acid-fast bacteria was suspended in a Thomas tissue grinder tube containing approximately 1 ml *0.01%* Tween *80* solution and further diluted until a uniform 1 mg/ml bacterial suspension was produced by comparison with a 1 mg/ml standard tube [39]. Hundred-fold serial dilutions of 10^{-3}, 10^{-5} and 10^{-7} were prepared by transferring a loopful bacterial suspension into a pipetted 1 ml aliquot of *0.01%* Tween *80* solution. Four drops of each of the bacterial suspensions were inoculated onto L.J. media in Petri dishes and evenly distributed. The dishes were sealed and placed in plastic bags. When mature individual colonies had developed they were examined under the stereoscopic microscope for shape, size, colour, ratio of big to small colony variants and emulsifiability.

Temperature Range, Growth Rate and Pigmentation: Mycobacterial suspensions were inoculated onto various media and incubated at different temperatures. One L.J., one N.A. and one (H.M.) culture was incubated at 22°C and another set at 37°C. One L.J. culture was incubated in a slanted position in continuous artificial light from a 25 watt bulb. All other cultures were incubated in the dark, including one L.J. each at 42°C, 45°C and 52°C. The cultures were checked at weekly intervals and strains showing good growth after five days were regarded as rapid growers and strains taking longer as slow growers. The

colonies were differentiated into the four groups of Runyon [1].

The following biochemical properties were examined.

Catalase Production: Catalase activity was tested by adding a few drops of H_2O_2 — Tween 80 reagent [39] directly to the L.J. slant of the culture at room temperature. The enzyme catalase liberates free oxygen which is seen as very small bubbles. The heat-stable forms of catalase (68°C for 20 minutes) were tested according to Kubica and Pool [44] modified by using 1 ml of a 1 mg/ml bacterial suspension in Sörensen phosphate buffer pH 7.0.

Tween Hydrolysis: This test was done according to Kubica and Dye [45]. It determines the presence or absence of lipase which hydrolyzes Tween 80 (a polyoxyethylene derivative of sorbitan mono-oleate) with the formation of free oleic acid. Neutral red is used as indicator. The tubes were observed daily for 21 days and compared visually with an uninoculated control tube of substrate.

Niacin Production: The formation of niacin was determined by the aniline-BrCN method [39] which is a modification of the niacin test of Konno [46]. The presence of niacin is indicated by the immediate production of a complex yellow-coloured compound in the König reaction according to which a pyridine compound reacts with cyanogen bromide and primary or secondary amines to produce coloured compounds. Only mature colonies were used, and the cultures were grown on well aerated L.J.

Arylsulphatase Production: The test used was essentially that of Kubica and Vestal [47], with a modification [48]. In this modification the substrate tripotassium phenolphthalein disulphate is used in two concentrations viz. a 0.001 M solution (tested after three days) and a 0.003 M solution (tested after 14 days) in a Dubos Broth Tween- albumin medium, which is tested with a 2N Na_2CO_3 solution for colour development at the stipulated periods. This test determines the presence or absence of the enzyme arylsulphatase which splits the bond between the sulphate group and the aromatic ring in the substrate by hydrolysis, thus liberating phenolphthalein which gives a red colour with alkaline sodium solutions. The intensity of the colour varies with the amount of enzyme produced viz. faintly pink to deep red.

Nitrate Reduction: This test was done according to a modification of Virtanen's method [48]. The amount of nitrate reduced to nitrite by nitrate reductase is determined by visual observation of colour change. The nitrite formed undergoes a diazotization reaction with sulphanilic acid which subsequently forms a pink-red coloured compound with the coupling agent N- (1-naphtyl)-thylenediamine dihydrochloride in acid medium.

Amidase Activity: This test is based on the original method of Bönicke [49]. The actual method followed was that of Juhlin's modification [50] but employed only the ten amides in the Bönicke series. In this test the presence of deaminating enzymes acting on the C - N linkages in certain amides is determined by testing for the NH_3 liberated by means of the phenolhypochlorite reagent which gives a blue compound with ammonia.

Pathogenicity Tests in Animals: A representative number of different strains were selected and injected into various species of animals. Fowls and rabbits were used most often but mice, guinea pigs and in some cases pigs, goats and sheep were also used. Albino mice weighing 10 g each were used in groups of eight. Fowls of the Leghorn breed were 10 weeks old when injected. Rabbits were adult and weighed an åverage of 3 kg. Young pigs of an average weight of 80 lb were used. Goats and sheep used were young adults weighing 30—40 kg.

The inoculum was prepared from L.J. cultures 3—4 weeks old, suspended in saline in a tissue grinder and standardized to contain 1 mg/ml. All species except guinea pigs were injected intravenously. When other routes are used the early onset of acquired immunity can interfere with the testing of pathogenicity and virulence. Mice were given 0.3 ml of the original suspension. The inocula for fowls were 0.01, 0.1 and 1.0 mg wet bacterial weight, and for rabbits 0.1 and 1.0 mg bacteria. Pigs were injected with 0.5 mg bacteria, or 1.0 mg for larger pigs. Goats and sheep were given a 1 mg inoculum into the jugular vein. The route for guinea pigs was varied, one animal was injected intramuscularly, one subcutaneously and one intraperitoneally. A 0.1 mg inoculum was used for true tubercle bacilli and 1 mg for atypical mycobacteria. Mice were kept for a period of four weeks and all other species for six weeks before being sacrificed.

Gross pathology was described and samples for culturing taken from suspicious ma-

terial. The lungs of small animals were examined under the stereomicroscope, and in mice the density of the lungs was determined according to Crowle [51].

Tissues removed for culture were dipped into alcohol, flamed and then decontaminated with 2% HCl for 30 minutes, washed and inoculated onto L.J. media.

The following tissues were used for reculturing: mice — lung; fowls — liver and spleen; rabbits — lung, liver, spleen and kidney; guinea pigs — necrotic or abscess material at the site of injection, regional lymph nodes, liver and spleen; pigs, goats and sheep — lung, liver, spleen, kidney, muscle (psoas minor) and either the bronchial or mediastinal lymph node, being the most likely location of mycobacteria which had been arrested in the lung and then transported to the regional lymph nodes. Smears were made of suspicious lesions and stained for acid-fast bacilli.

RESULTS

A. Animal Feed and Environment

The majority of feed mash samples contained a moderate number of non-chromogenic mycobacteria i.e. 1—25 colonies could be grown. Only one sample was negative and two were highly contaminated (30—250 colonies counted). Of the seven lucerne samples, either freshly cut or dried and milled, three were highly and four moderately contaminated. Three of five batches of fishmeal tested were negative but one contained a large number of Group III mycobacteria. Of four cuttings of dried or fresh grass, two were highly positive, one low positive and one negative. Of eight samples of wood shavings only one was negative and four contained large numbers of non-chromogenic mycobacteria. The other four highly positive samples consisted of soil collected outside a piggery and used as a trace element supplement for piglets, dust collected inside a pigsty, milled Sorghum tops, and meal made from maize cobs. It will be noticed that, except for the fishmeal, all highly contaminated materials were either dust, mixed with dust or continuously exposed to dust.

In Tables 1 and 2 results are set out according to farms. Both in animal feed and environment the number of non-chromogenic mycobacteria of Group III greatly exceeds that of pigmented mycobacteria of Group II. Almost no rapid growers (Group IV) were found, and none of Group I (*M. kansasii*).

In Table 1 it is shown that 70% of feed samples were positive for mycobacteria, the average number of non-chromogenic colonies per sample was 18 and the highest 250. The average number of pigmented colonies was 5 per sample and the highest count was 200. Typing has not yet been finalised.

Table 1: MYCOBACTERIA IN ANIMAL FEED

Samples of mash rations, mixed concentrate, lucerne, etc.

* = One sample contained 200 colonies.

Locality	Total No. Samples	No. Positive	Non-chromogenic colonies	Pigmented colonies
Halfway House	8	2	48	0
Bon Accord	5	5	59	7
Silverton	3	3	21	2
Pietersburg	5	4	56	0
Onderstepoort	17	11	211	224*
Garsfontein	4	4	254	9
Devon	3	3	51	17
Pyramid	3	3	8	0
Heidelberg	4	3	27	11
	52	38	735	270

Although fewer samples of bedding were examined (Table 2) the total colony count exceeded that for feed samples. The average number of colonies per environmental sample was 59 and the highest count was 300. Incubation of two samples of woodshavings at 37°C for a period of two weeks resulted in a 15-fold increase in colony count. Pigmented colonies were found at an average of three per sample and a maximum of 15. About 80% of environmental samples were positive for mycobacteria of Runyon's Group III.

Table 2: MYCOBACTERIA IN ANIMAL ENVIRONMENT

Samples of bedding i.e. wood shavings, dry grass, dust and soil.

Locality	Total No. Samples	No. Positive	Non-chromogenic colonies	Pigmented colonies
Halfway House	5	4	89	5
Halfway House*	1	1	38	0
Bon Accord	1	1	1	0
Silverton	2	1	9	0
Silverton**	1	1	107	15
Pietersburg	2	2	377	3
Onderstepoort	1	1	300	1
Pyramid	1	1	23	1
Heidelberg	2	2	183	14
	16	14	1127	39

* = Dust. ** = Soil.

Table 3: MAMMALIAN TUBERCLE BACILLI IN PORCINE LESIONS

	Total	Durban	Estcourt	Heidelberg	Vereeniging
No. of lesions studied	371	59	198	78	36
Positive for acid-fast bacilli (microscopy)	105 28%	16 27%	27 14%	43 55%	19 53%
Atypical mycobacteria (culture)	199 54%	27 46%	105 53%	45 58%	22 61%
Live tubercle bacilli:—					
a. on culture	16	5	3	2	6
b. in guinea pigs	21	7	5	3	6

B. Tuberculous Lesions in Pigs

The results of the microscopical, cultural and biological examination of 368 lymph nodes are given in Table 3. The emphasis was on the isolation by culture, and five media bottles each were used, while only one smear was made for Z.N. staining and one guinea pig injected for each specimen. This might in part explain the astonishingly low percentages (27% and 14%) of positive smears for samples from Durban and Estcourt. Furthermore the use of the surface active agent Desogen for the transport from Natal resulted in many poor smears. Light microscopy was not a reliable method for the detection of true tuberculous lesions, as 11 out of 18 cases were negative on smears. We expected to find mainly non-pathogenic *M. avium*-group strains. The object of guinea pig inoculation was to discover the rare cases of *M. tuberculosis* and *M. bovis*. Twenty-one cases (5.7%) were found where lesions had developed beyond the regional lymph nodes. Typing by colonial morphology, drug sensitivity, emulsifiability, pyromycic acid, hydrazide resistance and niacin production indicates that all 21 pigs were infected with human tubercle bacilli.

The lowest incidence of true tuberculosis was found in Estcourt and the highest in the Vereeniging abattoir. Together these 21 cases occurred on 15 farms. A double or triple isolation was done in five of the farms. In four cases the samples came from porkers and in the others from baconers. Sometimes very few tubercle bacilli were viable, consequently cultures were negative in five cases and the strain had to be recovered from the guinea pig. In ten instances the guinea pigs showed only a few lesions beyond the regional lymph nodes near the injection site, pointing again to a small number of tubercle bacilli present in the lesions. In most pigs only one lymph node, either in the head or in the intestines, was found infected. Both these findings also point to a low virulence of *M. tuberculosis* in pigs. Nine of the 13 piggeries were large, one was small and three belonged to speculators buying pigs in the Bantu homelands. The rate of condemnation was made known to us in 12 cases and in only one of these were animals totally condemned, i.e. out of a batch of 20 pigs where two condemnations occurred. The cultures revealed high colony counts of 100—400 in six cases, moderate counts of 10—50 colonies in eight, and low counts of three and six colonies in two cases. *M. tuberculosis* was found in all sizes and types of lesions which were indistinguishable from non-tuberculous lymphadenitis.

C. Non-Tuberculous Lymphadenitis in Pigs

Table 4 summarizes the bacteriological results on 350 samples containing necrotic lesions resembling tuberculosis, but no true tubercle bacilli. Microscopy revealed acid-fast organisms in only 29% but in 35 instances acid-fast bacteria were seen without culturing being possible. Positivity of cultures seems to be influenced by distance — the two nearby abattoirs had a rate of 58% positive cultures while the Natal abattoirs rated 45% and 53%. More colonies developed from Transvaal material. The quartenary ammonium compound Desogen is obviously detrimental to non-chromogenic mycobacteria when in contact for 3—4 days. However, the average rate of 65% samples confirmed as mycobacterial lymphadenitis compares well with overseas studies [12, 13, 18, 19, 33, 35]. The number of non-chromogenic colonies was very high indeed and this must be attributed to the pretreatment method and the number and variety of culture media used.

Of the 349 guinea pigs injected with diseased material (excluding true tuberculosis), 337 showed no lesions whatsoever. The 12

Table 4: MYCOBACTERIAL LYMPHADENITIS

Results of Bacteriological Examination of 350 lesions in Lymph Nodes

ABATTOIR	Durban	Estcourt	Heidelberg	Vereeniging	Total
TOTAL No.	52	193	75	30	350
MICROSCOPY:					
Total Pos.	14	27	43	16	100 (29%)
Micro. Pos. only	7	10	13	5	35 (10%)
CULTURE:					
No. Pos.	23	103	45	16	187
Non-chromogenic strains	12	76	41	14	143
Mixed strains	9	21	4	0	34
Orange strains	2	6	0	2	10
TOTAL No. OF COLONIES:	518	14677	12919	2647	30761
Non-chromogenic colonies	434	14073	12295	2445	29247
Yellow colonies	54	525	613	200	1392
Orange colonies	30	79	11	2	122
PATHOGENICITY IN GUINEA-PIGS:					
Negative	50	184	73	30	337
Local lesion	2	9	2	0	13
SAMPLES CONFIRMED AS MYCOBACTERIAL L.-ADENITIS:					
Number	30	117	59	22	228
Percentage	58%	61%	79%	73%	65%

lesions seen were all at the site of injection and could have been caused by the quantities of necrotic material injected rather than pathogenic mycobacteria, as there was no correlation to high colony counts on culture. The guinea pigs, however, fulfilled their role by indicating all cases of true tubercle bacilli.

In Table 5 the size of the lymph node lesions is correlated with the colony count (Table 5, A) and with the gross pathology in guinea pigs (Table 5, B). It is shown that the size had no influence on the number of colonies grown. Whether pinhead, rice grain or pea size, cultures were negative in a large number of samples. On the other hand, high colony counts were sometimes obtained from very small lesions. It is more probable that the age of the lesion determines the viability of mycobacteria but this could not be ascertained.

Gross lesions in guinea pigs were also independent of the size of the porcine lesion. About 28% of the lesions were pinhead size or smaller, 37% were about the size of a rice grain, and the others were of pea or cherry size. Non-chromogenic mycobacteria caused local abscesses in only 12 cases.

In Table 6 two pathological and two epizootological factors were related to culturability and colony counts. Culturability was only slightly influenced by the number of lesions found in the same pig. The majority of single lesions were negative, but when the head and the intestines were both involved

Table 5: CORRELATION BETWEEN SIZE OF PORCINE LESION AND BACTERIOLOGICAL FINDINGS

(A) IN RELATION TO COLONY COUNT	Size of Lesion	Pinhead size or smaller	Rice grain size	Pea size and bigger
COLONY COUNT	Negative	35	78	46
	Scanty (1—10)	23	29	25
	Moderate (11—99)	16	9	20
	Numerous (100—400)	20	11	27
(B) IN RELATION TO GUINEA PIG PATHOGENICITY				
GROSS PATHOLOGY IN GUINEA PIGS	No Lesions	84	117	107
	Local abscess (Index 1—8)	4	4	4
	True Tuberculosis (Index 10—64)	7	6	8

the majority were positive. Half of the cases with several lesions in one organ were negative. This indicates that several lesions in the same pig are not the result of spread from a primary focus, but that they are several primary foci emerging independently of each other and perhaps at different times. Only one lesion per pig was sent in and examined. However, a future study of all lesions could elucidate this problem.

Table 6: COLONY COUNT CORRELATED WITH PATHOLOGICAL AND EPIZOOTIOLOGICAL FACTORS*

Factors		COLONY COUNT			
		Negative	Scanty (1—10)	Moderate (11—99)	Numerous (100 or more)
Extent of lesions	One lesion per pig	56	16	7	9
	Several lesions	62	28	11	21
	2 different organs involved	10	5	4	6
Location of lymph node	Head	92	39	12	14
	Mesenteric	40	18	13	21
	Unknown	13	7	4	7
Incidence of disease (condemnations)	Hight, 10 or more cases	20	3	8	14
	Moderate, 2—9 cases	72	43	16	22
	Single cases	60	21	6	8
Abattoir	Estcourt	92	48	24	33
	Durban	32	18	7	2
	Heidelberg	21	6	7	13
	Vereeniging	14	5	7	10
	Total	159	77	45	58

*Figures represent individual specimens, excluding 31 late cases.

The site of the lesion in the body did not significantly influence culturability either. The majority of head lymph nodes were negative and the majority of mesenteric nodes positive. Fifty-six percent of the lesions examined were in the lymph nodes of the head and 33% in those of the mesentery.

The incidence of the disease was judged by the rate of condemnation of heads or whole carcasses within one batch. It was considered high when 10 or more pigs were partly or totally condemned, and moderate when 2—9 pigs were involved. A single case concerned an animal from which the lesion was derived where no other condemnation was reported from the same piggery in the same batch. Forty-five large "outbreaks" were investigated and almost half the lesions were free from live mycobacteria. This indicates that a larger outbreak is not the result of an increased virulence of the causative organisms but is obviously only the result of higher exposure to an outside source of infection. The word "outbreak" is therefore unsuitable and one should speak of "incidence". The rate of negative lesions among the single cases was rather high, and it could be deduced that exposure to infection was low or took place some time ago.

The incidence of negative lesions or lesions positive in varying degrees was evenly distributed in all abattoirs. When the origin of the pigs was traced back to districts there was no indication that a particular region in South Africa has a specially high incidence of mycobacterial lymphadenitis.

D. Comparison of Non-Chromogenic Mycobacteria of Different Origin

The majority of the non-pigmented isolates from animal feed, bedding and environment exhibited a striking similarity to isolates from pigs, and those previously isolated at Onderstepoort from cattle and man[7, 8, 9]. For inclusion in the similarity analysis presented in Tables 7 and 8, a strain had to show the following characteristics: no production of niacin; no pigment in the light or dark; slow growth; no virulence in guinea pigs; colonial morphology consistent with Group III organisms. Thus *M. tuberculosis, M. bovis, M. kansasii* and Groups II and IV were excluded. All 106 strains listed in Table 7 resembled the *M. avium*-Battey group, forming smooth, shiny, round, regular, flat or dome-shaped colonies of different shades from buff to pale yellow.

The ability to reduce nitrate was generally absent or weak (±) in the more pathogenic isolates from mammals, while the majority of plant isolates showed a strong nitrate reduction. All non-chromogenic mycobacteria except *M. gastri* are reported to possess heat stable catalase. With our test procedure a number of other species lost catalase due to heating, especially those occurring free in nature.

The 3-day arylsulphatase test was, as expected, negative for the *M. avium*-Battey strains isolated from mammals, but positive for the more saprophytic Group III types. After the 14-day period almost all non-chromogens were positive. *M. avium*-Battey mycobacteria cannot hydrolyse Tween, and this is borne out in Table 7, but the non-pathogenic *M. terrae* and *M. gastri,* and some plant and bedding isolates were positive.

In agreement with Bönicke and others we regard the presence of nicotinamidase and pyrazinamidase i.e. the amidase pattern 5—6, as a very significant characteristic in the delimitation of the *M. avium*-Battey group from the other non-chromogens. The 5—6 pattern generally matched very well with other test results indicative for *M. avium*-Battey strains, especially pathogenicity and sensitin specificity. The majority of isolates from mammals possessed the 5—6 pattern, and also 16 of the isolates from feed and bedding.

Most strains grew at 42°C, but at 45°C (the optimal temperature for *M. avium)* many strains from feed, bedding, human sputum and cattle did not grow. Sixteen strains from the environment did not grow at 22°C.

E. Pathogenicity Studies

The results of animal inoculations as presented in Tables 8 and 9 make it evident that the pathogenicity of non-chromogenic mycobacteria did not depend on their origin but on their relationship to *M. avium* as determined mainly by biochemical characteristics. Regardless of whether the strain of *M. intracellulare* was isolated from pigs, man, cattle or environment it was capable of producing lesions in fowls and rabbits if injected in high dosages. But only true *M. avium* could cause tuberculosis in fowls at the low dose of 0.01 mg i.v. In rabbits *M. intracellulare* could produce the acute Yersin type pathology with its affinity for mesenchymal tissue. Most strains of *M. intracellulare* iso-

Table 7: COMPARISON OF BIOCHEMICAL PROPERTIES OF GROUP III MYCOBACTERIA OF DIFFERENT ORIGIN**

Source of Strains	Nitrate Reduction				Catalase 68°C				Arylsulphatase								"Tween" Hydrolysis				Amidase Pattern			Growth temperature					
									3 days				14 days											42°C			45°C		
	+	±	−	*	+	±	−	*	+	±	−	*	+	±	−	*	+	±	−	*	5, 6	Others	*	+	−	*	+	−	*
Pigs 24 strains	6	13	5		9	5	7	3			24		24					1	23		21	3		24			21	3	
Plant material 25 strains	15	2	4	4		3	13	9	7	2	9	7	11	3	4	7	8	0	4	13	4	14	7	6	12	7	0	25	
Bedding (wood shavings or dust) 28 strains	0	2	26		4	4	20		6	1	21		20	5	3			5	23		13	15		24	3	1	17	9	2
Man 12 strains	6	2	4		6	2	4				10	2	6		4	2	5		7		5	3	4	4	8		3	9	
Cattle 16 strains	3	7	5	1	8	4	4		2		10	4	10	6			4		12		9	7		12	4		10	6	

*Not yet tested.

**Each figure represents the number of strains reacting in the specified manner in the various tests done.

Table 8: PATHOGENICITY OF **M. INTRACELLULARE, M. AVIUM** AND OTHER NON-CHROMOGENIC MYCOBACTERIA TESTED BY I.V. INFECTION OF FOWLS AND RABBITS*

*Figures represent the results of single tests of each strain in the respective animals at the specified dose.

No. of strains	Origin and type of strains	Gross Pathology	Fowl			Rabbit	
			1.0 mg	0.1 mg	0.01 mg	1.0 mg	0.1 mg
12	Pig,	+ + + − + + + +	9	2	0	2	2
	M. intracellulare	+ + − +	6	4	0	0	3
		Negative	1	10	9	1	6
19	Environment,	+ + + − + + + +	4	7		11	4
	M. intracellulare	+ + − +	2	6		5	11
		Negative	0	5		1	2
4	Man,	+ + + − + + + +	2	0		0	0
	M. intracellulare	+ + − +	1	1		2	2
		Negative	1	3		2	2
9	Cattle,	+ + + − + + + +	2	2			4
	M. intracellulare	+ + − +	2	0			1
		Negative	2	4			4
7	Fowl, **M. avium**	+ + + − + + + +	7	6	4		
		+ + − +	0	0	2		
		Negative	0	0	0		
13	Environment,	+ + + − + + + +	0	0		1	0
	Saprophytic	+ + − +	6	2		5	2
		Negative	7	10		7	11
7	Man,	+ + + − + + + +	0	0		0	0
	Saprophytic	+ + − +	2	0		0	2
		Negative	5	7		5	5
5	Cattle,	+ + + − + + + +	2	2		2	1
	Saprophytic	+ + − +	3	1		0	1
		Negative	1	1		0	2

lated from the environment caused extensive or moderate lesions in rabbits. They were all freshly isolated while some of the strains from pigs, man and cattle had been subcultured frequently.

Six goats and 6 sheep were i.v. infected with plant strains; none showed any symptoms of disease. They were killed 6 weeks after infection. No macroscopical lesions were caused in the goats and retro-cultures were negative except a few colonies from 1 spleen, 1 liver and 1 mediastinal lymph node. Five of the 6 sheep showed no visible lesions and 3 had negative retrocultures. One sheep injected with *M. intracellulare* showed miliary tubercles of the lung and positive retrocultures and one of the other culture positive sheep harboured numerous mycobacteria in spleen, liver and kidneys in spite of normal appearance of the organs. The muscle was

Table 9: PATHOGENICITY OF NON-CHROMOGENIC MYCOBACTERIA IN YOUNG PIGS

Infective dose 0.5—1.0 mg culture injected i.v.

	No. of strains injected	Strains causing tuberculous lesions			No visible lesion
		+ + +	+ + − +	±	
M. avium	6	2	1	2*	1*
M. intracellulare	18	2	2	1	13
Other non-chromogens	8	0	0	0	8

Comment Four **M. avium** strains originated from fowls, 1 from a pig, 1 from a bovine.
Four **M. intracellulare** strains originated from plants, 6 from pigs, 2 from cattle, and 6 from man.
Seven of the "other non-chromogens" came from environmental material and 1 from a bird.
* = Old laboratory strains.

free of cultivable mycobacteria in all sheep and goats.

Thirty-two pigs were injected i.v. with viable mycobacteria of the three different types within Group III. The results given in Table 9 prove that *M. avium* caused tuberculosis rather regularly in the experimental model chosen. Only five of 18 strains of *M. intracellulare* were pathogenic for pigs, but none of eight other non-chromogenic strains which were biochemically not closely related to *M. avium*. The great majority of our porcine isolates were *M. avium*-like mycobacteria, but not all caused lesions when injected i.v. into other pigs. Retrocultures from the internal organs of 15 pigs injected with plant strains were positive in 9 cases and negative in 6 cases. All 39 muscle samples were negative on retroculture except for 3 colonies from one pig infected with *M. intracellulare*.

F. Species of Mycobacteria Involved

The species determination of 96 mycobacterial strains identified so far is presented in Table 10. *M. avium* was found in 13% of porcine lesions, but Worthington[7] believes that the true incidence should be higher. Future identification of our newly isolated porcine strains will elucidate this point. As expected, no *M. avium* was present in the environment but many plant strains have still to be typed. The first case of human infection with *M. avium* in South Africa was discovered only recently. The one case of *M. avium* in a cow is also an exception when seen in relation to several hundred strains of *M. bovis* isolated from bovines in the past at Onderstepoort. All isolates from chickens and turkeys were naturally *M. avium*, and came from typical pathological material, the fowl being the normal habitat of *M. avium*

The majority of porcine and environmental strains were defined as *M. intracellulare*, formerly called the Battey organism[52]. The morphological, physiological and biochemical characteristics of the majority of strains isolated from pigs and many strains from the environment were identical to those of *M. avium* except for their low pathogenicity and virulence for fowls and rabbits; this distinguished them as *M. intracellulare*. Biochemically the only difference between our *M. intracellulare* strains and *M. avium* was that after a 14-day test period they were arylsulphatase positive while *M. avium* strains were negative. The four human strains of *M. intracellulare* were pathogenic in each particular person, but predisposing factors were involved[8]. Some investigators do not agree with a separate species designation and regard *M. intracellulare* as a variety of *M. avium*. The rarity of fowl tuberculosis in South Africa and the absence of any close association of the pigs in modern piggeries with fowls, however, make it unlikely that we are dealing with *M. avium* modified by the lower body temperature of pigs, man or cattle. More significant is the finding of the same mycobacteria in the feed and environment of animals and man.

M. terrae, *M. gastri*[53] and *M. xenopei*[54] are mycobacterial species which have only recently been described and studied. They differ from *M. avium* in several biochemical

Table 10: OCCURRENCE OF SPECIES OF NONCHROMOGENIC GROUP III MYCOBACTERIA IN ANIMALS, MAN AND ENVIRONMENT

Source	No. strains	MYCOBACTERIUM SPECIES			
		M. avium	M. intracellulare M. xenopei	M. terrae M. gastri V subgroup	Group III unclassifiable mycobacteria
Pig: lymph nodes	24	3	19	0	2
Wood shavings	42	0	21	17	4
Plant material	36	0	6	28	2
Dust	7	0	7	0	0
Man: sputum	13	1	4	5	3
Cattle: different tissues	16	1	9	3	3
Fowls: livers	10	10	0	0	0

characteristics and are non-pathogenic to laboratory animals, pigs, ruminants and man. However, *M. xenopei* is reported to be pathogenic for fowls [55] but of lower virulence than *M. avium*.

DISCUSSION

The two salient features of this study are that 94% of tuberculous lymph node lesions in pigs are caused by *M. avium*-like mycobacteria and that organisms similar or identical to those causing the lesions have been isolated with regularity from animal feed, bedding and environment. It is also shown that these plant and environmental isolates produced the same type of lesions in fowls, rabbits and one pig as those isolated from pigs, cattle and man. There is much circumstantial evidence to suggest that *M. intracellulare* is the responsible organism, that it is able to exist and even multiply outside the animal body and that pigs are continuously exposed to these organisms. The finding that 5.8% of lymphonodular lesions are caused by *M. tuberculosis* is not unexpected but it complicates the issue. The lesions caused by these two mycobacteria cannot be differentiated with the naked eye or microscopically and the authors cannot recommend a quick laboratory test for this purpose. Culturing of lesions and identification of colonies requires a specialized laboratory and takes 2—3 months. Biological testing using guinea pigs would be simpler and shorter i.e. 6 weeks, although rather expensive. Attendants in in large piggeries should be regularly screened for tuberculosis. One should also consider boiling all waste food from institutional kitchens used as pig feed. In the past and in this survey such swill was proved to be the source of *M. tuberculosis* infection. Pigs from smallholdings and those handled by speculators will be suspect for human infection as long as pulmonary tuberculosis of man is endemic. Fortunately *M. tuberculosis* is not very virulent in pigs and it is unlikely that organisms will enter the bloodstream and thereby infect the meat.

The absence of any *M. bovis* in the 368 samples is significant in that it shows that the education of pig farmers by veterinarians has been successful. The compulsory pasteurization of milk is a contributory factor.

The ubiquity of the non-chromogenic mycobacteria might force us to accept porcine mycobacterial lymphadenitis as a problem which may be with us for a long time. The authors, in accordance with medical terminology, suggest designating disease conditions caused by mycobacteria other than the classical tubercle bacilli as "mycobacterioses", and the specific lesion in pigs as "mycobacterial lymphadenitis".

The nature, source and pathological significance of many of the so-called atypical mycobacteria are still insufficiently understood. Some of them, however, can cause disease conditions indistinguishable from tuberculosis i.e. lymphadenitis in children and mycobacterioses of the human lungs. Both *M. avium* and the Battey type mycobacteria have specific biological characteristics that clearly separate them from mammalian mycobacteria. In contrast with *M. avium*, *M. intracellulare* shows no full parasitic adaptation to certain animal species and its mode of transmission and spread is different from that of classical tubercle bacilli. It is probable that the well-known prolonged survival of *M. avium* in the external environment is also a feature of *M. intracellulare*. The affinity for mesenchymal tissue after i.v. infection, the massive intracellular growth, emaciation and death of fowls with pathology of the acute Yersin type seen with *M. avium* could also be produced with *M. intracellulare* using doses which are 10—100 times as large. Both organisms however possess other properties which are similar to those of saprophytic mycobacteria: the tendency to pleomorphism; pigmentation under certain conditions; uniform smooth growth; the absence of cords; the high degree of resistance against anti-tubercular drugs and mycobacterial phages.

The properties of *M. intracellulare* are quite uniform whether isolated in the southern United States, Europe, Western Australia or South Africa. They present a considerable problem as a cause of pulmonary lesions in man in countries where human tuberculosis is relatively rare. Transmission from man to man and animals to man was not observed, but pigs, cow's milk, fowls, wild birds and even rodents were blamed as reservoirs or sources of infection. The consumption [56, 57, 58] of pork from pigs with local mycobacterial lymphadenitis constitutes no health hazard. Our negative retrocultures of muscle only 6 weeks after massive i.v. infection indicate the ability of the pig to remove the non-chromogens completely. Such a bacteriaemia is most unlikely to occur in pigs with necrotic

lymphadenitis of this nature.

The present investigation confirms the suspicion of other investigators[16, 29, 30, 42, 56, 57, 58] that the reservoir is probably the soil. Plants, woodshavings and feed mixtures are possibly contaminated via dust. Man and cattle are therefore continuously exposed to potentially pathogenic mycobacteria and there is already evidence of such infection. The inhalation of contaminated dust could be the major route of infection for man, while pigs and cattle ingest greater quantities of these mycobacteria with their food. We believe that these *M. avium*-like mycobacteria are responsible for the high incidence of skin sensitivity to avian tuberculin in man[59] and cattle[60] in South Africa.

With a yield of 18—250 colonies per feed sample one can deduce that a pig eating 3 kg of such feed per day would ingest 5 000—75 000 of these mycobacteria per day. It is therefore not suprising that some investigators found these organisms in the faeces of pigs. Mechanical irritation caused by woodshavings which are eagerly chewed by pigs would facilitate the penetration of mycobacteria through the tonsils and intestines.

It is unlikely that these organisms have only recently increased to a level where it became an economic problem. It is possible that modern farming methods have contributed to the increased incidence of lesions. Other likely reasons are that there is better inspection at abattoirs, increased awareness of the condition, and better procedures for isolating and typing these organisms which previously were mistaken for classical avian tubercle bacilli. An observation which merits comment is that piglets hardly ever develop mycobacterial lymphadenitis, and adult animals, mainly sows, are free of lesions. In the case of piglets the explanation could be that animals first have to be sensitized by continuous absorption of the mycobacteria before their lymph node tissue will react with the typical necrotic lesion recognised at meat inspection. The absence of lesions in adult pigs could be explained by the rather low virulence of the organisms and the high natural resistance of the pig which will overcome the infection resulting in self-cure and reabsorption of necrotic tissue. The absence of live mycobacteria in the lesion of many baconers points in this direction.

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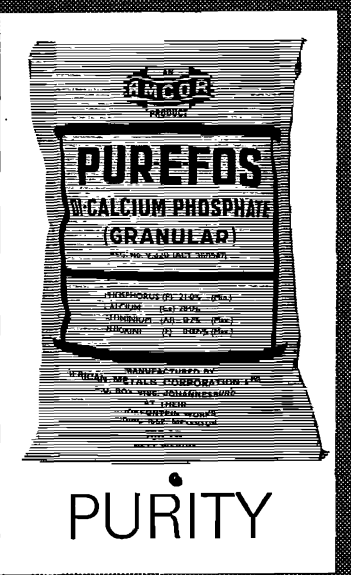

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Jl S. Afr. vet. med. Ass. 40 (3): 253-256 1969

PORCINE MYCOBACTERIAL LYMPHADENITIS : SOME EPIDEMIOLOGICAL ASPECTS *

R. K. LOVEDAY**

SUMMARY

The changing epidemiological pattern of mycobacterial lymphadenitis in South African pigs is discussed and compared with overseas findings. It is pointed out that the infection is now generally found to be caused by one of the opportunist avian-Battey group of mycobacteria and rarely by a mammalian organism. The disease is prevalent on a relatively small number of high-incidence premises, where the environment has often been found to be heavily contaminated by similar avian-Battey serotypes. Infection generally occurs via the gastro-intestinal tract, and may be established before weaning age. There is a marked tendency for self-cure and resolution of the gross lesions to occur in older animals. This changing pathological pattern influences the distribution of infected animals found at slaughter among the various liveweight grades of market pigs.

INTRODUCTION

Since the recognition early this century [1] of the pig's susceptibility to infection by all three types of classical tubercle bacilli, it became customary to regard the incidence of mycobacterial infection in this species as generally reflecting the presence of tuberculosis in the associated human, cattle or poultry populations. Outbreaks tended to be sporadic, and cleared up after identification and eradiction of the source of infection. Typing of 76 mycobacterial cultures from pig lymph nodes in 1956—58 by Robinson [2] showed 75% to be *Mycobacterium bovis* and the remainder *M. tuberculosis.*

During the past two years there has been reported [3] a tendency for the incidence of porcine mycobacterial lymphadenitis in a relatively small number of pig herds to rise to alarmingly high levels, with accompanying severe economic loss to the producer. These high-incidence premises were generally large and intensive, with usually a complete absence of any contact between pigs and cattle or poultry. In Great Britain the overall incidence of tuberculosis in pigs is stated [4] to have decreased from 3.6% in 1957 to 1.56% in 1966, although the level of infection in some individual herds is reported as high as 8%. This paper attempts to discuss some of the epidemiological complexities of the new situation in South Africa and to emphasise the notable change which has become evident in the nature and origin of the infection.

THE INCIDENCE OF INFECTION

The following table, showing the incidence of pig tuberculosis in various countries, indicates that the virtual eradiction of bovine tuberculosis in countries such as the United States and Great Britain has reduced, but not eliminated, the incidence of infection in pigs in these countries.

When comparing the South African figures, due allowance should be made for the following weighting effect. Among the various market grades of pigs, tuberculosis is diagnosed almost exclusively among baconers. This fact explains the higher incidence reported from the Estcourt Bacon Factory. The 1959 survey from this Factory, quoted above, was conducted on a sample consisting of 13 000 baconers. By contrast, baconers constitute approximately one quarter of the pigs slaughtered at the City of Johannesburg abattoir.

THE MYCOBACTERIAL SEROTYPE AND ITS SOURCE

In their important study of this aspect, Kleeberg and Nel [11] have provided an extensive review of the European literature, showing that the more recent studies attribute the majority of porcine lesions to infections

* Presented to Vet. Public Health Group, S.A.V.M.A. Congress, Onderstepoort, Sept. 1968.

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Table I

ORIGIN	Year	Total Incidence %	Generalised Tuberculosis %	Reference
U.S.A.	1922	16.38	0.20	5
U.S.A.	1962	2.25	0.008	5
England	1957	3.60		4
England	1966	1.56		4
Scotland	1967	5.00		6
City of Dublin	1967	0.016	0.008	7
Estcourt Bacon Factory	1959	7.66	0.52	8
	Year ending			
Estcourt Bacon Factory	30-6-1968	2.90	0.30	9
City of Johannesburg	1965—66	0.46	0.11	10
City of Johannesburg	1966—67	0.49	0.109	10
City of Johannesburg	1967—68	1.06	0.181	10

by avian-Battey group mycobacteria. In a British study [12] over 15 years, the incidence of *M. avium* infection in the first five years was 44% compared to 92% incidence during the final 5 years.

Summarising the results of earlier South African work, Kleeberg and Nel [11] emphasise that all the more recent typing results point to the fact that the majority of porcine lymphadenitis is being caused by mycobacteria other than *M. bovis* or *M. tuberculosis*. They cite unpublished data from Worthington (1965) who typed 54 mycobacterial porcine strains at Onderstepoort during 1961—65, and found 35 of these belonged to the avian-Battey group, 10 were *M. tuberculosis* and 9 were *M. bovis*.

Kleeberg and Nel [11] cultured mycobacteria from 180 of 339 tuberculous lymph node lesions from pigs from 4 abattoirs. No less than 90% of these cultures produced non-chromogens of the avian-Battey group — the remaining 10% being all *M. tuberculosis*. Of the 24 opportunist porcine strains they typed, 3 or 13% were found to be *M. avium*, the remaining 77% all being Battey bacilli. Typing of 25 British opportunist porcine strains [13] identified 22 as being *M. avium*, 2 as Battey strains and 1 as unclassified. Three *M. avium*, serotypes were described by these authors.

A large number of samples of dust, soil, sawdust and various feedstuffs from high prevalence piggeries were also cultured by Kleeberg and Nel [11]. No less than 70% of feed samples (including lucerne and even 1 sample of fishmeal) and 80% of environment samples (particularly soil and pine sawdust) were found to contain mainly avian-Battey group mycobacteria, frequently in large numbers. Their findings are in accord with those concerning soil and feed in the United States [14] and sawdust in Germany [15] and Australia [16].

Contrary to experience in the United Kingdom [17], wild or domestic birds have thus far not been shown to represent a significant reservoir of non-chromogenic mycobacteria in South Africa [11]. Avian-Battey type infection may also cause bovine mastitis and be excreted in the milk [18, 19]. A proportion of such organisms may survive pasteurisation and a survey of 329 random pasteurised milk samples in the United States [20] disclosed 9 samples contaminated by live opportunist mycobacteria, 8 of the 9 isolates belonging to the avian-Battey group.

Man himself may be a source of avian-Battey bacilli, since these organisms may cause pulmonary infection, particularly where previous lung damage has occurred. Such infection has been reported in South Africa [21] and a recent American report [22] listed 50 patients with opportunist mycobacterial infection, 49 of whom had positive sputa, of which 20 yielded avian-Battey bacilli. The incidence of opportunist infection in man has about doubled in Wales during the past 10 years [13].

THE ROUTE OF INFECTION

The gross lesions observed during meat inspection procedures generally represent an incomplete primary complex with lymphogenous spread to regional lymph nodes, the mucous membrane lesion being rarely observed or indeed sought. Intestinal lesions in pigs due to avian bacilli have been identified [5] while Jubb and Kennedy [23] consider ulceration of such a primary mucous membrane focus to be rare.

Since lymph node lesions occur predominantly in the mesenteric nodes [8, 10, 16], less frequently in the head nodes [8] and rarely in the bronchial nodes [8], it is apparent that infection occurs principally via the oro-pharynx or the small intestine, inhalation infection being rare. These findings are in accord with the gross environmental and feed contamination with mycobacteria found on high-prevalence farms.

Differences in pathogenicity have been disclosed by British workers [12] who found 48% of 62 bovine infections became generalised, whereas 11.7% of 256 avian type infections showed lesions in lungs, liver or spleen, the remainder causing lesions confined to head, neck or mesenteric nodes.

DISTRIBUTION OF INFECTION AMONG PIGGERIES

Contrary to expectation, pigs with mycobacterial lymphadenitis emanate from a remarkably small proportion of the suppliers to a particular market. All the animals with lesions at the Estcourt Bacon Factory in 1967—68 originated from only 7% of the suppliers to the factory [9], many exhibiting a distressingly high incidence. Similarly, Greathead [10], noted that 93% of the mesenteric mycobacterial lymphadenites at the Johannesburg abattoirs during the first 6 months of 1968 came from 4 producers, 6.7% of their slaughter pigs during this period being totally condemned for tuberculosis. A similar occurrence of high-incidence British premises has already been mentioned [4].

It is usual for slaughter lesions to occur for long periods in pigs from high-incidence premises, while the Estcourt Bacon Factory records for 1967—68 [9] also indicate that the great majority of producers supplying the factory never supply any slaughter pigs with gross tuberculous lymph node lesions. It is worth recording that excellent hygienic standards are usually maintained on these high-incidence premises, apparently with no appreciable effect on the incidence of the infection.

THE DISTRIBUTION OF INFECTION AMONG MARKET PIGS

An examination was made of the marketing records of two large and intensive pig farms, where mycobacterial lymphadenitis was occurring on a large scale. During an 11 month period from October 1967 to August 1968, 3 860 baconers were marketed from the first farm, 12.7% of these animals being either partially or totally condemned at slaughter for gross mycobacterial lymphadenitis. The loss resulting from these condemnations was estimated as R7 500 by the owner. During the same period this farm sent 773 porkers and 36 breeding animals to slaughter, but no gross lesions were found in any of these animals.

Precisely similar information was collected from the second, long-established producer, where a similar 12% condemnation rate among bacon pigs had suddenly manifested itself several months earlier. No husbandry changes could be traced which might have influenced this situation. On this farm, an insight into the rate of development and regression of the mycobacterial lymph node lesion was obtained through the co-operation of the owner in slaughtering several batches of bacon pigs at different liveweights, as set out hereunder:

Table 2

No. of pigs slaughtered	Average liveweight	Average deadweight	Approx. % with lesions
21	150	117	50
21	160	124	40
21	170	132	30
99	193	142	12

The Estcourt Bacon Factory has reported [9] that gross tuberculous lesions were found in only 16 of some 6 000 sows slaughtered during the 12 month period ending 30.6.68. All these sows originated from premises marketing baconers with mycobacterial lesions.

At the other end of the scale, Australian workers [24] have isolated mycobacteria from three 37 day old piglets and from a 6 months old baconer, none of which animals exhibited either gross or microscopic mycobacterial lesions.

DISCUSSION

The literature cited clearly reflects the changing pattern of porcine mycobacterial lymphadenitis in Europe, Australia and South Africa during recent times. Infection with avian-Battey group bacilli has supplanted mammalian infection to a very great extent. These opportunist organisms appear to enjoy an ubiquitous and free-living existence in nature, and may occur in profusion on high-prevalence premises, particularly in temperate and subtropical climatic zones [19]. While birds do not appear to be a reservoir in South Africa of mycobacteria, the well-known danger of infection from open cases of pulmonary tuberculosis among piggery workers has been re-emphasised by the latest South African investigations [11].

The obvious difficulties inherent in controlling or eradicating avian group infections originating from outside sources are formidable, and considerable further study of the mode of existence of Battey serotypes is urgently required. It is hoped that the survey presently being conducted to establish the location and nature of high incidence premises will contribute toward a better understanding of at least some of the many puzzling aspects of this whole problem.

ACKNOWLEDGEMENT

I have pleasure in thanking Mr. R. W. D. Stewart, Managing Director of Estcourt Bacon Factory, for his kind co-operation and willing supply of information.

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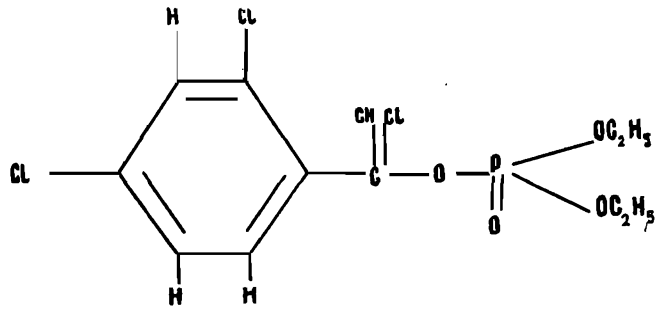

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PORCINE MYCOBACTERIAL LYMPHADENITIS : MEAT HYGIENE CONSIDERATIONS *

M. M. GREATHEAD**

SUMMARY

The lesions produced by mycobacteria in slaughter pigs are described and the relevant meat inspection regulations and judgement of infected carcasses outlined.

The increased incidence of the purely mesenteric form of porcine tuberculosis at the Johannesburg abattoir is recorded, the implications of this disease to the producer and the butcher are considered and the inadequacy of the present regulations is pointed out.

INTRODUCTION

In the past year at the Johannesburg abattoir there has been a pronounced increase in the incidence of mesenteric node tuberculosis, usually without lesions elsewhere in the body and mainly occurring in baconers emanating from well-managed herds. Previously the application of the regulations had proved straight-forward but the prevalence of these cases has made it necessary to review the standards for the evaluation of tuberculosis in pigs.

REGULATIONS

The Meat Inspection Regulations made under Section 115 of the Public Health Act (36/1919) came into effect on 1st January 1925.

Paragraph 14 prescribes the secondary examination which must be performed on a carcase in which evidence of tuberculosis is found: viz:—

(a) The viscera and associated lymph nodes must be carefully examined.

(b) Lymph nodes throughout the carcase must be incised.

(c In pigs, every carcase which shows any degree of tuberculosis infection must be split and the vertebrae and kidneys carefully examined."

Paragraph 15 is specific on the evaluation of swine tuberculosis:

"(a) If lesions exist in the submaxillary (mandibular) nodes and any other part of the carcase, the entire carcase and all organs shall be condemned.

(b) If tuberculosis lesions exist only in the submaxillary nodes, the head shall be condemned and the carcase passed."

PATHOLOGY

In an abattoir where a large number of pigs of different types are slaughtered, the lesions produced by or ascribed to mycobacteria vary in type and extent. The lesions are usually found in the mandibular, mesenteric and bronchial lymph nodes either singly or in combination. The bronchial nodes seem to be the least frequently affected. Occasionally, a generalised infection producing a more invasive type of lesion is found affecting the lymph nodes of the head and the carcase (e.g. precrural and superficial inguinal nodes). Very infrequently, tuberculosis of the vertebral column is found in carcases with long standing lesions in the lymph nodes of the body.

In the mandibular nodes lesions may be unilateral or bilateral and vary in diameter from one to ten millimetres. They calcify rapidly and are usually white in colour, though some may be yellowish. It general the lesions in older pigs are more extensive.

Mesenteric and bronchial nodes are usually affected with similar white semicalcified lesions which may be very small but are often large enough to be seen clearly through the intact peritoneum or pleura. Recently the incidence of mesenteric node tuberculosis has increased greatly and in many of the affected pigs no other sign of infection could be found. This type of lesion is found more frequently in baconers than in other types.

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** Dep. Director, Abattoir and Livestock Market Department, City Council, Box 1620, Johannesburg.

DIAGNOSIS

Without bacteriological culture of the affected material, a definite diagnosis of some lesions is difficult and it is impossible to determine the type of mycobacterium involved. Cultural methods are too slow for the results to be applied to the consignment involved but information so obtained may be used to identify lesions of a similar nature subsequently. Examinations of node lesion smears for acid fast organisms is frequently unsuccessful.

Judgement has to be based on the macroscopic appearance of the lesions and may lead to decisions erring on the safe side, with condemnation of heads or carcases in which suspicious node lesions are found. In general, however, abscesses caused by other organisms can be differentiated from mycobacterial lesions.

INCIDENCE OF TUBERCULOUS INFECTION IN SLAUGHTERED PIGS (JOHANNESBURG ABATTOIR)

Over a three year period from 1966 to 1968 the incidence of tuberculosis increased markedly as indicated in Table 1. This was particularly evident in 1968, when evidence of the disease was found in 1.06% of pigs compared to 0.49% in 1967. This was mainly due to an increase in mesenteric node tuberculosis as the percentages of whole carcases condemned on account of tuberculosis during 1967 and 1968 were 0.109 and 0.181 respectively. In 1968, 35% of infected pigs had lesions in the mesenteric nodes only and these animals constituted 0.36% of pigs slaughtered.

A dramatic increase in mesenteric node tuberculosis only, which accounted for an incidence of 0.62% of slaughtered pigs and 47.8% of infected pigs, appeared during the first six months of 1968. Mandibular node infection was found in 0.49% of animals slaughtered and in 36.9% of infected animals. (Table 2).

The heavy mycobacterial infection encountered in pigs from certain farms is shown in Table 3. Evidence of tuberculosis was detected in 659 (44%) of 1 492 pigs in consignments which contained infected animals from four farms. A total of 547 pigs (36.6%) had mesenteric node infection only and these comprised 83% of the infected animals.

Table 1: ANNUAL INCIDENCE

YEAR	SLAUGHTERED	INFECTED	CONDEMNATIONS		
			Carcases	Heads only	Viscera only
1965—1966	206 081	959 (0.46%)	227 (0.110%)	732	0
1966—1967	205 066	977 (0.49%)	224 (0.109%)	711	42
1967—1968	183 956	1962 (1.06%)	333 (0.181%)	949	680 (0.36%)

Table 2: INCIDENCE OVER SIX MONTH PERIOD IN 1968

MONTH	PIGS SLAUGHTERED	PIGS INFECTED	PER CENT INFECTION	NODES IN WHICH LESIONS WERE FOUND		
				Mandibular and others*	Mandibular only**	Mesenteric only***
January	15 810	112	0.7	18	94	—
February	14 936	74	0.4	19	55	—
March	15 194	177	1.1	25	72	80
April	15 199	282	1.8	32	83	167
May	16 189	363	2.2	62	92	209
June	13 017	174	1.3	22	47	105
TOTAL	90 345	1 182	1.3	178 (0.19%) (15.1% of infected pigs)	443 (0.49%) (36.9% of infected pigs)	561 (0.62%) (47.8% of infected pigs)

*Affected carcase condemned.
**Affected head condemned.
***Affected viscera condemned.

Table 3: INCIDENCE IN INFECTED CONSIGNMENTS FROM FOUR FARMS (SIX MONTHS PERIOD, 1968)

FARM	PIGS IN INFECTED CONSIGNMENTS	INFECTED PIGS	NODES IN WHICH LESIONS WERE FOUND		
			Mandibular and others*	Mandibular only**	Mesenteric only***
1	922	565	50	1	514
2	365	60	37	5	18
3	97	22	5	5	12
4	108	12	8	1	3
TOTAL	1 492	659 (44%)	100 (6.7%)	12 (0.8%)	547 (36.6%)

*Affected carcase condemned.
**Affected head condemned.
***Affected viscera condemned.

From Tables 2 and 3 it can be seen that for the same six month period 547 (93%) of the 561 recorded cases of mesenteric node tuberculosis were derived from four farms. These four producers lost 100 carcases (6.7%) as result of total condemnation.

EVALUATION

According to the regulations, the whole carcase must be condemned when lesions of tuberculosis are present in the head and elsewhere in the body, even if the lesions are fairly well calcified, circumscribed and appear localised. If infection is found in the head only, this is condemned.

The question which arises when lesions are found in the mesenteric or bronchial nodes only is whether the whole carcase, all the viscera, or only the affected organs should be condemned.

In practice, when the mesenteric node infection described above was first encountered, all the thoracic and abdominal viscers except the kidneys were condemned. After consideration and in the absence of bronchial node lesions, a less severe pudgement was imposed, the stomach and intestines only being condemned. In each case where the carcase was passed with condemnation of either the head or the viscera or the organs only, the vertebral column was split and examined for tuberculous lesions. In the 1962 carcases split during 1967—68, not a single lesion of vertebral tuberculosis was found.

A second question arises: is there any justification for splitting the vertebral column of a pig in which tuberculous lesions are localised in a single regional lymph node? To the author's knowledge, vertebral tuberculosis has been found in only three cases at the Johannesburg Abattoir in the past eight years. These cases were old pigs with such extensive lesions in other parts of the body that they would have been condemned even without splitting the spinal column.

ECONOMIC IMPLICATIONS OF SWINE TUBERCULOSIS

(a) To the producer

(i) *Total condemnation of the carcase.* In terms of the regulations, every carcase which has even the smallest lesions of tuberculosis in both the head and the carcase or viscera, must be condemned. This means a complete loss to the producer unless an insurance scheme is in operation.

(2) *Condemnation of head or viscera.* In these cases the carcase must be split after removal of the affected parts and this mutilation may depress the price paid by the factory or the butcher.

(b) To the factory and butcher

(1) *Baconer Carcases.* Bacon factories experience difficulty with handling, hanging and deboning carcases which have been split.

Unsplit carcases are deboned in the hanging position by the following method:—

Beginning at the tail the dorsal muscles are cut away from either side of the dorsal vertebral spines, exposing the transverse processes of the lumbar vertebrae and the costal articulation of the thoracic vertebrae. The body, central canal and dorsal spines of the vertebra are removed with a chopper, dividing the carcase into two with the transverse processes and ribs still in the meat. These are removed after curing.

The *M. longissimus dorsi* is the most important part of a side of bacon and mutilation

reduces the quality of the final product. Warm carcases are most easily cut up.

A split carcase is difficult to debone because it lacks rigidity and cannot be hung as easily as an intact carcase. The saw cut should be made carefully through the vertebrae immediately lateral to one side of the dorsal spines. After the muscles have been loosened on that side, the bodies of the vertebrae are still effectively divided for inspection for tuberculosis and the carcase can be neatly deboned. If the spines are divided the operation is more difficult to carry out and, if the cut is too lateral, the bodies of the vertebrae cannot be effectively inspected and the dorsal muscles are damaged.

Some factories hesitate to buy split baconer carcases and will not pay top prices for those which have been badly split.

(2) *Porkers and other types of carcases.* No particular problems are encountered with these because they are used for chops, sausages etc.. Damage to the loin muscles and badly split vertebrae will not materially affect the quality of the product. Neverthe less, split carcases of these types tend to command a price which is a cent or two per lb lower than intact ones.

CONCLUSION

With the isolation of new types of mycobacteria, *e.g. M. intracellulare,* from apparently fairly typical tuberculous lesions in slaughter pigs, the meat inspector is finding it more difficult to make a judgement which is fair to both producer and consumer. Further information is necessary regarding the pathogenicity of these organisms and their possible distribution in infected carcases.

New meat inspection regulations in respect of tuberculosis in pigs should be based on practical experience gained in South African abattoirs and must enable the inspector to make a logical judgement.

Abattoirs should co-operate with bacon factories by carefully splitting tuberculous carcases in such a way that effective inspection can be performed without mutilation of the product.

ACKNOWLEDGEMENT

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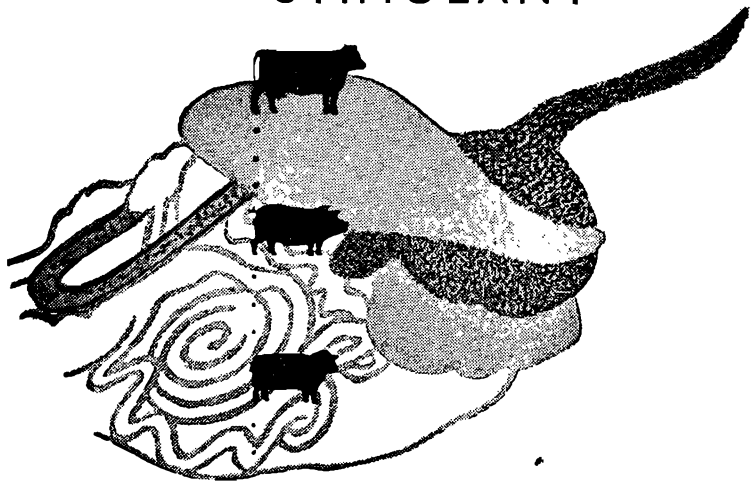

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BLOOD TRANSFUSION IN CATTLE WITH SPECIAL REFERENCE TO THE INFLUENCE OF BLOOD GROUPS

II Repeated Blood Transfusions

K. VAN DER WALT* AND D. R. OSTERHOFF**

SUMMARY

The results failed to identify a single blood factor or phenogroup which appeared to be responsible for transfusion reactions. No relationship between naturally occurring antibodies and transfusion reactions could be demonstrated. The present *in vitro* tests proved inadequate to predict transfusion reactions and practitioners' attention is drawn to the unreliability of these tests which only engender a false sense of security.

If a transfusion has been performed with out mishap, one or more transfusions can be given within the next five days. The same donor should be used and pooling of blood from different donors should be avoided.

The volume of blood transfused varied greatly and no correlation between it and the subsequent appearance of transfusion reactions could be demonstrated. An amount of 10 ml blood per lb body weight might be recommended.

The clinical observations on transfusion reactions were inconclusive, the severity of the symptoms could not be related to the eventual outcome in every case. No recommendations regarding the choice of donor can be made at this stage.

INTRODUCTION

In the first paper of this series[1] it was explained that this study was undertaken because of many conflicting opinions regarding clinical transfusion reactions and that the role of blood groups in producing such reactions was generally unknown.

MATERIAL AND METHODS

The apparatus used and techniques employed have been described in detail in the first paper[1]. The animals used were clinically normal and none had previously received any injection of blood. Particulars of the animals are given separately for each section of the experiment.

RESULTS

a) *Transfusions repeated with blood of the same donor after six days*

Five Afrikaner heifers 12 to 18 months old, with an average weight of 600 lb were used. Each animal received two litres of blood twice from the same donor; the duration of transfusion varied from 6 to 16 minutes.

The results of the transfusions are presented in Table 1.

Clinical observations did not show any striking differences between first and second transfusion reactions, except in heifer No. 1230 which appeared comfortable immediately after the second transfusion but 45 minutes later started moaning and was apparently in distress; she recovered completely within the next 45 minutes. The high titre of anti-A haemolysin in the serum of this heifer's donor, in contrast to the naturally occurring isohaemolysins of the other donors, could possibly account for this reaction.

The haematological values between and after the two transfusions followed the same general trend as shown in the previous paper[1] and therefore are not given in detail.

b) *Transfusion repeated with pooled blood after seven days.*

Five Afrikaner heifers 12 to 18 months old and weighing about 680 lb were used. At the first transfusion each recipient received two litres of blood from each respective donor. The second transfusion was given after seven days with blood pooled from all five donors, each recipient being given two litres. The blood was pooled directly after

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Table 1: TRANSFUSION REPEATED WITH BLOOD OF THE SAME DONOR AFTER SIX DAYS

Recipient (No. of Animal)	1261	1230	1264	1240	1242
Recipient's blood containing naturally occurring haemolysins	+ + + *	+ +	+ +	0	0
Donor's blood containing naturally occurring haemolysins	+ +	+ + + + **	+ +	+ + +	+ +
Expected immune haemolysins	F	S_2	GQ	Y_2	D′ Z′
Clinical reaction after first transfusion	none	none	none	none	none
Immune haemolysins demonstrated first day after transfusion	0	0	0	+	0
Third day	0	0	0	+ +	0
Fifth day	+ +	+	0	+ + +	±
Clinical recation after second transfusion	none	mild shock	none	none	none
Seventh day	+ + +	+	0	+ + +	±
Thirteenth day	+ + + +	+	+ +	+ + +	+ + +
Forty-third day	+ + +	±	+	+ +	+
Produced immune haemolysins	F	Unidentified (anti-A + ?)	G	Y_2	D′ + ?

*The amount of haemolysins produced or naturally occurring is given in the following way: + + + + = titre higher than 1/64, + + + = 1/32—1/64, + + = titre 1/8—1/16, + = 1/2—1/4, ± = trace reactions in low dilution, 0 = no haemolysins. In this and subsequent tables, the time intervals of haemolysin production are calculated from the first transfusion.

**The donor's blood contained unusual strong anti-A haemolysins which were naturally present.

Table 2: TRANSFUSIONS REPEATED WITH POOLED BLOOD AFTER SEVEN DAYS

Recipient (No. of animal)	1258	1263	1244	1257	1262
Recipient's blood containing naturally occurring haemolysins	+ +	+ + +	±	+	+
Donor's blood containing naturally occurring haemolysins	+ + +	+ + +	+ +	+ + +	+ + +
Expected immune haemolysins after first transfusion	Q D′FV	—	HDGPV SA_1 SA_3	IX_1S_1	$BGB'C_2SA_2$
Clinical reaction after first transfusion	very light shock	none	none	none	none
Immune haemolysins demonstrated first day after transfusion	0	0	0	0	0
Fourth day	0	0	±	±	0
Seventh day (before the second transfusion)	+ + +	±	+ + + +	+ +	+ +
Results after second transfusion (with pooled blood)	mild shock, died 16 hrs. after transfusion	shock	shock, died 15 hrs. after transfusion	Very severe shock, died 12 hrs. after transfusion	mild shock
Eighth day	+ +	+ +			+ +
Fortieth day		±			+ + +
Produced immune haemolysins	F	Unidentified (anti-J − ?)	$GVSA_3$	S + ?	Unidentified (anti-J + ?)

bleeding and stood for about one hour before transfusion. This procedure was initially undertaken to test a theory that *in vitro* reactions occur which materially diminish the incompatibility of the pooled sample. Moreover, the use of pooled blood under field conditions is a distinct possibility and more information on its use is needed. The results are given in Table 2.

Clinical observations on the five recipients after the second transfusion can be summarized as follows: Animals Nos. 1262 and 1263 showed mild reactions; thirty minutes after completion of the transfusions the respiratory frequency and pulse rate increased markedly but returned to normal within 45 to 60 minutes.

The three recipients which died 12 to 16 hours after the second transfusion showed varying symptoms immediately after the transfusion. No. 1257 remained normal during the procedure but shortly afterwards respiration increased from 35 to 65 per minute, salivation and coughing became evident, the animal moaned, the skin surface became cold to the touch and there were signs of marked general discomfort. Recipient No. 1244 also showed typical signs of shock although not as severe as No. 1257. In this case the reaction was delayed for a full 30 minutes after completion of the transfusion. No. 1258 showed a mild reaction only and the death of this animal was completely unexpected. In all five animals no untoward reactions were observed during or immediately after the transfusion.

Post mortem examinations revealed the following general signs in all three animals; the abdominal and pleural cavities contained up to one litre of clear red fluid, and the bladders were filled with brown cloudy urine. There were small epi- and endocardial haemorrhages and small haemorrhages also occurred in the colons. The most outstanding changes, however, were present in the lungs, which were greatly swollen, severely congested and oedematous, with prominent gelatinous infiltration of the septae. Emphysematous areas were present along the borders and small subpleural haemorrhages were seen on the surfaces. Histopathological examination of the lung tissue revealed severe oedema with infiltration of lymphocytes and eosino-

Table 3: TRANSFUSIONS REPEATED WITH BLOOD OF THE SAME DONOR AFTER 24 DAYS

Recipient (No. of animal)	1233	1237	1243	1265	1232
Recipient's blood containing naturally occurring haemolysins	0 J-subst. in serum	0	0	0	+ +
Donor's blood containing naturally occurring haemolysins	+ + +	0	+ +	0	+ +
Expected immune haemolysins	HO_3L	PA′	H	BPA′	HA′
Clinical reaction after transfusion	none	none	none	none	none
Immune haemolysins demonstrated first day after transfusion	0	0	0	0	0
Third day	0	0	0	0	0
Sixth day	0	±	0	0	±
Eighth day	+ + +	±	0	0	±
Twelfth day	+ + + +	0	±	0	±
Twenty-fourth day	+ + +	+	±	0	±
Clinical reaction after second transfusion	none	mild shock	shock	none	none
Twenty-eighth day	+ + + +	+ + + +	±	0	±
Fifty-second day	+ +	+	±	0	±
Produced immune haemolysins	A	Unidentified	H	none	H + natur. anti-J

philes, similar to changes normally observed in anaphylactic shock.

c) *Transfusions repeated with blood of the same donor after 24 days.*

This experiment was part of a series of transfusions undertaken at different intervals to study the possibility of shock reactions being caused by antibodies stimulated by the initial transfusion.

A group of five heifers similar in age and body weight to the previous groups was used. In both the initial and subsequent transfusions two litres of blood were given, the average duration of all transfusions being nine minutes. The results are shown in Table 3.

The initial transfusion stimulated about the same level of immune antibodies in both animals (Nos. 1243 and 1232). At the second transfusion, however, only No. 1243 showed a shock reaction. This animal had no naturally occurring isohaemolysins but No. 1232, which did not show shock, had isohaemolysins in a titre of 1/8 to 1/16. Both animals received the same amount of blood containing donor isohaemolysins in about the same concentration.

Recipient No. 1233 produced a high concentration of isohaemolysins after the initial transfusion and also did not show the slightest sign of shock at the second transfusion.

Haematological values were studied right through the period until well after the second transfusion. The tendency to decrease (see Figs. 2, 3 and 4 of previous paper [1]) after the first transfusion was again evident and after the second transfusion values dropped even lower. Normality was only reached 40 days after the second transfusion.

d. *Transfusions repeated with blood of the same donor after 77 days*

A group of Afrikaner heifers similar to the previous groups was used. The difference between these animals and those in the preceding experiment was that all, or nearly all, the immune antibodies stimulated by the initial transfusion had already disappeared at the time the second transfusion was given 77 days later (Table 4).

Table 4: TRANSFUSIONS REPEATED WITH BLOOD OF THE SAME DONOR AFTER 77 DAYS

Recipient (No. of animal)	1247	1251	1253	1254	1236
Recipient's blood containing naturally occurring haemolysins	+ + +	0	+ +	0	+ + +
Donor's blood containing naturally ocurring haemolysins	+ +	0	+ + +	+ + + + *	+ +
Expected immune haemolysins	P SA_1	O_3D' SA_3	GB'D'VL	C_2X_1 SA_3	$HE'_3O'Y'C_1V$
Clinical reaction after first transfusion	none	none	none	shock	none
Immune haemolysins demonstrated first day after transfusion	0	0	0	0	0
Third day	0	0	0	0	0
Fifth day	0	+	+	0	0
Eighth day	+ +	+ + +	+ + +	+ +	±
Twentieth day	+ +	+ + +	+ +	+ +	+
Fiftieth day	+ only anti-J	+ +	+ +	±	+
Seventieth day	only anti-J	±	±	0	0
Clinical reaction after second transfusion	none	severe shock	mild shock	none	none
Eighty-fifth day	+ + +	+ + + +	+ + + +	+ + +	+ + +
Ninety-fifth day	+ + +	+ + + +	+ + + +	+ + +	+ + +
Produced immune haemolysins	J SA_1 + ?	O D'SA_3	GLVJ	C_2X_1 SA_3	J, V + ?

*This donor had, besides anti-J, also naturally occurring anti-Y_2 in its serum.

For the first time in the whole series we observed one animal (No. 1254) which showed a clinical reaction after the first transfusion but remained normal at the second transfusion.

Prior to the second transfusion a slight haemolysin titre could still be demonstrated in animals Nos. 1251 and 1253. Both received two litres of blood at the same rate at the second transfusion and both reacted. No. 1251 showed alarming symptoms of severe shock in contradistinction to a very mild reaction in No. 1253. Haematological values showed the same tendencies as were observed in the previous group.

e. *Transfusions repeated several times with blood of the same donor*

The six heifers in this experiment were in body weight and age similar to the previous groups. The animals were divided into two recipient groups of which the first received blood from a donor with high titre haemolysins as a result of a previous transfusion. The donor of the second group possessed only weak naturally occurring haemolysins.

Each animal received four transfusions from the same donor; only one litre was transfused on each occasion, the transfusion time averaging four minutes. The time interval between the first, second, third and fourth transfusion was two, two and four days respectively. The results are shown in Table 5.

No shock symptoms were observed in any of the three animals transfused with blood containing a high titre of immune haemolysins. Shock, however, occurred in all three animals belonging to the group receiving from the donor with weak naturally occur-

Table 5: TRANSFUSIONS REPEATED SEVERAL TIMES WITH BLOOD OF THE SAME DONOR

Recipient (No. of animal)	1239	1269	1246	1235	1256	1267
Recipient's blood containing naturally occurring haemolysins	+ + +	0	+ +	0	+ +	+ + +
	One donor with immune haemolysins			One donor with natural haemolysins		
Donor's blood containing haemolysins	+ + + +	+ + + +	+ + + +	+	+	+
Expected immune haemolysins	GQY_2C_2F	GQY_2C_2Z	GQY_2X_2	GQE'_3C_2	GQE'_3C_2F	GQE'_3C_2FL
Clinical reaction after first transfusion (23rd Oct.)	none	none	none	shock	none	none
Haemolysins demonstrated the second day after transfusion	±	0	0	0	0	±
Clinical reaction after second transfusion (25th Oct.)	none	none	none	shock	none	none
Haemolysins demonstrated on fourth day after first transfusion	+ +	+ +	+ + + +	+ + +	+	+ +
Clinical reaction after third transfusion (27th Oct.)	none	none	none	none	none	none
Haemolysins demonstrated on eighth day after first transfusion	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Clinical reaction after fourth transfusion (31st Oct.)	none	none	None. Died next day	shock	shock*	shock
Haemolysins demonstrated 14 days after first transmusion	+ + + +	+ + + +		+ + + +	+ + + +	+ + + +
Haemolysins demonstrated 18 days after first transmusion	+ + +	+ + +		+ + +	+ + +	+ +
Produced immune haemolysins	Y_2	QY_2	Y_2	GC_2	C_2F	G

*This animal showed heartblock following the transfusion.

ring haemolysins. These results are discussed more fully at a later stage.

Heifer No. 1246 suffered from *Anaplasma marginale* infection which was only diagnosed at the time of the second transfusion. She was kept in the experiment on the assumption that the transfusions could be of therapeutic value, but the small amount of blood used each time proved inadequate and she died of the disease on the ninth day.

f. *Transfusions repeated in monozygous twins.*

In this series four pairs of monozygous twins were used. Two pairs were Jersey cows, three and a half years old, in different stages of gestation. Three of these animals were lactating. The other two pairs consisted of Red Poll heifers, twenty months old and Drakensberger bulls, about ten months old.

In these experiments it was intended to elucidate some of the results obtained before. In a previous experiment pooled blood at the second transfusion produced disastrous results. In this experiment therefore pooled blood was administered to several of the twins at the initial transfusion. Details and results obtained in two pairs of twins are given in Table 6.

T 165 and T 166 were lactating but T 166, which had calved only six weeks previously, gave 23.5 lb of milk compared to 10.5 lb for T 165, which was six months pregnant. Both animals received exactly the same treatment throughout the experiment. It may be assumed that any differences in the clinical reaction are due to their different physiological status. Compared with the consistently severe reactions observed with pooled blood at the second transfusion, the administration of 1½ litre pooled blood at the first transfusion had no effect on T 165 (six months pregnant) but caused shock in T 166 (high yielding animal).

Seventeen days later 950 ml of blood from one donor was transfused into each twin. In both animals respirations increased from 24 to 78. Before the start of the second

Table 6: TRANSFUSIONS REPEATED IN MONOZYGOUS TWINS USING POOLED BLOOD IN THE INITIAL TRANSFUSION

Recipient (No. of animal)	T 165 (Jersey)	T 166 (Jersey)	T 171 (Jersey)	T 172 (Jersey)
Recipient's blood containing naturally occurring haemolysins	0	0	0	0
Donor's blood containing naturally occurring haemolysins	0	0	0	0
Donor's blood used for first transfusion	Blood of 3 donors pooled		One donor	4 donors pooled
Expected immune haemolysins	22 diff.	22 diff.	8 diff.	24 diff.
Clinical reaction after first transfusion	none	mild shock	mild shock	shock, severe later
Haemolysins demonstrated on second day after transfusion	0	0	0	0
Haemolysins demonstrated on seventh day	+ +	+ +	+	+ + +
Haemolysins demonstrated on fourteenth day	+ + + +	+ + + +	+ + + +	+ + + +
On the seventeenth day administration of second transfusion with the same blood in all cases (one donor). Clinical reaction immediately after:	shock heartblock*	shock	none*	none
Three—four days later	severe shock	severe shock	none	shock
Two days later	aborted	very ill**	normal	oedema
No further tests performed.				

*Nephritin prophylaxis acc. to Marx [2].

**Two days later the following treatment was given: An infusion of 250 ml "Myrilos" (B.W.) containing Calcium-borogluconate, Phosphorous, Magnesium, and Dextrose was given after the injection of five cc. Meticorten (aqueous suspension of prednisone 40 mg/cc (Sherag).

transfusion T 165 was treated prophylactically with Nephritin (Marx[2]) but this did not minimise the state of shock which developed. The symptoms in T 165 were in fact more severe than in T 166. In both animals clinical symptoms persisted and four hours later they were still severely ill. T 165 aborted two days after the transfusion. T 166 was kept alive with great difficulty. Milk production ceased completely in both animals, whereas the first transfusion of pooled blood had had virtually no effect on latation.

The other Jersey twins, T 171 and T 172 were also given two transfusions at a seventeen day interval. T 171 was two months pregnant while T 172 was in milk but not in calf. T 171 received blood from a single, although different, donor on the two occasions, while the blood of four donors was pooled for the initial transfusion in T 172, followed by blood from a single donor at the second transfusion.

The single donor blood administered to T 171 contained eight different red cell antigens and produced signs of a mild reaction. T 172 reacted very severely to the pooled blood containing 24 differing red cell antigens.

No immediate reaction was observed at the time of the second transfusion when both animals received blood from the same donor. T 171 was treated prophylactically with Nephritin and remained normal throughout. T 172, however, suffered from a delayed reaction and oedematous swelling of the lower abdominal wall, which persisted for about two weeks.

Two donors chosen specifically for the great number of differing red cell antigens (15 and 16 respectively) were used for transfusions into twin Red Poll heifers A and B (Table 7).

The same amount of blood (1100 ml) was used for both animals at the initial transfusion without causing any clinical effect.

Seven days later the transfusions were repeated using the same donor. Twin B received 1500 ml and developed very severe shock with all the symptoms of collapse. In spite of treatment by 50 mg Phenergan (R) (M&B) and one litre of physiological saline, the animal died during the night. On observing the severe reaction of this animal,

Table 7: TRANSFUSIONS REPEATED IN MONOZYGOUS TWINS USING VARIOUS DONORS

Recipient (Animals Marked)	Twin A (Red Poll)	Twin B (Red Poll)	Twin C (Drakensb.)	Twin D (Drakensb.)
Recipient's blood containing naturally occurring haemolysins	0	0	0	0
Donor's blood containing naturally occurring haemolysins	0	0	0	0
Donor blood used for first transfusion	One donor	one donor	one donor	pooled blood
Expected immune haemolysins	15 different haemolysins	16 different haemolysins	8 different haemolysins	31 different haemolysins
Clinical reactions after first transfusion	none	none	none	none
Immune haemolysins demonstrated first day after transfusion	0	0	0	0
Third day	+ +	+ +	0	+
Sixth day	+ + +	+ + + +	+.	+ + + +
Administration of second transfusion, the same donors for twin A and B, the same pooled blood for C. and D. Clinical reaction:	shock	severe shock, oedema*	none	none
Immune haemolysins demonstrated tenth day after first transfusion	+ + + +		+ + + +	+ + + +
Third transfusion administered ten days following the second transfusion with blood from the same single donor; transfusion reaction:	severe shock— died next day			

*Treatment: 50 mg Phenergan to one litre phys. saline. Very severe, died overnight.

the transfusion on twin A was stopped after administering 1200 ml of blood. This animal also suffered severe shock but survived. The impression was gained that she would have died had the total amount (1500 ml) of blood been given. Ten days after the second transfusion 1000 ml of blood from the same donor as on the previous occasions was given to twin A. She developed shock within two minutes of starting the transfusion, showing trembling, dyspnoea, urination, lacrymation, grunting and diarrhoea. The animal died within 24 hours.

The Drakensberger twins were used to study the effect of possible breed resistence to the incidence of reactions since Drakensbergers are known to be very hardy animals.

The twins received one litre of blood at three consecutive transfusions at a rate of about 300 ml per minute. The second transfusion was given seven days after the first and the third after an interval of ten days.

Twin C initially received blood from a single donor differing in eight red cell antigens. The second transfusion consisted of pooled blood from five animals. No reaction was observed. For the third transfusion another single donor was used. A moderate reaction developed shortly after the transfusion but the animal recovered completely within a short time.

Twin D was the only animal in the whole series to receive pooled blood at the first and second transfusions from three and five donors respectively. No reaction developed. When receiving blood from a single donor at the third transfusion this animal reacted similarly to his twin.

A summary of results of all repeated transfusions is given in Table 8.

DISCUSSION

Immunological aspects.

Complete blood group determinations were undertaken in these experiments. Careful scrutiny of the results failed to identify a single blood factor or combination of factors (groups) which appeared to be responsible for or relatively more important in transfusion reactions. Such reactions can be expected to occur when the donor possesses strong naturally occurring antibodies and the recipient strong antigens on the red cells alone, or on the red cells and in the serum, or *vice versa*.

Much attention was paid to the J system in cattle blood groups. The latter is the only one of the 11 systems known in which the antigen is detected by naturally occurring antibodies; a situation similar to the ABO-blood group system in man. In South Africa naturally occurring antibodies were demonstrated in 22.7 per cent of all bovines and 82.7 per cent of these were anti-J (Osterhoff [3]).

Here again, the present studies failed to yield conclusive results. In 41 initial transfusions where either the donor's or recipient's blood contained natural antibodies, only five transfusion reactions resulted. The same holds true for the effect of the level of produced antibodies on the incidence of reactions. As example, recipient 1233 shown to possess strong antibodies with a titre higher than 1 : 64, had no reaction after repeated transfusion, but recipient 1243 on which the haemolysis test produced only trace reactions, developed severe shock following a repeated transfusion.

Neimann-Sorensen [4] similarly failed to demonstrate any correlation between the occurrence of anaphylactic reactions and demonstrable antibodies. This adds further weight

Table 8: SUMMARY OF RESULTS OF REPEATED TRANSFUSIONS

	No Transfusion	Shock or death of animals	Total
Transfusion repeated before the fifth day following the initial transfusion	11	1	12
Transfusions repeated after the fifth day up to two months following the initial transfusion	12	7 (1 dead)	19
Transfusion repeated with pooled blood	0	5 (3 dead)	5
Transfusion repeated in lactating cows (twins)	1	3 (1 abort.)	4
Transfusion repeated three and more times	2	7 (2 dead)	9
Total	26	23	49

to our conclusions that the presently known blood group antigens alone are not the only reason for post transfusion shock. We believe that hitherto undetected antigens may be partly or wholly responsible for transfusion reactions. These antigens might give rise to antibodies simultaneously with those which can be demonstrated with present techniques.

The above supposition emphasises the unreliability of the present *in vitro* tests and an *in vivo* test such as the "biologische Vorprobe" described by Marx[2]. The "trial injection" (Braend[5]; Wujanz & Rittenbach[6]) seems to be the safest and most practical at present. The basis of these tests is the injection of a small amount of blood (100—200 ml.) into the recipient and waiting from 10 to 30 minutes to see if any signs of shock develop before proceeding with the rest of the transfusion.

It is evident that further research work on the interaction of the different blood constituents and characteristics between the donor and recipient must be carried out to elucidate the causes of transfusion reactions. The best approach would be to separate the erythrocytes from the plasma and perform transfusions of the constituents of blood separately in comparatively large amounts.

Incidence of transfusion reactions.

In the series of 85 young animals[1], 11 showed a reaction after the first transfusion (three severe) which yields an incidence of about 13 per cent. Compared to this, nine cases occurred in 22 first transfusions given to pregnant cows; the incidence here being 41 per cent.

Apart from this, shock occurred in a high yielding monozygous twin while the other twin, six months pregnant, did not show a reaction. This observation supports the statement of Schmid[7] about the increased incidence in high yielding cows.

From the practitioners point of view these results indicate that, without any prior tests for compatibility, about one out of eight or nine first transfusions is likely to result in a reaction, and the risk is greatly increased in high yielding cows.

Interval between transfusions.

The interval for the appearance of isoimmune antibodies after the first transfusion is variously given as 3 to 5 days[8], one week[9], 10 days[10] and 3 to 10 days[11].

In the present experiment the antibodies appeared at an average of 5.9 days, with a negligible variation.

It is commonly accepted that the appearance of antibodies significantly increases the risk of transfusion reactions but that repetition of transfusions before their appearance is safe. These ideas are supported by the present results. Repeated transfusions before the fifth day after the initial transfusion resulted in one reaction in 12 procedures: a yield of about eight per cent which corresponds closely to the 13 per cent found in the overall group of first transfusions. In transfusions repeated after the fifth day, the incidence rose to 35 per cent where the same donor was used; one fatal reaction occurred in 19 cases.

If the animals were given repeated transfusions after the fifth day with pooled blood, or from different donors, reactions were observed in 47 per cent of cases, with five fatalities in 18 procedures.

After the appearance of antibodies, there is a slow disappearance occupying from 36 to 116 days. It was clearly demonstrated, however, that the disappearance of antibodies had no effect on the once sensitized body; for that matter the presence of demonstrable antibodies is not necessary for the appearance of reaction symptoms, and a prolonged interval after the first transfusion does not diminish the incidence of reactions at subsequent transfusions.

From these results it is concluded that, provided the first transfusion was performed without mishap, repeated transfusions from the same donor can be given up to five days after the initial procedure without fear of a reaction. After this period the procedure becomes unsafe and remains so, in all probability for the rest of the animal's life.

Choice of donor.

Ferguson[9] stated that if a cow be immunized with the blood of her daughter, the resulting immune serum would be a relatively simple one (consisting of only a few antibody types) and the post transfusion reaction in such cases would be generally mild or even completely lacking.

In the present experiments on monozygous twins evidence to support this statement was inconclusive. One Drakensbereger twin received blood with 8 incompatible antigens compared to 31 in the other twin. The latter responded with a marked elevation in anti-

bodies by the sixth day whereas the other showed only a slight concentration. The clinical reactions were identical however, i.e. both showed shock after the third transfusion. The difference in reactions between twins 165 and 166 receiving the same number of different immune haemolysins can be ascribed to high milk production in the one reacting more severely. In fact no significant difference was demonstrated which could be ascribed to the number and complexity of incompatible antigens affecting the incidence or severity of reactions:

An interesting approach to the problem would be the use of donors with only a few demonstrable antigens on their red cells. It is doubtful if a search after "universal donors", the blood of which could be stored according to modern methods, would be successful, but the matter demands consideration. At this stage no recommendations regarding the ideal donor can be made.

Speed of infusion.

Schermer [8] recommended that blood should be infused at the rate of one litre in 3—4 minutes while Simpson *et al* [12] administered at any desired rate. Sen'Kov [13] transfused 60—65 ml/min. and Irwin [10] 100 ml/min.

Ryksen [14] thought that the administration of one gallon of blood in two hours was so fast that it resulted in the death of the animal and Lotze [15] recommended stopping the transfusion for 15—30 min. after every two litres to enable the circulation to adjust. Archer *et al.* [11] emphasised that large volumes of blood should be given slowly (1 litre/10 min.) but Hall *et al.* [16] commented on this statement and pointed out that the speed of administration depended essentially on the individual case.

In the present series the amount of blood infused varied from 50 ml to more than 250 ml per minute. The recipients tolerated these procedures without showing any signs of shock or circulatory overloading, but in ill animals caution is necessary and the infusion speed should be regulated according to the needs and reaction of the individual animal.

Haematological observations.

Details on haematological observations were given in the previous paper [1]. It was shown that a drop in haematological values occurred in many cases and that in practice a sufficiently big transfusion to save the animal's life entails a possible anaemia up to 14 days after the transfusion. As a second transfusion after five days becomes dangerous, every possible method should be employed to stimulate haemopoiesis to counteract the possible fall in haematological values.

Clinical observations on transfusion reactions.

The clinical picture observed was similar to those described by Ferguson [9], Marx [2], Sen'Kov [13] and Schmid [7].

In the transfusion reactions noted there was a significant increase in respiratory rate from an average of 42 to 60 respirations a minute. This increase occurred during the procedure and the onset was evident after the first 200 to 400 ml blood had been transfused. Body temperatures remained constant and the pulse rate did not increase significantly although a few cases of intermittent heart block were observed.

In the cases ending fatally the severity of the symptoms could not be related to the eventual outcome; some fatal cases hardly showed any clinical signs of shock. Haemoglobinuria was, however, observed in all the fatal cases as well as in cases which were ill for several days. The pigmented urine was sometimes passed before the end of the transfusion.

Treatment.

Ferguson [9] observed that transfusion reactions were decreased in some cattle by the intravenous injection of 2 to 10 ml solution of 1 : 10,000 adrenalin. Marx [2] also found that the number of reactions were decreased after the subcutaneous administration of 10 ml of 1 : 4,000 Nephritin solution, although this treatment was not successful in every case. Schmid [7] recommended the same treatment and mentioned the possibility of using Rutin (1000 mg/Kg) as it influences the permeability of bloodvessels. Schutte [17] used 3 to 4 ml of a 1 : 1000 solution of adrenalin subcutaneously or intravenously and added 90 mg Methedrine (Burroughs Wellcome) intramuscularly.

In the single case where Nephritin was used prophylactically, the evidence was inconclusive. On Sen'Kov's [13] findings on the persistence of pathological lesions in spite of amelioration of symptoms with treatment, it seems unlikely that treatments presently in vogue will contribute materially to the outcome of post transfusion shock.

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BOOK REVIEW

DISEASES OF THE CANINE EYE

F. G. STASTUP

Baillière, Tindall and Cassell, London 1969. Pp vii+387 Figs. 272+50 in Colour. £6.50.

It is indeed satisfying to find that there is an ever increasing number of textbooks concerned with well defined specialities to grace the bookshelves of those seeking specialist knowledge in any field. The book under review is a worthy addition to the existing works on veterinary ophthalmology but its subject matter is confined to the dog. With the very extensive bibliography the interested reader can browse further afield. Although the contents of the book will be fairly familiar to those having access to good libraries, it is recommended to everybody requiring the latest information on ophthalmology of the dog.

Besides the customary outline of the anatomy and examination of the eye, medical treatment as well as the required operating theatre and ophthalmic instruments are described. The anatomy and physiology of individual parts of the eye and their diseases and treatment are then dealt with. The illustrations are clear, those in colour being particularly informative.

Some conditions merit more attention, e.g. the keratitis peculiar to German Shepherds is barely mentioned and the special operation not at all, although this condition certainly deserves a heading to itself.

The reviewer also wonders whether more detailed reference to the pathology, supported by a few histopathological illustrations should not be contemplated in a future edition. Similarly the extra-ocular physiology could be presented in somewhat greater detail. It is possible that a grouping together of discussions of ocular signs pointing to non-infectious disease elsewhere for instance, is mentioned. There are other signs which point to lesions in specific parts of the brain.

The above remarks, however, must not be interpreted as detracting from the essential value of the book.

C.F.B.H.

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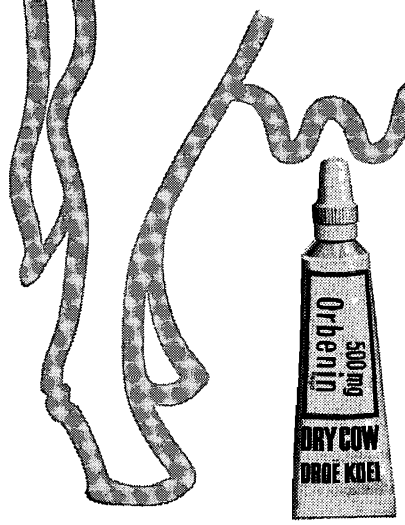

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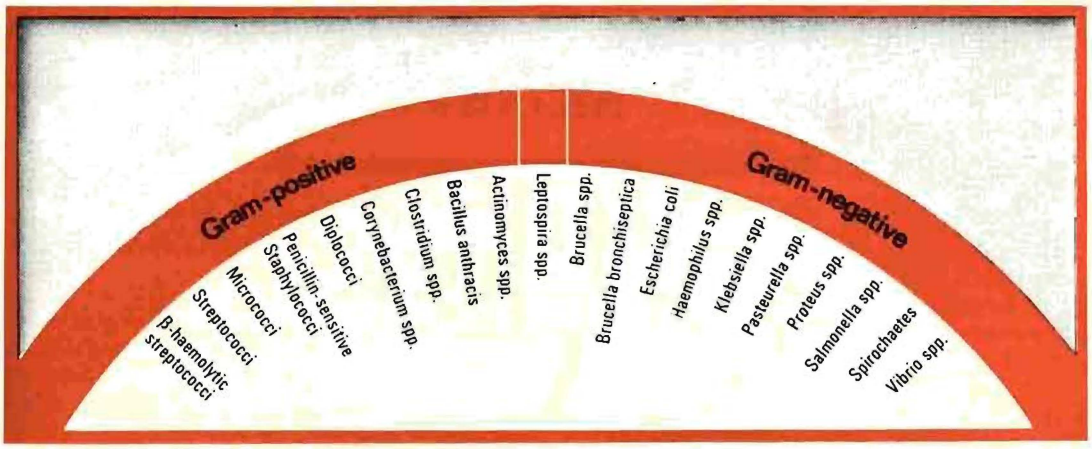

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Jl S. Afr. vet. med. Ass. 40 (3): 279-283 1969

STERILITY IN A BULL, CO-TWIN TO A FREEMARTIN *

W. H. GERNEKE**

SUMMARY

An infertile bull with bilateral hypoplastic subcutaneous testes and co-twin to a "freemartin" is described. When killed at 21 months of age it had a chimeric ratio of 77 per cent 2A-XX cells versus 23 per cent 2A-XY cells in its haemopoietic tissues. Although alluring, chimerism cannot be accepted without further evidence as the primary aetiological factor of the incomplete testicular descent. Adrenocortical and hypophyseal changes indicating a hormonal imbalance and the possibility of a fortuitous coincidence of a genetically determined cryptorchid are complicating aetiological factors. This is the first case on record of sterility in the male co-twin of a freemartin.

INTRODUCTION

It is a well known fact that in about 90% of heterosexual bovine twins the heifer is sterile and accordingly known as a "freemartin"[1]. Both individuals always reveal chimerism[2] as a result of the allantochorionic anastomoses established between the twins when the CR length is as little as 3.75mm[1]. Such dizygotic twins are also tolerant to homografts[3,4] and possess identical red blood cell antigens and transferrin types[1]. Allantochorionic anastomoses also occur between similarly sexed twins but without detrimental effect.

Chimerism is diagnostic for early freemartinism[2]. XX/XY-chimerism can, however, also occur in various tissues not as a result of chorionic anastomoses but following on dispermic fertilization and subsequent fusion of two ova or fertilization of an ovum and its non-extruded polar body[5]. Such animals are hermaphrodites.

At birth chimerism is usually present and approximately on a 50 : 50 basis, but during the adult stage the relative percentages of male and female cells may gradually become increased or decreased in either individual, probably as a result of chance alone. Although two cases have been reported of the male co-twin having more female than male haemopoietic cells soon after birth[2,6], no single case has ever been reported of the male co-twin being sterile.

In this article chimerism and certain other aspects of an infertile Ayrshire male co-twin to a freemartin are described.

MATERIAL AND METHOD

In the course of routine blood group determinations by Prof. D. R. Osterhoff of the Dept. of Zootechnics, it was revealed that a certain pair of purebred Ayrshire heterosexual twins had identical blood groups. The heifer was accordingly expected to be a freemartin but the owner was unwilling to part with it. Subsequent chromosome studies on both the twins established chimerism (see Table 1) and also revealed that the male had a greater percentage of female than male cells in its bone marrow. As a result of this discrepancy the male co-twin was obtained for further chromosome studies. The method used for these studies was that described previously[1].

Table 1: INDICATING GRADUAL SHIFT IN FAVOUR OF FEMALE MYELOID CELLS IN THE MALE CO-TWIN

Animal	Age	Percentage ♀ spreads	Percentage ♂ spreads	Total number of spreads counted
Heifer	1 month	57	43	83
Bull	1.5 months	58	42	170
	4.5 months	55	45	30
	12 months	67	33	58
	20 months	74	26	137
	21 months	77	23	222
		77	23	230

* Paper read at the Annual Conference of the Anatomical Society of Southern Africa held in Durban, May 1969.

** Dept. of Anatomy, Faculty of Veterinary Science, University of Pretoria, P.O. Onderstepoort.

The bull was kept under observation for 21 months after which it was killed and material for histological study taken from the sexual and endocrine organs.

OBSERVATIONS

At three months post-natal age both testes had only reached the caudal end of the inguinal canal (Fig. 1, B & C). At twenty months the animal was tested for fertility and found completely sterile with very little libido. It preferred sucking teats to mounting cows on heat!

Although relative percentages of male and female cells at approximately one month of age were only slightly in favour of female cells in the male, later determinations indicated a gradual increase in female cells and a decrease in the number of male cells (Table 1). On slaughtering the animal at 21 months of age its final chimeric ratio was 77 per cent 2A-XX/23 per cent 2A-XY cells.

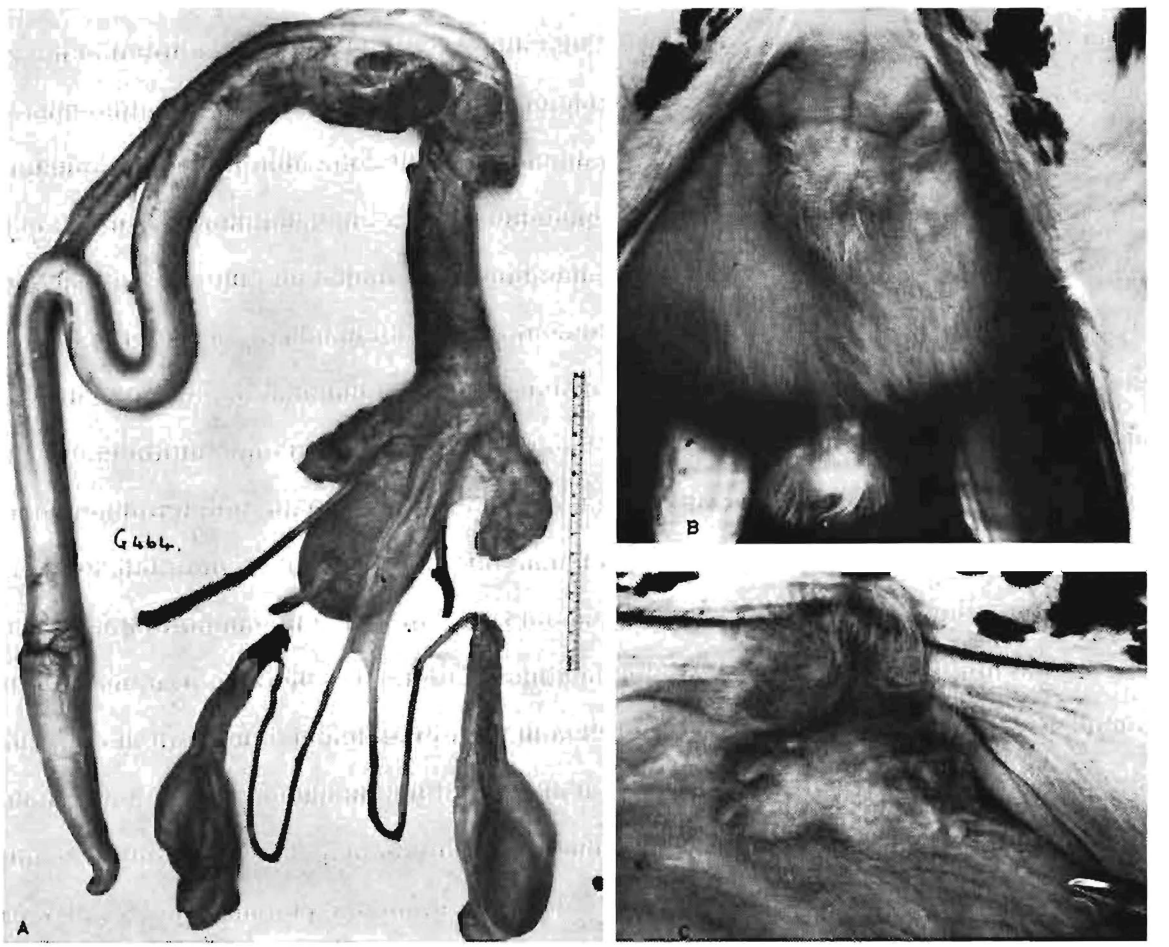
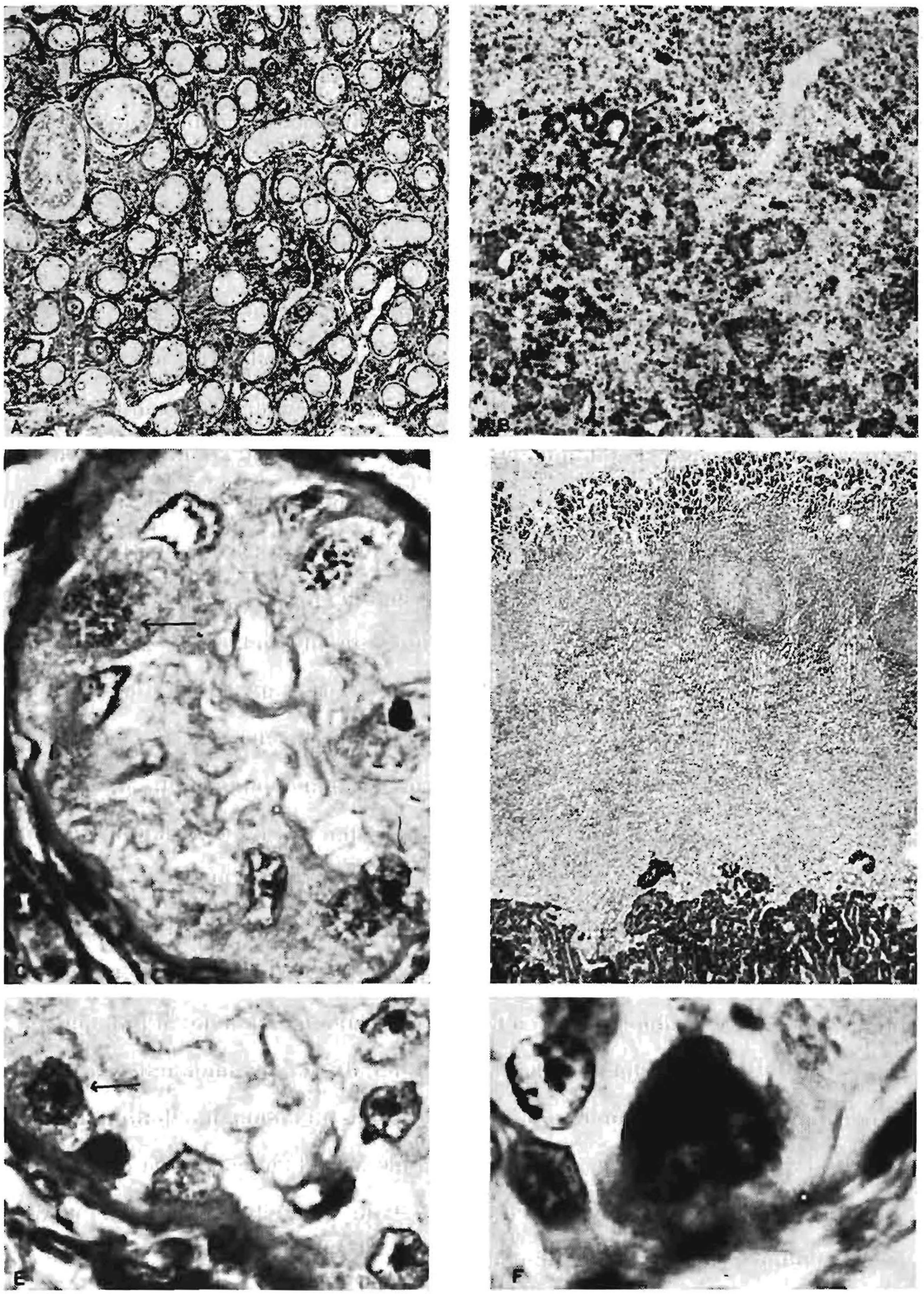


Fig. 1 A. Genital organs of the male co-twin.
B. Caudal view with the testicles latero-dorsal to the scrotum.
C. Ventral view, with teats and right testicle visible at the point of the pair of scissors.

Fig. 2 A. Hypoplastic seminiferous tubules with interstitial cells of Leydig relatively increased X75 H. & E.
B. Hyperplasia and follicle formation of Alpha cells of the anterior lobe of the hypophysis X75 M.Azan.
C. A primary spermatocyte as seen in a hypoplastic seminiferous tubule. The basal lamina is hypertrophied. X 1200 H.E.
D. The adrenal cortex revealing the focal normal to hypertrophied areas amid a generally atrophic cortex. X30 H. & E.
E. A spermatogonium revealing its extremely basophilic nucleolus X1200 H. & E.
F. A spermatocytic giant cell surrounded by Sertoli cells. X 1200 H. & E.



At the time of its death the animal still had a relatively small build which closely resembled that of a female with the exception of the horns which were typically male. The scrotum was comparatively small but the four teats were each about an inch long (Fig. 1C). It had quite a docile nature.

The testes revealed bilateral hypoplasia in an extreme form (Fig. 2A). They were flaccid, dry on sectioning and without any sign of spermatogenesis. Most seminiferous tubules were reduced in diameter, with a thickened and often folded basement membrane (Fig. 2C) and were lined by only a few Sertoli cells and very rare spermatogonia or primary spermatocytes (Fig. 2, C & E). The latter occasionally revealed meioses but no stage beyond the first meiotic division was ever encountered. Instead, degenerative changes such as eosinophilia and vacuolization of the cytoplasm, and margination of the chromatin as well as pycnosis and karyolysis were present in the majority of these cells. Spermatocytic polyploidy and giant cell formation (Fig. 2F) were encountered in a few tubules. The spermatogonia were easily differentiated from the Sertoli cells by their more basophilic nucleoli (Fig. 2E).

The interstitial cells of Leydig were relatively increased in number, some of them revealing mitotic figures while others in contrast, revealed degenerative changes such as pycnosis and condensation of cytoplasm. The accessory male glands were well developed, but quite inactive. The penis was well developed.

With the exception of scattered nodular to polygonal areas of the adrenal cortex which contained normal or slightly hypertrophic cells the rest of the cortex revealed a greater or lesser degree of atrophy (Fig. 2D). The pars distalis of the hypophysis revealed excessively degranulated basophils in its central area. The basophils gave a positive aldehyde-fuchsin reaction, were PAS-positive and revealed an occasional polyploid nucleus. Some resembled the so-called "castration cells". The alpha cells were tightly packed with granules and revealed some hyperplasia and follicle formation (Fig. 2B). No discrepancies were observed in the fusion of the epiphyseal cartilage plates of the long bones.

Polymorphic sexing revealed only four atypical drumsticks and 30 sessile nodules in 2 500 neutrophils counted.

DISCUSSION

The question to be answered here is whether the incomplete descent of the testes was primarily caused by the predominance of female myeloid cells or perhaps by other factors such as an autosomal recessive gene[8], prenatal vitamin deficiency[7] or some other unknown cause.

Ohno *et al*[6] and Kanagawa *et al*[2] have each mentioned a case of female cell predominance (76 per cent 2A-XX/24 per cent 2A-XY and 62 per cent 2A-XX/38 per cent 2A-XY, respectively) in a male co-twin without any abnormalities occurring. Both, however, were only studied soon after birth. As the descent of the testicles in the bovine has just about been completed in a foetus of 3 months (31.5 cm CR length)[9] and the approximate chimeric ratio at this stage would at the most be 50 : 50 (assuming that free mixing of blood through chorio-allantoic anastomoses had taken place) it can be assumed that many more sterile male co-twins would be born if the descent could so easily be retarded by excess X-chromosome enzymes. This retarding influence would have been exerted before the foetal age of 3 months at which age female cell predominance would not have been so evident as at the post-natal age of 21 months. As no previous cases of sterility in a male co-twin have ever been reported one cannot without further confirmatory evidence consider female cell predominance as the primary aetiological factor especially not if Ohno's and Kanagawa's findings are taken into account. On the other hand it can be argued that although the Y-chromosome has a suppressing influence on the X-chromosome (compare XXY — i.e. Klinefelter syndrome) the reciprocal effect viz. that extra X's present have a retarding influence on the expression of the Y-chromosome genes is also true. In this case only extra X containing cells were present and their only effect could have been a retarding influence on body growth but not on testicular descent which should have been retarded at a much earlier age.

Aspermatogenesis in the case under review can be ascribed to the position of the testes in the caudal extremity of the inguinal canal where the temperature differential essential for mammalian spermatogenesis was absent. The histological picture, however, revealed more advanced hypoplasia than would be expected in a cryptorchid testis. Degeneration of the spermatogonia was so obvious

that the impression was gained that, had the animal lived longer spermatogonia would have completely disappeared.

The owner most emphatically denied the existence of cryptorchidism in his herd, thus, although it seems unlikely that this was a possible cause of the degenerative changes in the spermatogonia and primary spermatocytes the rare chance of a spontaneous mutation having occurred must be considered. The fortuitous existence of a cryptorchid as the co-twin is thus a rare possibility.

An explanation of the possible aetiology of the condition is further complicated by the adrenocortical and hypophyseal changes. Although no hormonal assays were undertaken, the histological picture clearly indicated an hormonal imbalance but whether as a cause or effect is uncertain. Degranulation of the beta cells in the hypophysis can be ascribed to the push-pull mechanism of ACTH secretion whereas the lack of active secretion in the hyperplastic alpha cells tightly packed with granules might have been a contributory factor to the rather small build of the animal. Of special interest is the fact that the focal lesions of the adrenal cortex closely resemble the focal hypertrophy encountered in the adrenal cortex of the intersexual pig described by Gerneke[1]. Could this possibly have a functional significance?

The only explanation that can be given to the absence of typical drumsticks in neutrophils notwithstanding the high percentage of female cells present, is that the Y-chromosome enzymes must to some extent have had a suppressing influence on drumstick formation which resulted in the presence of a considerable number of sessile nodules. The latter in the bovine are also a positive indication of the female sex[1].

Until further comparative evidence becomes available the only conclusion to be drawn at this stage is that a hormonal imbalance was responsible for the condition and that female cell predominance could have been the effect thereof rather than the cause.

ACKNOWLEDGEMENT

I wish to thank Dr. R. I. Coubrough of this Faculty for conducting the fertility tests and for his general interest throughout.

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LETTER TO THE EDITOR

With reference to the article on "Blastomycotic mastitis in South Africa" by W. H. Giesecke, Ellen E. Nel and L. W. van den Heever in the *Jl. S. Afr. vet. med. Ass.* 39(3): 69—85, I wish to report that the new species of Candida to which reference is made in table 5 and which was isolated from a case of bovine mastitis has now been verified as a *sp. nova* and named *Candida steatolytica* by Dr. Yarrow 1969 *Antonie van Leeuwenhoek* 35 : 24—28. The organism has strong fat hydrolyzing characteristics.

W. H. GIESECKE
Veterinary Research Institute Onderstepoort

Jl S. Afr. vet. med. Ass. 40 (3): 284-289 1969

A BIOCHEMICAL EXPLANATION FOR RESISTANCE TO ORGANOPHOSPHATE IXODICIDES SHOWN BY A STRAIN OF BLUE TICK *(BOOPHILUS DECOLORATUS)* FROM SOUTH AFRICA

R. J. HART AND P. BATHAM*

SUMMARY

The activity and reactions of cholinesterases from organophosphate susceptible and resistant Blue ticks *(Boophilus decoloratus)* from Southern Africa have been investigated *in vitro*. The cholinesterase of the organophosphate resistant strain of *B. decoloratus* was found to differ from the normal enzyme in possessing a lower total activity and by containing at least two and probably three enzyme types which differ in their reaction with organophosphates. These changes were similar to those found in two strains of organophosphate resistant cattle ticks *(B. microplus)* from Australia.

It was concluded from these laboratory findings that the resistant Blue tick would show approximately the same tolerance in the field as the less resistant of the two Australian cattle tick strains, and that the South African ticks would not present as great a problem in control as the very highly resistant ticks now found in Australia.

INTRODUCTION

An acquired resistance to organophosphate treatment was found in the Cattle Tick *(Boophilus microplus)* on a property in Queensland (Australia) and reported in 1964[1]. A second strain showing a much greater degree of resistance was later discovered in another part of the State[2]. Biochemical investigations led to the conclusion that the primary cause of the resistance was a change in the character of the tick cholinesterase, this enzyme being the principal target for the organophosphate, and its inhibition causing directly or indirectly the death of the parasite[3, 4].

Following a report of the occurrence of organophosphorous resistance in a strain of Blue tick *(Boophilus decoloratus)* in South Africa[5], the opportunity arose to examine the cholinesterase of this and other strains of *Boophilus decoloratus* from Southern Africa for enzymic changes which could account for the resistance. The characteristics of the *in vitro* reactions between the tick enzyme and organophosphates are reported and compared with results obtained with the various susceptible and resistant strains of *B. microplus* from Australia.

MATERIALS AND METHODS

(a) *Tick strains*

Cultures of all the tick strains used in this work are maintained in these laboratories. The origins of *B. decoloratus* — Berlin (D) strain have been reported by Shaw *et al*[5]; briefly this is a field strain from South Africa which has shown resistance to control by organophosphates. Its distribution is restricted to a relatively small area of the Eastern Cape Province, north of the Buffalo River.

With the movement of cattle from the area during the drought of 1967—68, this strain must be expected to spread to other areas of the Republic (Thompson and Baker —personal communication). The strain of *B. decoloratus* designated ACR showed resistance to treatment with arsenic and chlorinated hydrocarbons, and is similar to if not identical with the specimens described by Whitehead in 1959[6]. The wild strain of *B. decoloratus* designated P strain was brought from Lesotho where it had had no known contact with insecticides and is presumed therefore to be susceptible to all ixodicides.

The strains of Cattle Tick *(B. microplus)* have been described by Shaw[7, 8]. Strain Z is the wild strain from Australia (brought from the laboratories of the C.S.I.R.O.), normally susceptible to organophosphorous ixodicides. Strains M (Ridgelands) and L (Biarra) are both field strains from Queensland

* Cooper Technical Bureau, Berkhamsted, Herts. U.K.

showing resistance to organophosphorus compounds, Ridgelands (M) strain has a factor of resistance to dioxathion of approximately 25 times compared with normal Z strain and Biarra (L) strain shows a factor of approximately 75 times [8].

For quick reference the strains are summarised:

D — *B. decoloratus* resistant to organophosphorus compounds.

ACR — *B. decoloratus* susceptible to organophosphorus compounds; resistant to arsenicals and chlorinated hydrocarbons.

P — *B. decoloratus* normally susceptible.

L — *B. microplus* very resistant to organophosphorus compounds.

M — *B. microplus* resistant to organophosphorus compounds.

Z — *B. microplus* normally susceptible.

(b) *Experimental*

In order to avoid interference from enzymes in the bovine blood meal of the adult tick, unfed larvae were used in these experiments. The larvae, reared under controlled conditions, were used 10—20 days after emergence.

Larvae were anaesthetized, weighed in bulk and homogenized in deionised water (at 5 or 10 mg larvae per ml) in an ice cooled, all glass, tissue grinder.

Homogenates were used within an hour of preparation, cholinesterase being assayed by the highly sensitive method of Ellman, Courtney, Andres, and Featherstone [9]. The reaction mixtures were made up of: 0.4 ml of enzyme homogenate, 0.1 ml of 0.01M 5,5'dithiobis-2-nitrobenzoic acid (Sigma) and 0.02 ml of 0.075M acetylthiocholine iodide (Sigma) and 2.5 ml of 0.0375M phosphate buffer (pH 7.2) and change in absorption with time was followed at 412 $m\mu$ (at 37°C). Change in optical density was corrected for non-enzymic hydrolysis of substrate and for the natural colour of the homogenate before calculation of enzyme activity.

Various concentrations of inhibitors, in 0.1 ml of water, were added to the incubating reaction mixtures at the appropriate interval before the addition of acetylthiocholine. Inhibition stopped on the addition of substrate.

For comparison, some assays of enzyme activity were also carried out using the residual acetylcholine method of Hestrin [10].

The inhibitors used were:

1. Paraoxon, diethyl 4 - nitrophenyl phosphate — Cooper Technical Bureau.
2. Coroxon, diethyl 3-chloro-4 methyl-coumarin - 7 yl-phosphate — Cooper Technical Bureau.
3. Supona (Chlorfenvinphos), diethyl 2 chloro-1-(2,4 dichlorophenyl) vinyl phosphate — Shell Research Ltd.
4. DDVP, dimethyl 2 : 2 dichlorovinyl phosphate (Dichlorvos) — Shell Research Ltd.

The compounds selected are all direct inhibitors of cholinesterase.

(c) *Analysis of results*

The regressions of log residual enzyme activity on time were fitted by a least squares method using an ICL 1904 computer. The method used for calculating bimolecular rate constants (K_2) from the slope of the regression is as follows:

From the general case (based on Aldridge [11]),

$$R_t = R_o e^{K_2 It}$$

Where R_t is the percentage residual activity, R_o the antilog of the abcissa intercept, I the molar concentration of inhibitor, and t the time (in minutes) of incubation with inhibitor.

For a given inhibitor concentration,

$$R_t = R_o e^{-Ct}$$

Where C is the slope of the regression fitted

hence $K_2 It = -Ct$

and $K_2 = -C/I$

The use of a computer was of particular value where there were two enzymes which were inhibited at different rates as in the case of those from the resistant ticks; additionally, confidence limits could be established.

RESULTS AND DISCUSSION

The uninhibited cholinesterase activities of the resistant and susceptible strains of *B. decoloratus* are given in Table 1. The susceptible P strain hydrolysed acetylcholine and acetylthiocholine faster than the organo-

phosphate resistant D strain. The reactions between cholinesterase from the three strains of *B. decoloratus* and the four organophosphates, expressed as the log residual activity were all linear with time of inhibition indicating that these reactions obeyed first order kinetics and were essentially irreversible. With the two organophosphate susceptible tick strains the plot projected to zero inhibition time indicated 100% residual activity (see Fig. 1); similar projections for the resistant tick cut the abcissa at the 50—60%

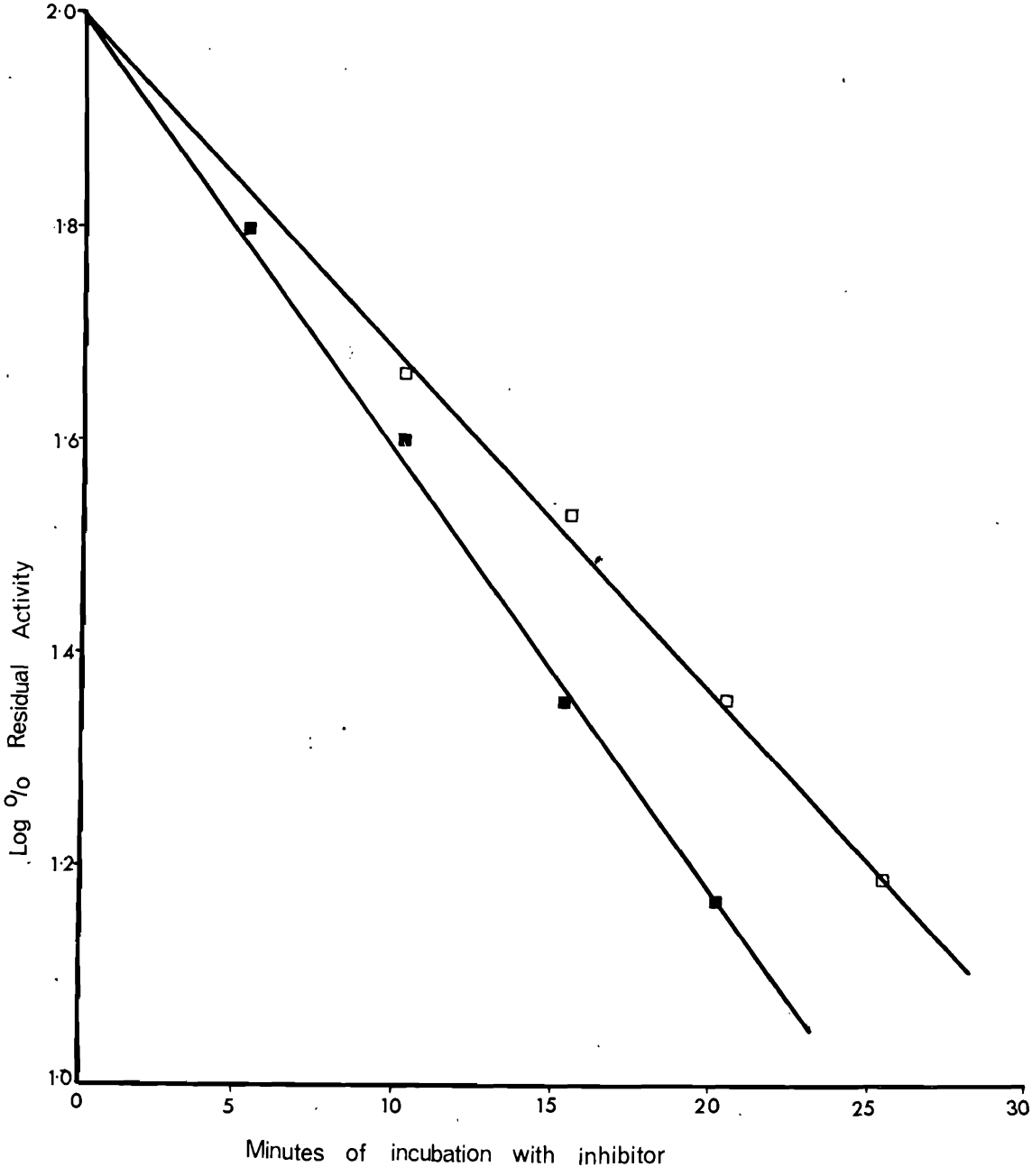

Fig. 1 Examples of reactions between P and ACR strain cholinesterase and organophosphate inhibitors.

Reaction type A (normal)
■—■—P strain and DDVP 10^{-7} Molar
□—□—ACR strain and Paraoxon 5×10^{-7} Molar

level (see Fig. 2). In the case of a single enzyme, or a mixture of enzymes with identical reactions, 100% residual activity at zero inhibition time would be expected; however the results obtained with organophosphate resistant ticks indicate that there is a mixture of at least two enzyms which react differently with organophosphates; one enzyme or group of enzymes is rapidly inhibited and the remainder are affected more slowly. The results obtained for the intersection of the abcissa, and the rate constant for the reaction be-

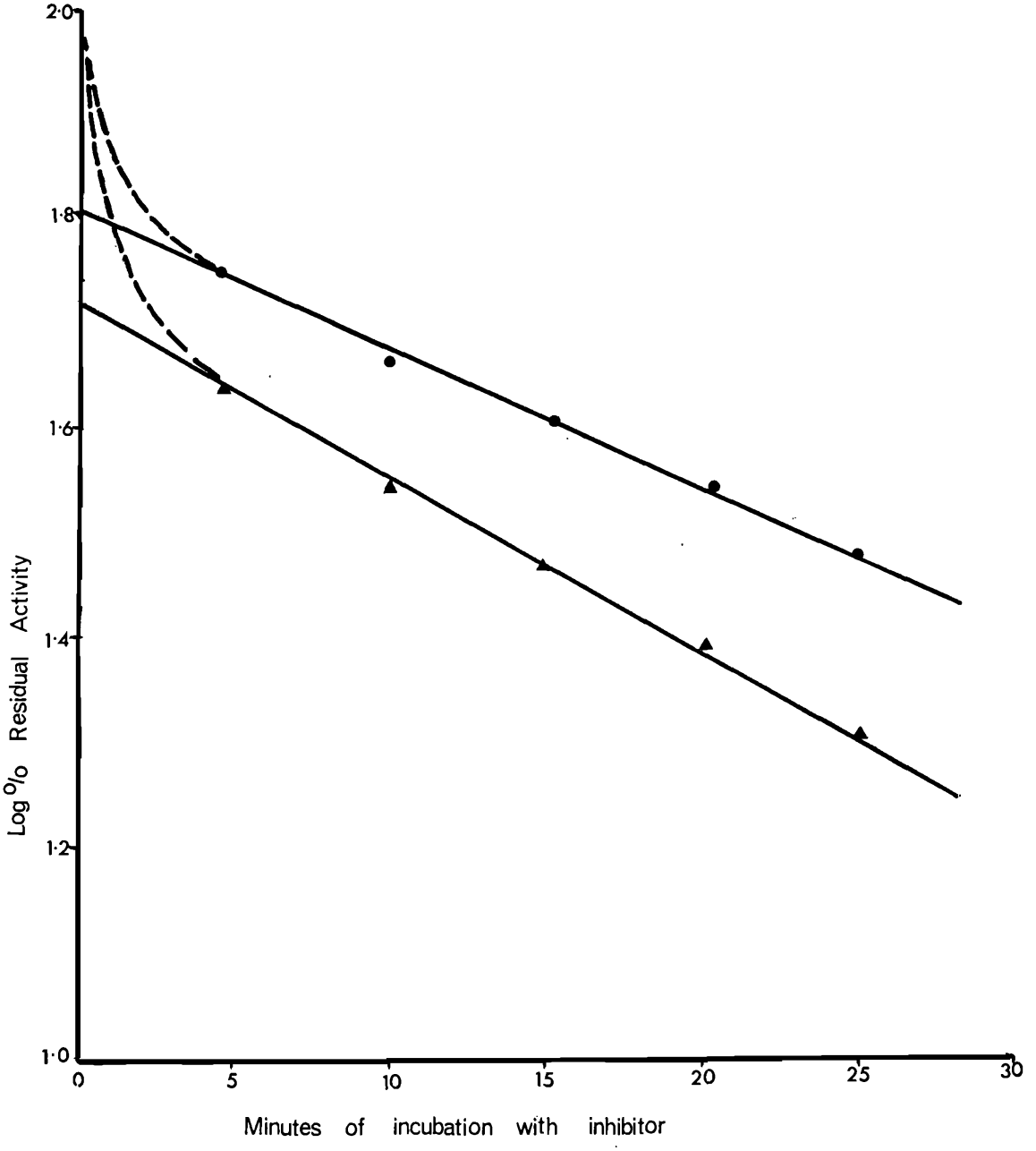

Fig. 2 Example of reactions between D strain tick cholinesterase and organophosphate inhibitors.
●—●—Reaction type B—D strain and Paraoxon 5×10^{-7} Molar.
▲—▲—Reaction type C—D strain and DDVP 5×10^{-7} Molar.

Table 1: TOTAL CHOLINESTERASE ACTIVITY OF B. DECOLORATUS

(μ Moles substrate hydrolysed per g. live ticks per hour at 37°C)

Tick Strain	Ellman method		Hestrin method	
	mean	S.E.	mean	S.E.
D. phosphate resistant	96	4.75	58	7.9
ACR phosphate susceptible	313	7.2	338	4.7
P phosphate susceptible	272	13.1	335	1.5

tween the four organophosphates and the enzymes of the two susceptible strains of *B. decoloratus* and the resistant enzyme component of the resistant Blue tick are given in Table 2. Rate constants for reactions between the inhibitors and the cholinesterase of other susceptible and resistant ticks are given in Table 3 for comparison.

From the estimates of total cholinesterase activity in Table 1 it is apparent that the enzyme system in the organophosphate resistant strain has undergone a change reflected by the decreased overall activity when compared to the other strains. A lowered total cholinesterase activity has been noted previously from organophosphate resistant *B. microplus*[3,4]. This does, however, only indicate that a change has taken place and cannot be used experimentally as an indicator of resistance as the change may also affect the substrate specificity of the enzyme and in our experience the rate of hydrolysis of different choline esters varies disproportionately from strain to strain.

Table 3: RATE CONSTANTS (K_2) FOR REACTIONS BETWEEN B. MICROPLUS CHOLINESTERASE AND ORGANOPHOSPHATES

Inhibitor	Z Normal strain	M Ridgelands strain resistant	L Biarra strain very resistant
Coroxon	1.5×10^6*	1.2×10^5*	2.8×10^2 l/mole/min.
Paroxon	3.7×10^5*	1.7×10^4*	6.0×10^2
Supona	4.8×10^4	—	4.2×10^2
DDVP	8.0×10^5*	2.2×10^5*	1.6×10^2

*Results from Lee and Batham[3] included for comparison.

The unusual kinetics for inhibited cholinesterase in *B. decoloratus* shown in Fig. 2 occur in reactions between organophosphates and cholinesterase from resistant strains of *B. microplus* and indicate, in our experience,

Table 2: REACTIONS BETWEEN B. DECOLORATUS CHOLINESTERASE AND ORGANOPHOSPHATES

Tick Strain	Compound.	% residual activity at abcissa intercept		Rate constant (K_2) (as 1/1mole/min.)	
		mean	S.E.	mean	S.E.
Organophosphate resistant					
D	DDVP	49.5	2.6	1.32×10^5	0.29
D	Coroxon	50.1	3.9	2.0×10^5	0.22
D	Supona	61.9	5.4	8.6×10^4	0.09
D	Paraoxon	62.9	3.9	7.0×10^4	0.04
Organophosphate susceptible					
ACR	DDVP	100	—	7.8×10^5	1.76
ACR	Coroxon	100	—	7.3×10^5	0.82
ACR	Supona	100	—	4.4×10^5	1.08
ACR	Paraoxon	100	—	1.8×10^5	0.34
P	DDVP	100	—	6.8×10^5	0.44
P	Coroxon	100	—	4.3×10^5	0.4
P	Supona	100	—	2.0×10^5	0.26
P	Paraoxon	100	—	2.0×10^5	0.27

that a proportion of the enzyme is fully inhibited quickly (more quickly than the normal cholinesterase from susceptible strains) but a larger proportion is inhibited much more slowly. The use of a computer enabled us to estimate more accurately the proportions involved and it was seen that the inhibitors DDVP and Coroxon consistently caused the plot of the reaction to cut the abcissa at a lower point than with Supona and Paraoxon. From the data given in Figure 2 and Table 2 it appears that when inhibited by DDVP or Coroxon, approximately 50% of the enzyme was quickly eliminated (Reaction type C) whereas with the other two inhibitors, 40% is eliminated rapidly (Reaction type B). It could be concluded from these figures that the organophosphate resistant D strain of *B. decoloratus* has at least three different cholinesterases, one (about 40% of the total) very susceptible to all four inhibitors, one (about 10%) very susceptible to DDVP and Coroxon but resistant to Supona and Paraoxon and one (about 50%) resistant to all four compounds.

The reactions between the four inhibitors and the organophosphate insensitive cholinesterase of D strain all proceed two to six times more slowly than between the inhibitors and the enzyme from the two organophosphorus susceptible strains of *B. decoloratus* (P and ACR). This difference in rate is of the same order as the difference in reaction rates between normal (Z strain) and the insensitive cholinesterase of Ridgelands (M) strain resistant *B. microplus*, although the difference is slightly greater with the Australian strain. Neither of these examples approaches the difference in reaction rates between the cholinesterases of normal (Z) and the very resistant (L) strain *B. microplus* which have reaction rates differing by a factor of at least one hundred. It would be expected from these results that Berlin (D) *B. decoloratus* and Ridgelands (M) *B. microplus* would show approximately the same degree of resistance in the field whereas Biarra (L) *B. microplus* would be much more tolerant of ixodicidal treatment.

There is no evidence to suggest that the two stages of resistance shown by Australian cattle ticks are related and it can be hoped that no more highly resistant strain will occur in Southern Africa.

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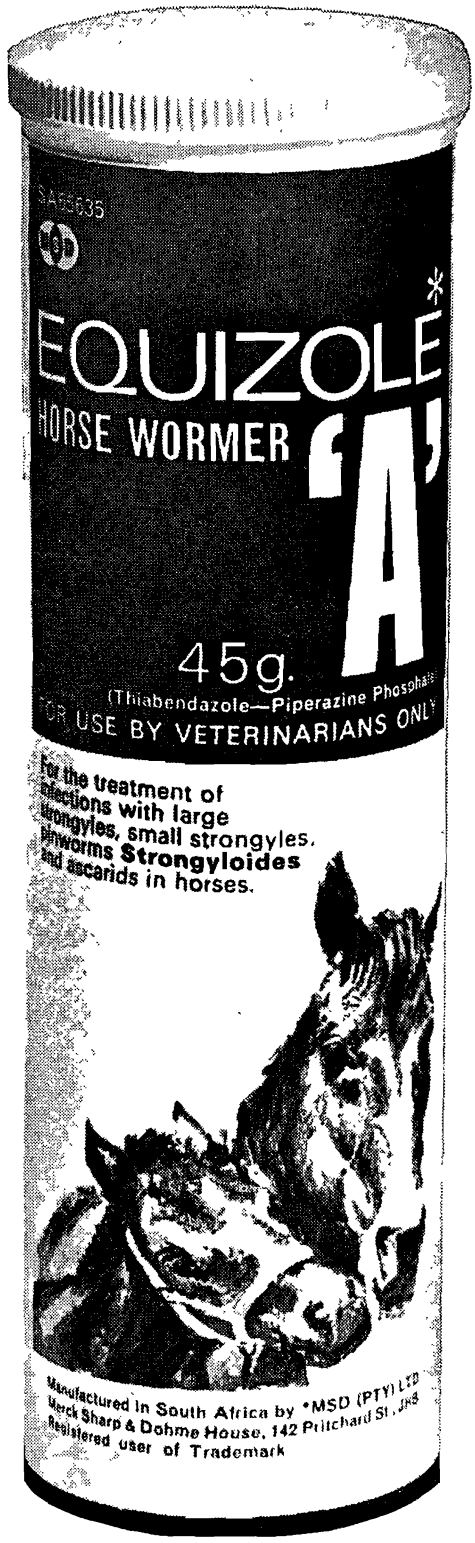

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THE USE OF AMPROLIUM* IN THE TREATMENT OF COCCIDOSIS IN DOMESTIC RUMINANTS

I. G. HORAK†, S. M. RAYMOND† AND J. P. LOUW†

SUMMARY

Experiments are described in which amprolium was used in the treatment of naturally acquired coccidial infections in sheep, goats and calves.

Amprolium administered to sheep and calves at a dosage level of 50 mg/kg liveweight for four consecutive days and to goats at 100 mg/kg liveweight for the same period, rapidly reduced faecal oocyst counts, while at the same time clinical improvement was observed.

In toxicity studies in sheep, goats and calves a dosage level several times in excess of the therapeutic level produced no visible signs of toxicity.

INTRODUCTION

The use of amprolium for the control of coccidiosis in experimentally infected cattle and sheep has been described by Peardon *et al.*[1], and Hammond *et al.*[2,3] while Fitzsimmons[4] has described its use in goats naturally infected with several *Eimeria* species.

The present experiments were conducted to determine the efficacy of amprolium in the treatment of coccidiosis in naturally infested sheep, goats and calves.

SHEEP

History

A farmer in the Johannesburg district of the Transvaal had been purchasing sheep in poor condition from various parts of the country since November 1965. These sheep were transported to his farm where they were kept, 15 to a pen, in a battery system on earth floors covered with wood-shavings for six weeks.

At the end of September 1966 a parcel of young Dorper sheep from the Bultfontein district of the Orange Free State were placed in the battery where they were mixed with young Merino sheep.

Neither group thrived and on 1st December diarrhoea was noticed. The first death occurred six days later and three more sheep died during the following two days. The cause of death was diagnosed as acute coccidiosis.

MATERIALS AND METHODS

Forty-five young sheep were divided into three equal groups on faecal oocyst counts and bodyweights. One group was treated orally with a five per cent solution of amprolium at 44 to 58 mg/kg liveweight for four consecutive days, another was treated orally with a ten per cent solution of sulphadimidine** at 100 to 125 mg/kg liveweight for four consecutive days and the third group was kept as untreated controls.

Faecal oocyst counts, obtained by a modification of the McMaster technique for worm egg counts[5], and bodyweights were recorded on the first day of treatment (hereafter referred to as Day O) and periodically thereafter.

A morphological species differentiation was made on the oocysts present in the faeces of three sheep[6].

RESULTS

The average faecal oocyst counts of the three groups are illustrated in Fig. 1.

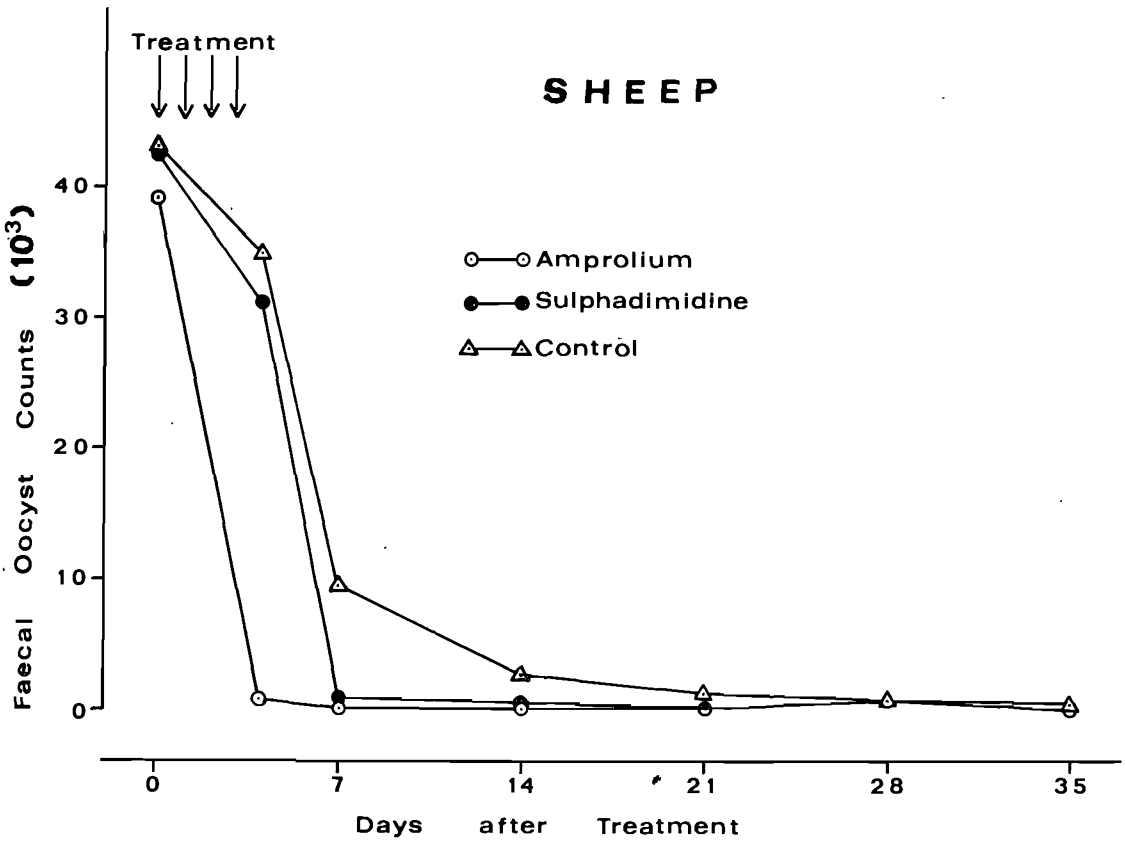
The initial average faecal oocyst count in the group treated with amprolium was 39 300 o.p.g. (oocysts per gram). This decreased to 770 o.p.g. on Day 4 and to zero on Day 14.

The initial average faecal oocyst count in the sulphadimidine group was 42 600 o.p.g. — this was reduced to 31 300 o.p.g. on Day 4 and to 470 o.p.g. on Day 7, reaching zero on Day 22. Both this group and the amprolium group had low average faecal oocyst counts on Day

* AMPROL 20% Soluble Powder, MSD (PTY) LTD.

† MSD (PTY) LTD., 142 Pritchard Street, Johannesburg.

**SULPHAMEZATHINE, I.C.I. South Africa (Pharmaceuticals) Limited.



28, due to a few sheep with positive counts. A single oral treatment with amprolium at 100 mg/kg liveweight on Day 29 reduced these counts to zero.

The initial average faecal oocyst count in the untreated control group was 43 000 o.p.g. This had decreased to 35 600 o.p.g. on Day 4. A marked drop was noted on Day 7 when the average count was 9 400 o.p.g. and thereafter a gradual decrease to 200 o.p.g. at the conclusion of the experiment occurred.

A more rapid clinical improvement was noticed in the sheep in both treated groups than in those in the untreated control group.

The three groups all lost weight during the first week following treatment but thereafter bodyweight increased. The group treated with amprolium gained 4.8 kg; the sulphadimidine group 3.8 kg and the control group 4.6 kg when compared with their original average bodyweights.

The species differentiation of the oocysts present in the faeces of three sheep is presented in Table 1.

Table 1: MORPHOLOGICAL DIFFERENTIATION OF OOCYSTS FROM SHEEP

Sheep No.	Eimeria arloingi	E. ninakohlyakimovae	E. parva	E. faurei	E. granulosa
404	45%	30%	20%	5%	0%
480	63%	20%	10%	0%	7%
487	52%	22%	14%	10%	2%

The dominant species in the sheep was *Eimeria arloingi*, followed by *E. ninakohlyakimovae* and *E. parva*.

Symptoms.

The following symptoms were observed in affected sheep: listlessness, unthriftiness, anorexia, staring coat in Dorpers and a break in the wool in some of the Merinos, a submandibular oedema in two Dorpers; some sheep had a severe, fluid, mucoid diarrhoea containing streaks of fresh blood, while in others no diarrhoea was present; prostration and eventually death.

Autopsy.

The carcasses were emaciated. Coccidial foci were present throughout the length of the small intestine and were apparent from the serosal surface of the intestine. The intestinal blood vessels were congested and the walls of the small and large intestine were hyperaemic and oedematous. Numerous petechiae were present in the epithelium of the small intestine, while echymoses were more common in the large intestine. Large numbers of oocysts were found in scrapings of both the small and large intestine.

DISCUSSION

Although treatment with amprolium or sulphadimidine rapidly reduced faecal oocyst counts, a similar but more gradual reduction occurred in the group of untreated sheep. It would therefore appear that the crisis of the outbreak had passed and that the sheep were already recovering when treatment commenced. The advantages of medication in this case were a more rapid clinical improvement while at the same time the spread of infection was limited by the rapid reduction in faecal oocyst output.

GOATS

Experimental Animals

Forty Angora goats were purchased in the Graaff-Reinet district of the Cape Province and transported to a farm in the Hennops River district of the Transvaal, where they were housed in concrete-floored pens which were cleaned daily. Faecal oocyst counts were performed on these goats and they were found to be infected with *Eimeria* spp.

MATERIALS AND METHODS

Experiment 1

Twenty-five goats were divided into two groups according to body weight and faecal oocyst counts. One group consisting of 12 goats was treated orally with a five per cent solution of amprolium at 50 mg/kg liveweight for four consecutive days. The other group, consisting of 13 goats, was maintained as untreated controls.

Faecal oocyst counts were done on the day prior to treatment and periodically thereafter, while bodyweights were taken at weekly intervals and morphological species differentiations[6] on oocysts recovered from the faeces of two goats were carried out.

A temparary reduction in oocyst counts occurred in the treated group, but thereafter faecal oocyst counts rose to high levels and the experiment was discontinued. At a later date 23 goats were included in Experiment 2.

Experiment 2

Twenty-three goats were divided into two groups according to bodyweight. One group consisting of 12 goats, which were treated orally with a five per cent solution of amprolium at 100 mg/kg liveweight for four consecutive days; the other group consisted of 11 goats which were given no treatment.

Faecal oocyst counts were done on the day prior to treatment and periodically thereafter and bodyweights were obtained at weekly intervals.

RESULTS

The average faecal oocyst counts of the goats in Experiments 1 and 2 are illustrated in Fig. 2.

Experiment 1

The initial oocyst count in the treated group was 17 333 o.p.g. This decreased to 1 573 o.p.g. on the fourth day of treatment, but thereafter rose steadily to reach a final average of 123 272 o.p.g. The average bodyweights of these goats increased by 0.7 kg during the experiment.

The initial oocyst count in the untreated controls was 12 308 o.p.g. This dropped to 5 975 o.p.g. four days later and then rose rapidly to 121 154 o.p.g. These goats gained 0.3 kg during the course of the experiment.

The species differentiation of the oocysts recovered from the faeces of two goats is given in Table 2.

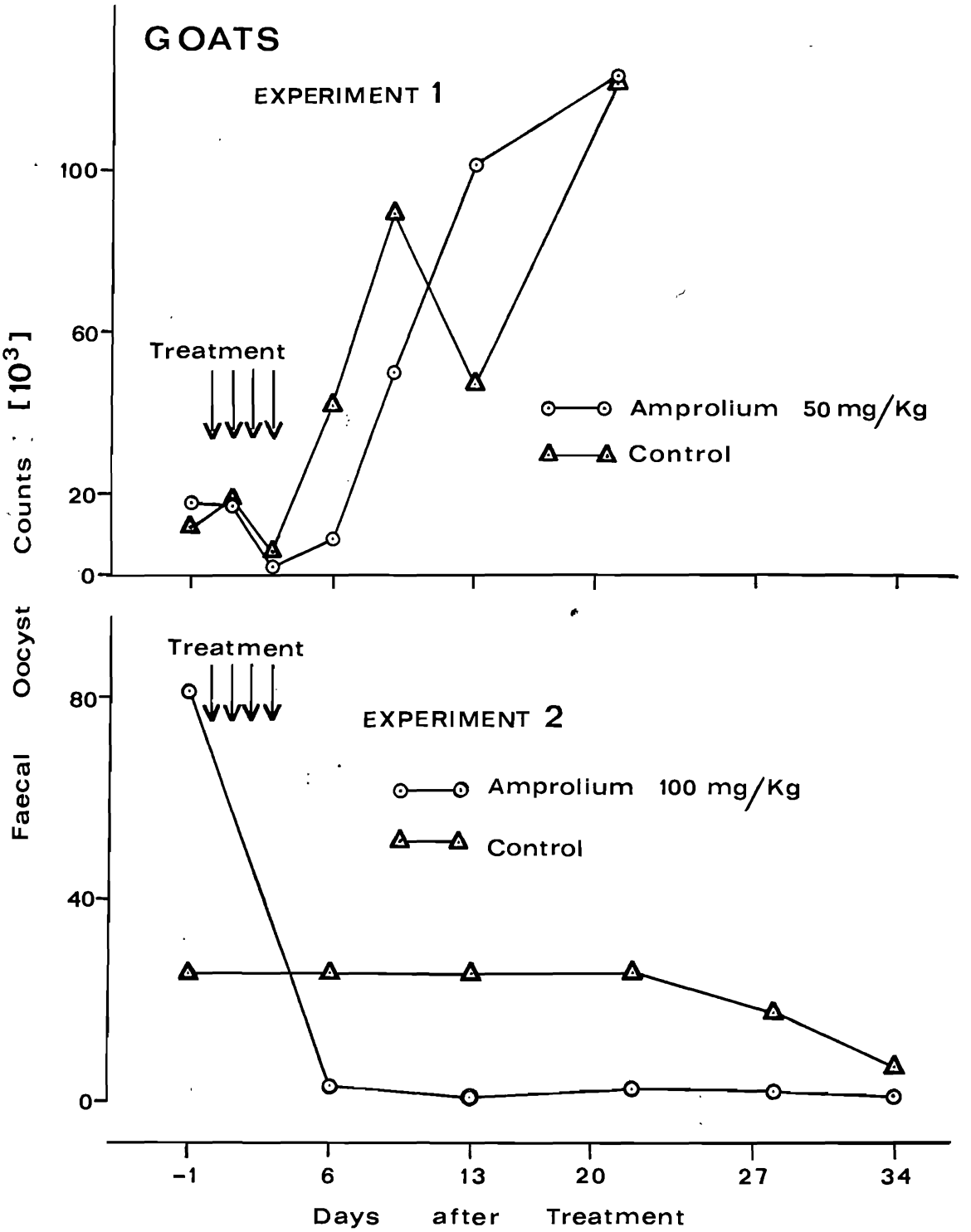
Table 2: MORPHOLOGICAL DIFFERENTIATION OF OOCYSTS FROM GOATS

Goat No.	Eimeria arloingi	E. ninakohlyakimovae	E. parva	E. faurei
87	68%	22%	2%	8%
100	62%	32%	2%	4%

The dominant species was *E. arloingi* followed by *E. ninakohlyakimovae.*

Experiment 2

The initial oocyst count in the treated group was 81 583 o.p.g. This decreased to 3 083 o.p.g. three days after the cessation of treatment and remained at a low level for



the remainder of the experiment. The goats gained 2.7 kg during the experimental period.

In the control group the initial oocyst count was 25 673 o.p.g. This remained at a fairly constant level until the conclusion of the experiment when it had decreased to 6 991 o.p.g. These goats gained 2.4 kg during the course of the experiment.

DISCUSSION

Unfortunately the goats did not suffer from clinical coccidiosis during the course of the two experiments so that no estimation could be made of the efficacy of amprolium when used in clinical coccidiosis of goats.

Individual goats had faecal oocyst counts as high as 1 776 000 o.p.g. and yet did not lose weight or develop diarrhoea. Other Angora goats, not in the experiments, died of coccidiosis with faecal oocyst counts below 20 000 o.p.g., indicating that mere faecal oocyst counts are not a reliable method of assessing the severity of coccidial infections and that they must be considered in conjunction with the species involved and the clinical picture in the animals.

Similar observations have been made by Marlow [7] during field outbreaks of coccidiosis in goats.

The failure of the faecal oocyst count to remain at a low level after the cessation of treatment with amprolium at 50 mg/kg is similar to the observations made by Fitzsimmons [4]. He found that faecal oocyst counts in goats rose again even after prolonged daily treatment with amprolium at 50 mg/kg.

In the present experiments faecal oocyst counts remained low until the experiment was concluded four weeks after treatment with amprolium at 100 mg/kg liveweight.

CALVES

Experimental Animals

Dairy calves in the Heidelberg district of the Transvaal were weaned at two weeks of age and thereafter bottle- and supplement fed as well as having access to teff and lucerne hay. Ten to twenty calves were kept in each open camp and during a period of extremely wet weather developed diarrhoea and pneumonic symptoms and were generally unthrifty; coccidiosis was considered a complicating factor.

MATERIALS AND METHODS

Thirteen calves were selected on faecal oocyst counts. These calves were divided into three groups with reasonably similar faecal oocyst counts.

One group of five calves was treated orally for four consecutive days with a five per cent solution of amprolium in water at a dosage level of 50 mg/kg liveweight. The second group of four calves was treated similarly with amprolium at 100 mg/kg liveweight, while the remaining group of four calves was maintained as untreated controls. Faecal oocyst counts were done periodically in all the animals.

RESULTS

The average faecal oocyst counts of the three groups are illustrated in Fig. 3.

The initial average faecal oocyst count of the group treated with amprolium at 50 mg/kg liveweight was 12 300 o.p.g. This increased to 20 400 o.p.g. on the day that treatment commenced. On the third day of treatment the oocyst count had dropped to 3 700 o.p.g. and this decreased gradually to 30 o.p.g. by Day 16. One calf in this group died on Day 8 from severe debilitation coupled with bacterial infections unresponsive to terramycin therapy.

The initial average faecal oocyst count in the group treated at 100 mg/kg liveweight was 12 300 o.p.g. This had increased to 17 100 o.p.g. on the first day of treatment. By the third day of treatment oocyst counts had fallen to 300 o.p.g. and were negative by Day 9.

The average faecal oocyst count in the control group was 6 400 o.p.g. at the start of the experiment and remained reasonably constant till Day 9 with a reduction to 3 500 o.p.g. on Day 16.

Clinical improvement with cessation of diarrhoea occurred in both treated groups soon after treatment, while the control group took longer to recover.

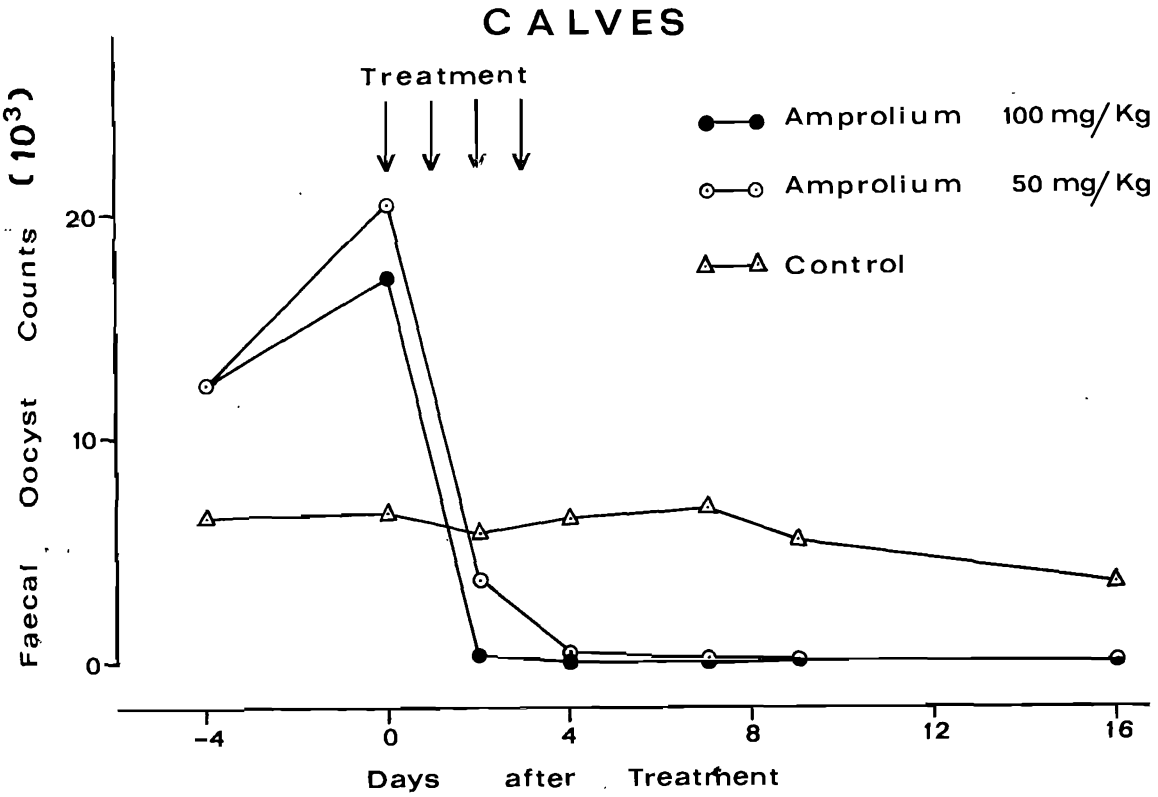
DISCUSSION

Amprolium at both 50 and 100 mg/kg liveweight rapidly reduced faecal oocyst counts in both treated groups, while very little reduction occurred in the average faecal oocyst counts of the control group. The reduction in oocyst counts after treatment at 100 mg/kg liveweight was slightly more rapid than at 50 mg/kg liveweight, but no difference in clinical improvement could be seen between the two treated groups.

TOXICITY

Experimental Observations

Five sheep were dosed *per os* with a five per cent solution of amprolium at a dosage



rate of 200 mg/kg for four consecutive days. No visible signs of toxicity were seen in any of the sheep; seven days after initial treatment their average bodyweight was 0.5 kg less than their original average weight, but thereafter increased normally.

Two sheep were dosed orally with a five per cent solution of amprolium, one at 500 mg/kg and the other at 750 mg/kg liveweight. Both these sheep gagged during drenching, probably due to the low pH (circa 2) and the large volume of the solution and some of the drench entered their lungs. They died immediately after drenching from asphyxia caused by drowning, as confirmed at autopsy.

One sheep was dosed by means of a stomach tube with a five per cent solution of amprolium at a dosage rate of 750 mg/kg for four consecutive days. No symptoms of toxicity or loss of bodyweight were recorded.

Two Angora goats were dosed by means of a stomach tube with a five per cent solution of amprolium, one at a dosage rate of 500 mg/kg liveweight and the other at 750 mg/kg liveweight for four consecutive days. During the week prior to drenching these goats had spent five days in rail transit, both developed severe diarrhoea and lost considerable weight after drenching, but so did other non-drenched goats from the same group. Both goats survived.

Six calves were drenched orally for four consecutive days with a five per cent solution of amprolium, four at a dosage rate of 200 mg/kg and two at 400 mg/kg liveweight. No symptoms of toxicity or depression of bodyweight were recorded.

DISCUSSION

Amprolium appears to be non-toxic for ruminants at several times the therapeutic dosage level, the only observed effect being a possible temporary weight depression.

Solutions containing more than five per cent of amprolium should not be used for

drenching as the acid pH may cause gagging, which could be followed by drowning or subsequent foreign body pneumonia, should the mis-dosed animals survive for any length of time.

ACKNOWLEDGEMENTS

We would like to thank Messrs. A. F. Fleming and S. G. Anema for the use of their sheep and calves and the assistance given by their staff during the experiments.

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BOOK REVIEW

CHECK LIST OF THE HELMINTH PARASITES OF AFRICAN MAMMALS OF THE ORDERS *CARNIVORA, TUBULIDENTATA, PROBOSCIDEA, HYRACOIDEA, ARTIODACTYLA* AND *PERISSODACTYLA.*

ROUND, M. C.

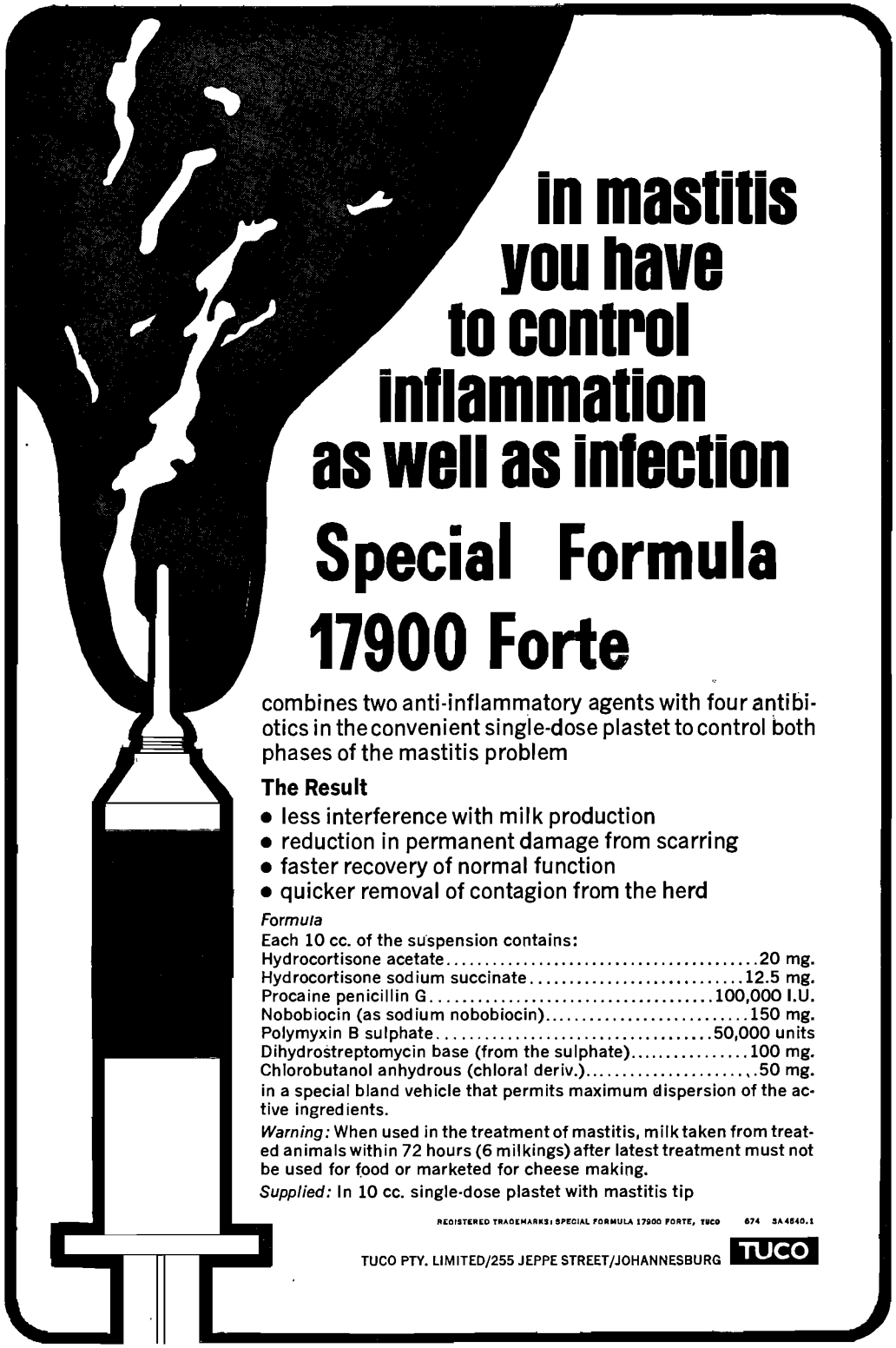
Technical Communication No. 38 of the Commonwealth Burea of Helminthology, St. Albans. Commonwealth Agricultural Bureaux, 1968. pp. vi+252.

The main part of this book consists of the following checklists: Parasite/Host, General Host/Parasite and Host/Parasite by countries. Records from hosts whose geographical distribution includes Africa, but are not with certainty known to be from Africa are included in a separate list.

After browsing through this book and using it for quick reference while doing taxonomic work, one has nothing but admiration and praise for the meticulous way it has been compiled; the records are not only listed but there are also comments on their veracity when this is doubtful. The only error noticed in this checklist is the locality of the record of *Schistosoma margrebowiei* Le Roux, 1933 from *Kobus leche* Gray, 1850; this is given as the Republic of South Africa but should be South West Africa. When Ortlepp („'n Oorsig van Suid-Afrikaanse helminte veral met verwysing na die wat in ons wilde herkouers voorkom." *Tydskr. Natuurwet.* 1, 203—212, 1961) recorded this parasite he treated these two localities as well as Swaziland as a unit; in this region the lechwe occurs only in South West Africa.

It is hoped that Dr Round will complete this task by also compiling a checklist of the helminths of the Insectivora, Chiroptera, Primates, Pholidata, Lagomorpha, Rodentia and Cetacea of the African continent.

A.V.



in mastitis you have to control inflammation as well as infection

Special Formula 17900 Forte

combines two anti-inflammatory agents with four antibiotics in the convenient single-dose plastet to control both phases of the mastitis problem

The Result

- less interference with milk production
- reduction in permanent damage from scarring
- faster recovery of normal function
- quicker removal of contagion from the herd

Formula

Each 10 cc. of the suspension contains:

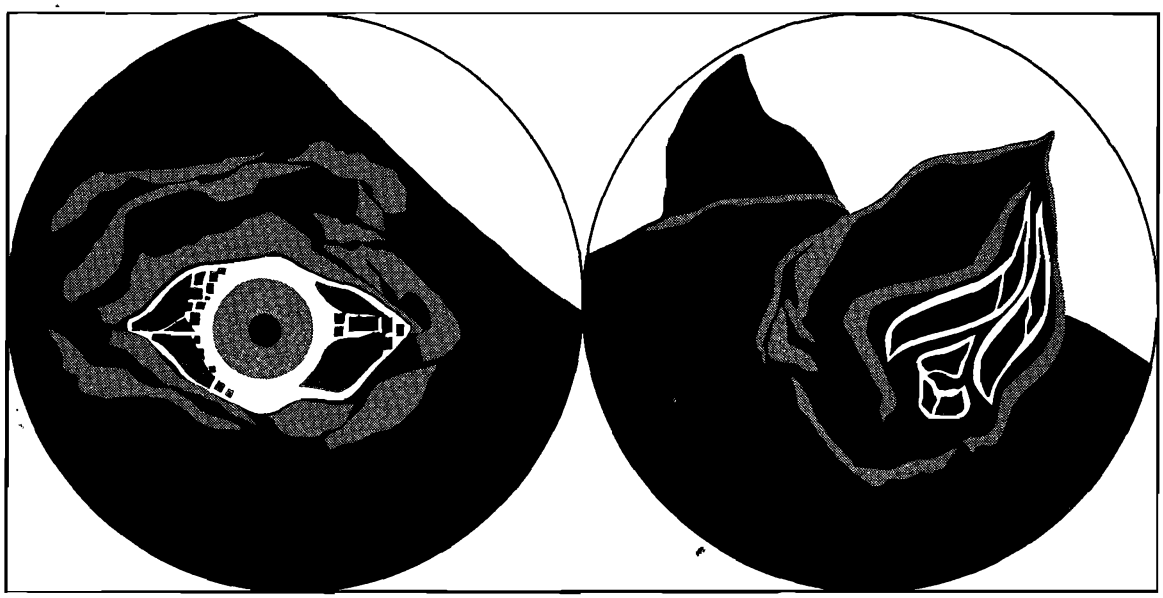
Hydrocortisone acetate	20 mg.
Hydrocortisone sodium succinate	12.5 mg.
Procaine penicillin G	100,000 I.U.
Nobobiocin (as sodium nobobiocin)	150 mg.
Polymyxin B sulphate	50,000 units
Dihydrostreptomycin base (from the sulphate)	100 mg.
Chlorobutanol anhydrous (chloral deriv.)	50 mg.

in a special bland vehicle that permits maximum dispersion of the active ingredients.

Warning: When used in the treatment of mastitis, milk taken from treated animals within 72 hours (6 milkings) after latest treatment must not be used for food or marketed for cheese making.

Supplied: In 10 cc. single-dose plastet with mastitis tip

In superficial eye and ear conditions of domestic animals...



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eye-ear ointment
the triple-action therapeutic that

1 Controls bacterial infection with Neomycin
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Eye-Ear Ointment

Each gram contains:
Neomycin Sulphate....5 mg. (0.5%)
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Base designed for application, adherence and dispersion at body temperature.

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Jl S. Afr. vet. med. Ass. 40 (3): 303-311 1969

THE ADRENAL CORTEX AND DEVELOPMENT OF CORTICOSTEROIDS FOR VETERINARY THERAPY

MISS O. UVAROV*

In the therapeutic revolution which has taken place in the last twenty years, the discovery of cortisone and related compounds may well rank amongst the greatest achievements. These compounds have a scope of action and versatility "wider than that of any other therapeutic agent so far evolved" [1]. An extensive literature now exists on the use of these compounds, but their action in different diseases requires further investigation. In this paper an aatempt is made to:—

(1) Review some of the important functions of the adrenal cortex;

(2) Discus some pharmacological properties of corticosteroids;

(3) Discuss the development of corticosteroids and some conditions for which these preparations are used, as well as some of their known contra-indications.

Diseases of the adrenal glands in animals are not well documented [2] and it is accepted that one is not using corticosteroids for treating disease of the adrenal glands, but merely for their pharmacological effects.

ADRENAL CORTEX

The secretion of the adrenal cortex is influenced by the adrencorticotrophic hormone (ACTH) and more than 50 steroids have been isolated from the adrenals, some of which may be artefacts without physiological activity.

In general, more is known about the effects of corticosteroids than their function and a reminder of their basic physiological action is often appreciated by the veterinarian.

The function of a gland may best be studied by observing the effect of its removal from the live animal. Ingle [3] described the effect of adrenalectomy, and showed that the removal of the gland had effects on circulatory, growth, digestive and renal functions, and on the resistance of the animal. Conversely, when a corticosteroid is used in an animal with an intact adrenal gland, the same systems may be affected.

Corticosteroids are important in many physiological processes, in the very maintenance of life, ability to work, to resist stress and to maintain normal carbohydrate levels etc. [2]. It is usual to divide biologically active steroids into:—

(A) Glucocorticosteroids — which affect carbohydrate metabolism and have an anti-inflammatory effect;

(B) Mineral corticosteroids — which influence the metabolism of sodium and potassium and regulate the electrolyte and water balance;

(C) Sex steroids — which will not be considered in this paper.

The Effects of Administration of Corticosteroids: Corticosteroids can influence:—

(A) The metabolism of:
   a. carbohydrate: causing glycogenesis from protein or fat;
   b. protein: producing a rise in protein breakdown;
   c. fat: causing acceleration of breakdown and mobilisation of fat;
   d. electrolyte and water balance, with possible sodium and chloride retention accompanied by increased potassium excretion.

(B) The mesenchymal tissue — including connective and lymphoid tissues, blood vessels and synovial membranes — leading to a decrease in circulating lympho-

* Glaxo Labs. Ltd., Greenford, Middlesex, England.
(Paper presented at the Annual Congress of the S.A.V.M.A. Pretoria, 1968).

cytes, eosinophils and the phagocytic activity of the reticuloendothelial system, thus producing a depression of all the elements of the inflammatory process.

(C) Capillary resistance, producing a decrease in permeability of serous membranes and vessels and an increase in capillary resistance.

(D) Tissue regeneration, since corticosteroids cause inhibition of granulation tissue.

(E) Allergic and hypersensitivity effects, causing their depression.

Adrenal Steroids and Stress: Corticosteroids play an important part in the phenomenon of stress. The adrenals are affected by many stimuli such as heat, starvation, infection, trauma, fatigue etc., and the response to these stimuli (also known as stressor factors) may result in an increased secretion of hormones. These stimuli are said to alter the adrenal steroid secretion which affects homeostasis within the body, leading to the phenomenon of 'stress'. Selye[4] postulated the theory of stress after exposing experimental animals to several unrelated stimuli, for example starvation, heat, cold, increased exercise and the injection of different substances. He found that irrespective of the type of stress, certain constant changes in some organs were produced. He called this non-specific systemic reaction "the general adaptation syndrome", defining it as "the sum of all non-specific systemic reactions in the body which ensues upon long continued exposure to stress". Thus, it was postulated that some disease conditions may be classified as "diseases of adaptation". Subsequently, this theory fell into disrepute. The hypothesis of 'stress' and general disease adaptation is still of interest, even if the physiologists disagree about it. Conditions are encountered in veterinary medicine in which it is possible to postulate that some specific as well as non-specific causes of disease cause ill health. Further research into the function of the adrenal cortex and its effect on different systems in the animal body could finally elucidate the importance of adaptation and the phenomenon of stress as related to health.

DEVELOPMENT OF CORTICOSTEROIDS FOR CLINICAL USE

All identified adrenal cortical hormones belong to the steroid group. These and semi-synthetic hormones are collectively called corticosteroids. The first hormone to be isolated was cortisone, derived from animal adrenals. Only minute quantities could be isolated and a search for other sources of this steroid ultimately led to the discovery that waste product from sisal manufacture contained a steroid called hecogenin which became the starting material for large scale production of corticosteroids. Subsequently, several corticosteroids were produced in an attempt to develop preparations with more selective action than the parent compound — cortisone. The structures of some of the more important ones are illustrated in Figure I.

The more important preparations now used in veterinary practice in the U.K. are:—

(A) Betamethasone (16-β-methyl-9-αc-fluoroprednisolone). This is 40 times more potent than cortisone and approximately 5 to 8 times more potent than prednisolone.

(B) Dexamethasone (16-αc-methyl-9-αc-fluoro-prednisolone). This is approximately 30 times as potent as cortisone and 6 times as prednisolone.

(C) Prednisolone — This is approximately 4 times more potent than cortisone.

Clinical use of Corticosteroids

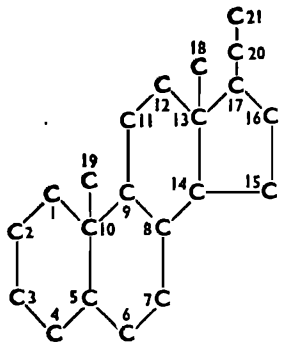
Corticosteroids do not cure disease, they merely reverse symptoms, suppress lesions (if these are still reversible) and at times prevent extension of disease into a more chronic form[2]. They often reduce duration of illness and enhance other treatments. It can be said that these substances exert a supportive action. Clinical use of corticosteroids falls into two main groups:—

(A) Anti-inflammatory and anti-allergic;

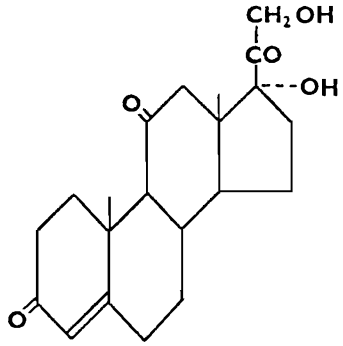
(B) Replacement therapy in the treatment of a variety of conditions.

Since corticosteroids exert powerful hormonal effects in the body it is essential to safeguard their use by differential diagnosis and selection of the right preparation for each case. In view of their ability to suppress all phases of the inflammatory process it is essential to consider conjoint use of antibiotics

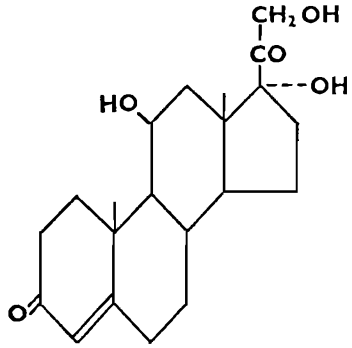
FIGURE I



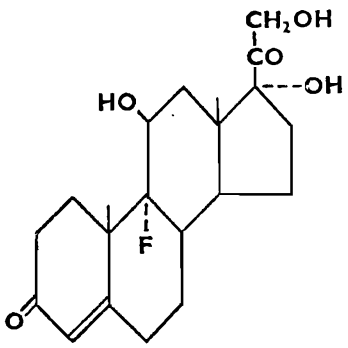
PREGNANE



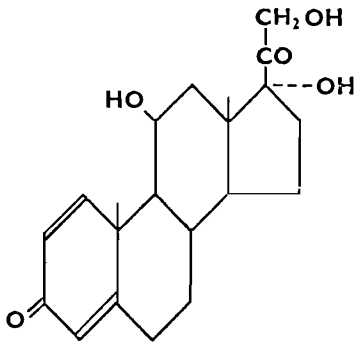
CORTISONE



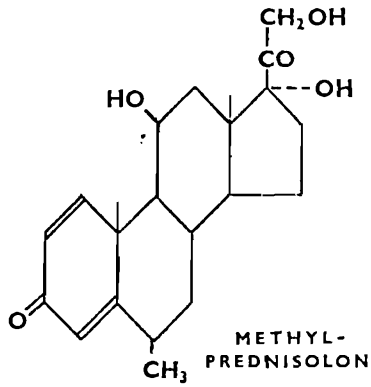
HYDROCORTISONE



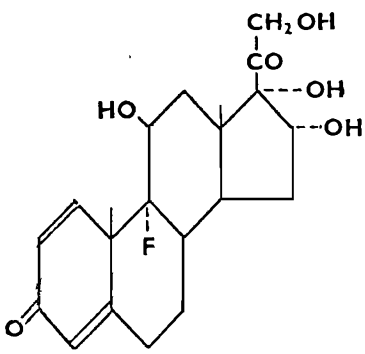
FLUOROCORTONE



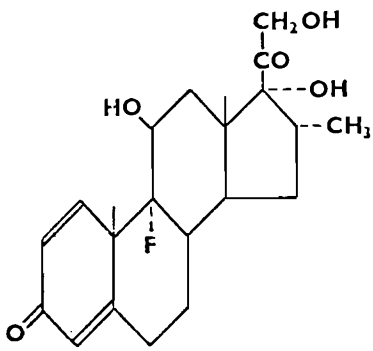
PREDNISOLONE



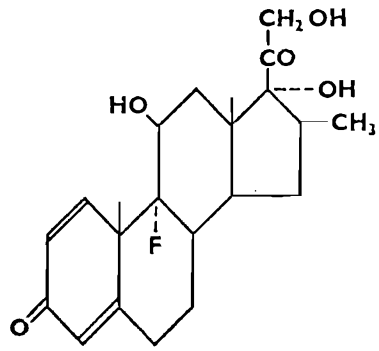
METHYL-
PREDNISOLONE



TRIAMCINOLONE



DEXAMETHASONE



BETAMETHASONE

if infection is present, or even suspected. In such instances the antibiotic must be used for a longer period than the steroid. It is also necessary to:—

(A) Consider the route of their administration which can be external, local, (i.e. intra-articular, peri-articular, subconjunctival and external,) or systemic, by injection using the intravenous route for solutions and the intramuscular route for suspensions, and oral for tablets or boluses.

(B) Consider the type of preparation to use. There can be solutions for emergency intravenous use, suspensions for intramuscular and local use, tablets for small animals, and ointments, lotions, eyedrops, etc.

Diseases in which Corticosteroids are Used

Skin conditions — these include urticaria, non-specific and allergic dermatitis, eczema (including interdigital and pustular forms in the dog, and miliary form in the cat), and "sweet itch" in the horse. Clinicians expect to obtain a quick initial response (12 to 24 hours) and success in over 90% of cases treated.

Eye Diseases — corticosteroids suppress the exudative phase of inflammation, with decrease of fibroblastic proliferation, and in suitable cases enable healing to take place with reduced scar formation. Great caution must be exercised in selecting suitable cases for corticosteroid therapy because excessive suppression of the inflammatory response may predispose perforation and collapse of the eyeball. Diseases treated include conjunctivitis, keratitis, iritis, periodic opthalmia, *some* corneal ulcers, interstitial corneal vascularisation and oedema of the membrana nectitans. They may also be used for symptomatic relief pending operation, entrophion in the dog. The subconjunctival route is used for suitable cases where once-weekly injection using approximately 0.5 ml of a suitable corticosteroid preparation can produce good results. It is essential to include an antibiotic in most eye preparations, or, better, the antibiotic can be given separately to enable it to be used for a slightly longer time than the steroid.

Ear Diseases — Several ear conditions are treated with corticosteroids to reduce inflammation, pain and swelling. The necessity for including chemotherapeutics (antibiotics or parasiticides), or the removal of wax and hair, still remains in appropriate cases.

Orthopaedic Uses — Many conditions are now treated with corticosteroids. Amongst these are arthritis (early cases), tendonitis, synovitis, stifle joint lameness (in the dog), periostitis, bursitis, navicular disease in the horse. Additional uses are being evolved. It is necessary to consider a number of aids in the treatment of orthopaedic disease. These include:—

a. Necessity to establish the site of lameness, by clinical examination or X-ray, or by nerve block.

b. If synovial fluid is present in a joint, to aspirate and examine it bacteriologically.

c. During treatment, anaesthesia and asepsis are essential.

d. Following corticosteroid treatment it is necessary to rest the animal and to provide very light and gradual exercise after the course of treatment.

The intra- or peri-articular routes may be used in the treatment of:—

a. Early cases of arthritis;

b. "Joint ill" (mostly in young animals). Corticosteroids relieve pain and may prevent irreversible damage to articular cartilages;

c. Navicular disease. In this disease good response to treatment may be only 50 to 60% of animals becoming sound. Yet even this prolongs their life and usefulness for some years.

The techniques of intra-articular injections are similar in most species and require asepsis, restraint of the animal, and anaesthesia. The knowledge of anatomical structures involved enables the practitioner to perfect this method of treatment which to some extent has been documented in references 5 to 11, but a more extensive clinical appraisal of this therapy is required.

Corticosteroids as Supportive Therapy

There are many conditions in which corticosteroids are used singly or as an adjunct to other treatment as supportive therapy. In some cases their effects may be non-specific, for example a sense of well-being or an increase of appetite is often detected early during treatment. In others, for example acetonaemia in cattle, the rationale for using these substances is based on their glucogenic effect. A brief description is here included of these uses.

Corticosteroids in Acetonaemia (Bovine Ketosis

Amongst the many reports available on the use of corticosteroids for this condition are references 2 and 12 to 16. It is known that corticosteroids, when administered to cattle, have a marked effect on gluconeogenesis. The concept of acetonaemia, as an endocrine disturbance [17] and based on Selye's hypothesis [4] has now been discounted. Several corticosteroids have been used clinically. Burns [18] was able to compare the glucogenic effect of several substances administered to cattle. This included glucose by the intravenous route, glycerol and potassium chlorate by the oral route and several doses of prednisole alcohol, prednisolone trimethylactate and betamethasone alcohol by the intramuscular route and bethamethasone disodium phosphate and prednisolone disodium phosphate by the intravenous route.

Burns carried out blood glucose and liver glycogen level estimations before and after the use of these corticosteroids and compared them to those produced by glucose (given as a single intravenous injection) and glycerol and potassium chlorate given orally. He found that using glucose, the increase in blood sugar and liver glycogen levels was of short duration compared with the increase recorded after the administration of prednisolone or glycerol. He also showed that betamethasone produced a much greater glucogenic response than prednisolone, and that the raised blood sugar levels were maintained for several days, see Figures II and III. Burns *et al.* [19] also found that the glucogenic effects produced in these experiments were consistent with those noted in field cases of acetonaemia, and this work made it clear that it is necessary to maintain raised blood glucose levels for several days in the treatment of this disease. Since the number of corticosteroid preparations is still increasing, Evans *et al.* [20] studied the effect of a number of additional compounds in cattle. Amongst these were betamethasone, dexamethasone and a number of esters of both compounds. The bethamethasone esters given intra-muscularly were 17-butyrate; 17,21-dipropionate and 21-cyclohexyl -acetate. Betamethasone disodium phosphate, given intravenously, was also included. This work showed that, with a possible exeption of betamethasone 17,21-dipropionate, none of the esters was better than betamethasone itself. In a further experiment using betamethasone 17,21-dipropionate, betamethasone 17-butyrate, and betamethasone itself, it was confirmed that betamethasone was as active as the esters. The effect of betamethasone 21-disodium phosphate on blood glucose was similar to that recorded by Burns in his comparative studies of several corticosteroids. Since at least 24 therapies are available for the treatment of ketosis (Kronfeld[12]) the practitioner has a wide choice, but the glucocortiroids with potent glucogenic effects have an established place in the treatment of acetonaemia [18]. It is accepted that in this disease it is necessary to pay attention not only to the treatment of the individual cow, but also to the nutritional status of the patient and of the herd. Additional information is also required on the intermediary metabolic processes in ruminants which are probably important in the aetiology of this disease.

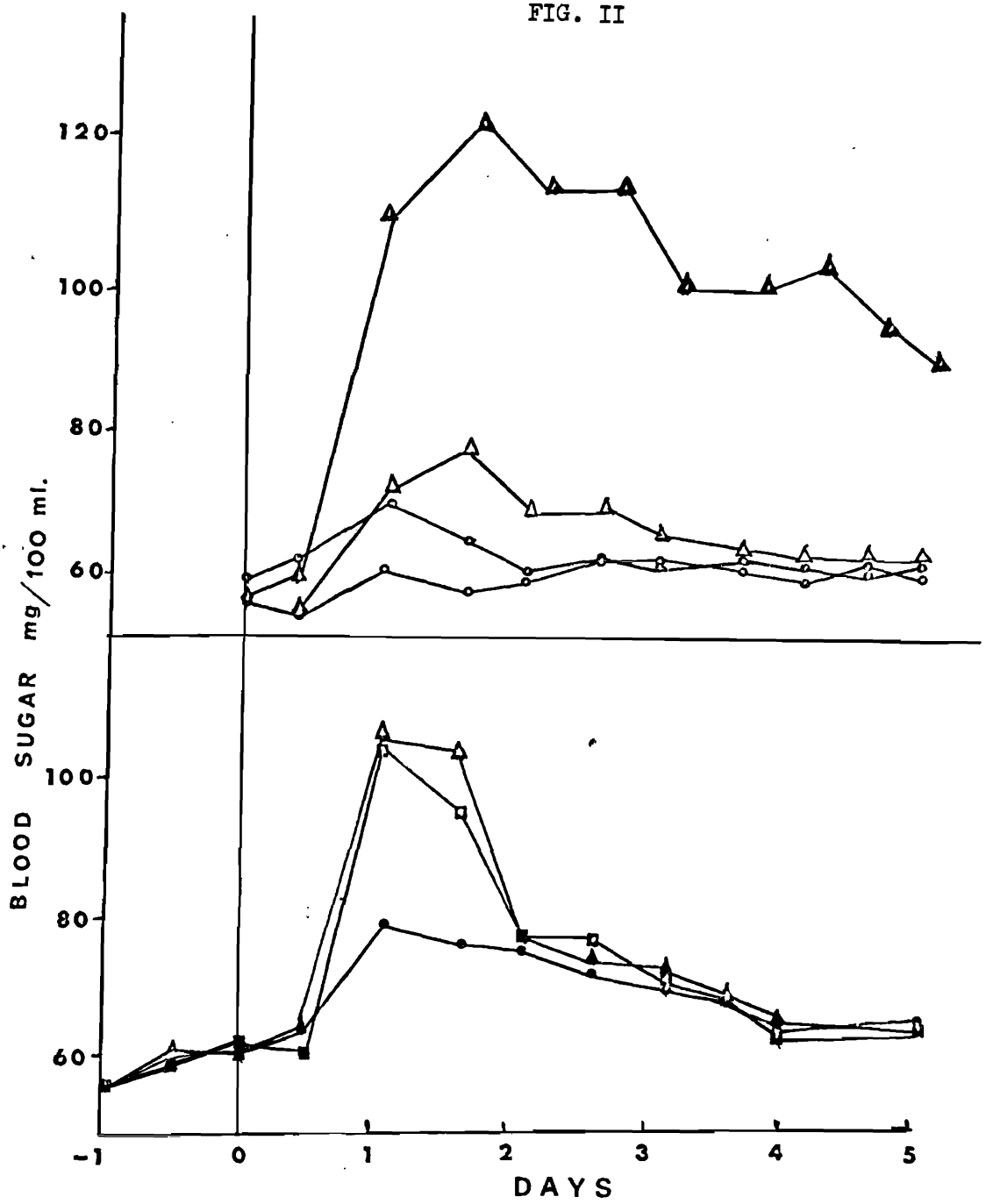
Corticosteroids in Other Diseases —

Corticosteroids are used in a wide variety of other diseases, amongst which are azoturia in horses, pneumonia, eosinophilic myositis, peritonitis, enteritis, colitis, burns, shock, infections, rodent ulcers (cats) and other conditions. In most of these it is necessary to include antimicrobial agents and to continue these for longer than the use of the steroid. Clinical reports indicate that quicker symptomatic improvements are noted if corticosteroids are used with antibiotics, and this has been also noted in human medicine [21, 22]. In veterinary practice additional information should be collected and published on the use of corticosteroids as conjoint therapy by comparison to a control group, as has been reported by Wagner *et al.* [22] in man. Since the use of corticosteroids is now so extensive it is almost inevitable that some differences of opinion should exist between workers in the laboratory and in practice. For example, when dealing with different aspects of the disease aetiology and clinical results of corticosteroid therapy, opinions vary on the usefulness of these substances in pregnancy toxaemia in sheep, mastitis in cattle, their possible effects on development of immunity during vaccination and their effects on pregnancy.

All these topics would require a separate presentation. In brief it is the author's opinion that:—

a. Until much more is known about the aetiology of pregnancy toxaemia in sheep,

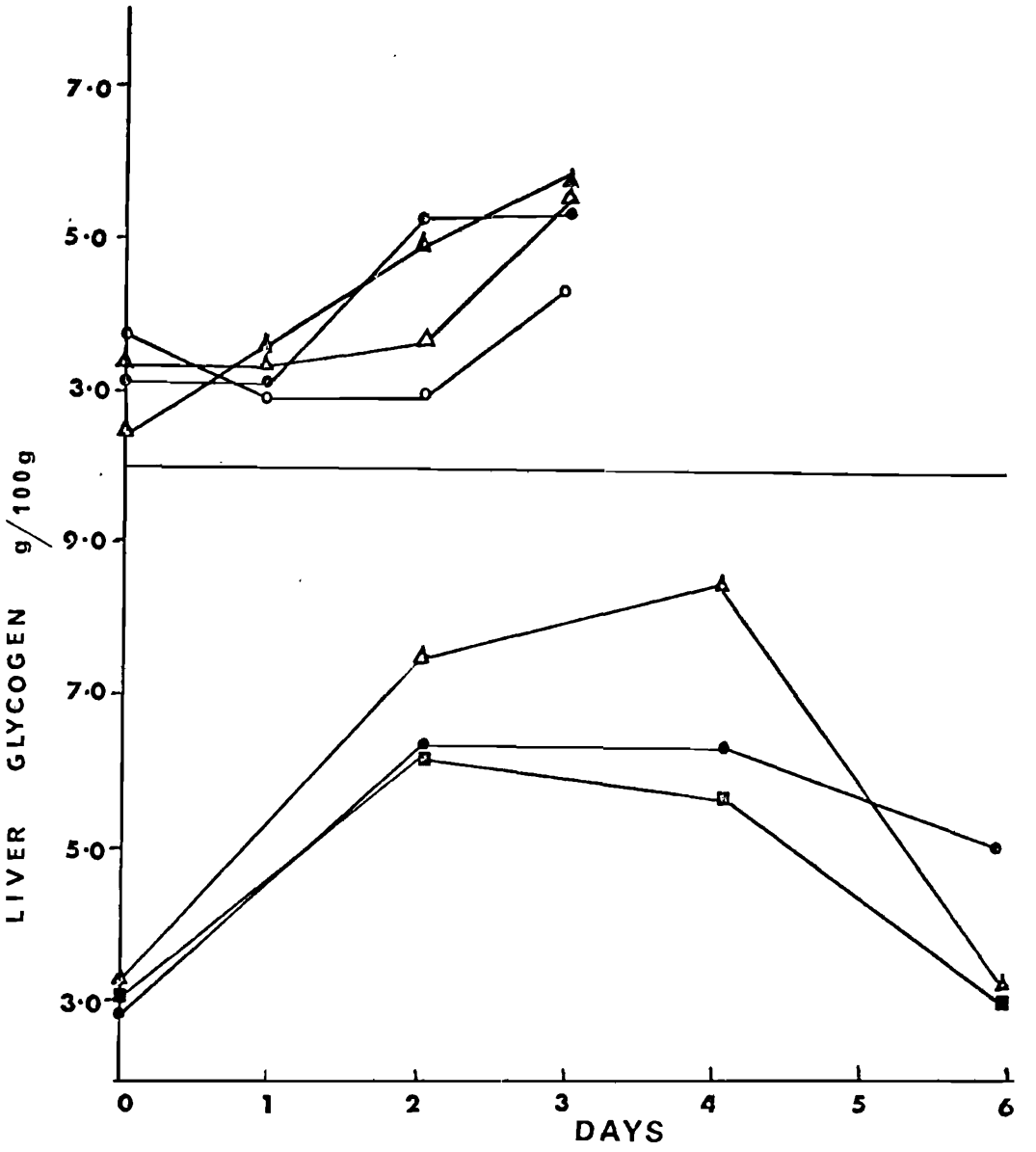
FIG. II



Mean blood sugar levels following treatments.

●	Prednisolone	100 mg.	intramuscularly
○	" TMA	100 mg.	"
△	Betamethasone	20 mg.	"
▲	"	100 mg.	"
■	" phosphate	20mg.	intravenously

FIG. III



Mean liver glycogen levels following treatments.

- ● Prednisolone 100 mg. intramuscularly
- ○ " TMA 100 mg. intramuscularly
- △ Betamethasone 20 mg. "
- ▲ " 100 mg. "
- ■ " phosphate 20 mg. intravenously

corticosteroids should be avoided in this disease. It appears that in some cases these substances may produce abortion, or death of the ewe. Reid [23] in Australia has carried out some work on a group of ewes with this disease using corticosteroid treatment in some and not in others. He recorded a number of deaths in the corticosteroid treated group, which confirms the author's clinical unpublished findings.

b. In mastitis in cattle; the reasons for including corticosteroids in intramammary preparations appear to vary. In some countries it is thought that these substances are beneficial in reducing the inflammation whilst in other countries it is admitted that corticosteroids in intramammary preparations are necessary for the reduction of "chemical irritancy" of the antibiotic preparations. It is expedient to use only drugs which are acceptable to the bovine udder [24] and although some early reports [25, 26] suggest some benefits from the inclusion of corticosteroids for intramammary therapy, these claims have not been substantiated by other workers [27, 29]. The necessity for restricting the number of substances infused into cows which are later destined to be found in milk, and the different infections which are generally not diagnosed bacteriologically until possibly after the initial treatment, all suggest that local installation of these substances into the udder is to be discouraged. However, the author knows many veterinarians who use corticosteroids by the parenteral route for the treatment of acute cases of mastitis. This leads to a general improvement (i.e. the non-specific sense of well-being) of the animals and an apparent reduction in teat hardness and pain of the udder. In each instance a conjoint use of an antibiotic is also included and frequently is extended for a longer period than the use of the steroid.

c. In a small experiment, Greenwood, Evans and Uvarov [30] were unable to show any immuno-suppressive activity whilst using one corticosteroid at therapeutic levels, in dogs which had been vaccinated against distemper.

d. Effect of corticosteroids in pregnancy. The administration of drugs during pregnancy is very occasionally discussed in relation to the use of corticosteroids. It is appreciated that during pregnancy, all drugs should be used with caution until further evidence is available on their effect on pregnancy. The author has not been able to reproduce abortion in normal cattle when using some of the modern corticosteroids in conventional and double the therapeutic doses during different stages of pregnancy, or when corticosteroids were administered by the intravenous route. Since corticosteroids in human medicine have been used to prevent abortions, it is obvious that much more work is required to compare the effects of these substances in different species and stages of pregnancy. The very extensive use of corticosteroids in animals and the lack of reports on abortions suggests that this is not a great hazard.

Side Effects from Corticosteroid Usage

It is generally accepted that if side effects do occur, the are mainly reversible. In small animals excessive thirst and polyuria have been associated with an overdose of corticosteroid. Diarrhoea and shaking in cattle has been noted very occasionally, but cannot be related to one particular drug or dose. A reduction of milk yield is known when these drugs are used in normal animals, but this is difficult to measure in sick animals where milk yield is generally depressed.

Contra-Indications

Veterinarians seldom report any contra-indications for these drugs, but the following are the more obvious.

Degenerative diseases of the eyes; Cardiac and renal impairement; Infections — unless full antimicrobial therapy is included and is used for longer than the steroid; Diabetes mellitus in the dog; Pregnancy toxaemia in sheep; Lameness of unknown origin; Inappetance — if no differential diagnosis is present.

It is essential to work out a maintenance dose of corticosteroids and to reduce the dose gradually in all instances where these drugs are used for a prolonged period.

The Selection of Corticosteroid Products

In selecting a corticosteroid product, the veterinarian should first and foremost be concerned with the evaluation of the drug in different species. The information on these

drugs should be based on species evaluation and not on results obtained from laboratory animals or man, and transposed for veterinary use. This is particularly important in bovine practice. The route of administration and the dose for each preparation must also be clearly defined for each species. Published references on evaluation add greatly to the veterinarians confidence in the use of one of the most important drugs so far evolved for use in animals and man.

ACKNOWLEDGEMENT

I thank all the veterinarians who have, and continue to, evaluated corticosteroids produced for veterinary practice. I am grateful to the Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food in the U.K. for their help in the interpretation of the use of these drugs in metabolic disease, and to my own colleagues for diligently accepting the necessity of evolving drugs for veterinary practice.

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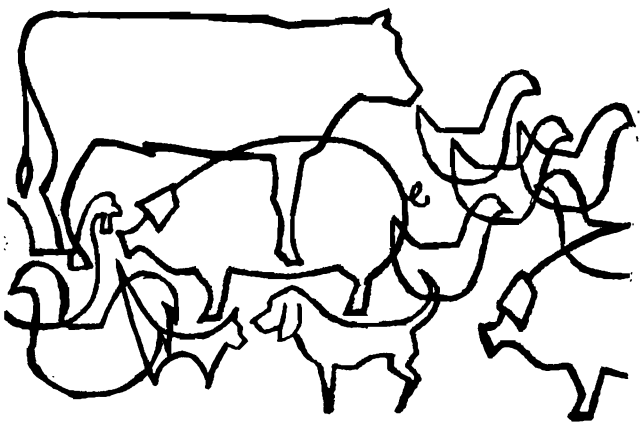

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THE BLUE WILDEBEEST AS A SOURCE OF FOOD AND BY-PRODUCTS:

The production potential, parasites and pathology of free living wildebeest of the Kruger National Park

E. YOUNG, L. J. J. WAGENER AND P. J. L. BRONKHORST*

SUMMARY

This report furnishes evidence concerning the live and dressed carcase weights, dressing out percentage, and component weights of blue wildebeest of the Kruger National Park. The incidence of parasites and pathological conditions encountered in the course of various surveys and which may render their carcases and organs unfit or commercially inferior, is also recorded.

INTRODUCTION

Since 1966, blue wildebeest, *Connochaetes taurinus*, were culled in the Kruger National Park. In order to obtain an indication of the food production potential and the significance of parasites and pathological conditions of this species in the Park, carcases and organs of some of these animals were examined for parasites and lesions; some were also weighed for the calculation of mean production figures.

MATERIAL AND METHODS

The animals were shot throughout the year in the central and southern districts of the Kruger Park. The carcases and organs of 33 adult wildebeests were weighed and examined immediately after exsanguination, dressing and evisceration. From the live weight and weight of the dressed carcases their dressing percentage was calculated.

Pienaar (1969) determined the live weight of 260 wildebeest of known age and sex and also calculated the age and sex composition of 296 herds in the park [1]. This information (Table 1) was used in the calculation of the live weight of an average sized wildebeest. With random culling of game, the average weight of individual wildebeest destroyed may be expected to closely approximate this figure. The dressing percentage of the weighed carcases was taken into consideration in the calculation of the expected average dressed carcase weight of randomly selected wildebeest of the Kruger National Park.

Complete examination of all the body components of every individual animal was impossible and the incidence of parasitic and other pathological conditions was therefore estimated on a percentage frequency basis. A total of 344 animals was examined for cysticercosis and 91 for pentastomiasis and lungworm infestation.

RESULTS

The mean weight of the 33 full-grown, adult blue wildebeest and their dressed carcases amounted to 506.93 and 293.06 lb respectively, the carcase yield thus being 57.7 per cent.

The average herd composition of wildebeest in the Kruger National Park, the average live weight of the different groups and the calculated live and dressed carcase weight of an average sized wildebeest are provided in Table 1. Mean weights of the different organs and other components of adult animals are summarized in Table 2.

The incidence of the three most significant parasitological conditions of wildebeest carcases and the frequency with which different parts of the body were affected with macroscopically visible pathological conditions are provided in Tables 3 and 4.

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Table 1: THE HERD COMPOSITION AND AVERAGE LIVE WEIGHT OF DIFFERENT AGE AND SEX GROUPS OF BLUE WILDEBEEST AND CALCULATED LIVE AND CARCASE WEIGHTS OF AN AVERAGE SIZED INDIVIDUAL

Age/Sex	% of herd	Av. body wt. (lb)	Relative biomass (lb)
Adult bulls (>2 years)	24.59	549.87	13521.30
Adult cows (>2 years)	38.37	473.77	18178.55
Juveniles (1—2 years)	9.41	371.30	3493.93
Calces (under 1 year)	27.63	234.17	6470.12
Total biomass (100 representative individuals)			41663.90
Body weight of average sized wildebeest (lb)			416.64
Dressed carcase weight of average sized wildebeest (lb) (57.7% of total body weight)			240.40

Table 2: MEAN WEIGHT OF DIFFERENT ORGANS AND OTHER COMPONENTS OF ADULT WILDEBEEST CARCASES

Heart	2.24 lb	Lungs (without trachea)	5.38 lb
Liver	5.27 lb	Spleen	1.17 lb
Kidneys (both)	1.06 lb	Tongue	1.21 lb
Skin	40.03 lb	Tail	0.90 lb
Free fat, stripped from the carcase and internal organs			1.42 lb
Head and distal extremities of limbs, i.e. below carpal and tarsal joints			49.18 lb
Tripe (cleaned stomachs and intestines)			27.28 lb
Yield of blood, directly after shooting			14.00 lb

Table 3: INCIDENCE OF CYSTICERCOSIS, LUNG WORM INFESTATIONS AND PENTASTOMIASIS IN CARCASES AND EDIBLE OFFAL OF WILDEBEEST

Parasitic condition	Incidence of infestation
Cysticercosis	77.03%
Pentastomiasis	75.82%
Lungworm infestation	64.92%

Table 4: FREQUENCY OF PARASITES AND PATHOLOGICAL CONDITIONS IN WILDEBEEST CARCASES, TISSUES AND ORGANS

Organs	Frequency (%)	Parasites/Pathology
Carcase (musculature)	±77	**Cysticerci.**
Heart	±35	**Cysticerci.**
Lungs	±77	**Cysticerci,** lungworm, focal disseminated pneumonia and emphysema, or calcified parasitic nodules.
Liver	±71	**Cysticerci,** pentastomes, cloudy swelling, biliary cirrhosis, focal disseminated necrosis, ulceration of capsule, haemorrhages or peritonitis.
Spleen	0	None.
Kidneys	± 2	**Cysticerci.**
Pericardium	± 7	**Cysticerci** or pericarditis.
Pleura	± 8	**Cysticerci.**
Peritoneum	±33	**Cysticerci,** or chronic fibrous peritonitis.
Skin Superficial lymph nodes	± 2 100	**Cysticerci,** lymphoid hyperplasia, oedema or haemorrhages.

DISCUSSION

The mean weights of the 33 adult blue wildebeest (these animals were represented by 27 males and six females) and their dressed carcases, correspond very well with Hitchins[2] mean weights for adult wildebeest bulls.

The mean dressing out percentage of 57.7 per cent for our wildebeest also compares very well with his findings as well as with the corresponding range of 50—63 per cent for "African game animals"[3]. It also approximates closely the mean value of 57.4 per cent for impala of the Kruger National Park[4].

The mean weights of parenchymatous organs, fat, blood, tongues, tails, skins and offal were calculated to determine the potential economic value of an entire wildebeest. In order to make such calculations of more practical value, it was attempted to estimate the frequency of pathological conditions in carcases and different organs.

Seventeen different parasites have so far been found in blue wildebeest of the Kruger National Park[5] but only six may significantly affect the commercial value of wildebeest

carcases. Carcases or organs affected with these parasites may acquire a repulsive appearance and may be rendered commercially inferior as a result of such infestations. The most important of these seem to be the two lung-worms, *Dictyocaulus viviparus* and *Protostrongylus* sp., an unidentified pentastone or "tongue-worm" and *Cysticercus regis*.

Although two lung-worm species have been found in wildebeest [6], no attempt was made at distinction at meat inspection. The parasites were not always found but the characteristic lesions were sometimes used as an indication of infestation. Parasites could sometimes be seen in the bronchi of affected wildebeest and the most conspicuous macroscopic lesions consisted of whitish, well-demarcated patches of emphysema, especially on the dorsal surfaces of the diaphragmatic lobes, and more rarely of focal disseminated areas of pneumonia, while in some cases calcified nodules were also present. The mesenteric lymph nodes were frequently enlarged, possibly due to migrating stages of lungworms. Lesions were found in wildebeest of all ages; infested animals were usually in good condition. Because of the low commercial value of lungs, the high incidence of infestation and the tendency of parasites to be present other than in the characteristic lesions, condemnation of all affected lungs as unfit for human food is considered advisable. Such lungs should preferably be used for by-products.

Immature pentastomes occurred in 64.92 per cent of the livers examined. They are typically tongue-shaped parasites and are, as a general rule, easily found in the larger hepatic veins, but may be found in the liver substance and are sometimes seen lying under the liver capsule. Small, almost round openings were often observed in the liver capsule and it is believed that these openings were caused by the pentastomes after the death of the host. The trimming of affected livers is not considered advisable and they should preferably be used for by-product manufacture. The parasites were also found in the atria and ventricles of the heart as well as some of the larger bloodvessels. Infested hearts could, however, be rendered fit for human consumption, by flushing out the parasites with water. McCully *et al.* [7], have similarly recorded pentastomes in wildebeest. Immature pentastomes were also found encountered in buffalo [8] but rarely in impala of the Kruger National Park [2].

Cysticerci or "measles" occurred in 77.03 per cent of the 344 wildebeest examined for this parasite. *Cysticerus regis* is the only species which has so far been identified in wildebeest of the Kruger Park [9]. Cysts were observed in almost all organs, and were frequently found in the cardiac musculature; 35.16 per cent of 91 hearts were found to be infested. Most of the cysts were seen in the superficial layers of the skeletal muscles and under the serous membranes of the body cavities and viscera. The latter phenomenon and chronic fibrous inflammation largely accounted for the relatively high incidence of pathological lesions of the serous membranes (Table 4). In this Table some of the other localities of *Cysticerus* are also indicated.

Because *Cysticercus regis* is not transmissible to man and is the only species encountered in wildebeest, only severely affected and aesthetically objectionable carcases were condemned as being unfit for human consumption. An insignificant number of carcases were severely affected; they were of course, suitable for the production of carcase meal. The cysts were found in almost every part of the cardiac musculature and affected hearts had to be sliced in order to determine the total distribution of the parasites. Trimming of affected hearts is therefore impractivable. In the other parenchymatous organs and the body cavities, the cysts were almost always situated superficially, and this facilitated their removal.

Oesophagostomiasis has also been diagnosed in local wildebeest [6] but no significant macroscopic lesions were attributed to this infestation.

Mange, caused by *Sarcoptes scabiei*, can cause significant damage to the hides of wildebeests in certain areas in winter. The incidence of mange was found to be as high as 15 per cent in a large herd on one occasion. In most instances it is less than one per cent. The general condition of severely infested individuals may also be detrimentally affected [10]. Such animals are very prone to predation and may also die as a direct result of the infestation.

The incidence of cutaneous papillamatosis is generally very low in blue wildebeest and the lesions are usually isolated and more commonly confined to the skin of the forequarters.

Most of the wildebeests came from an area where hydatidosis had frequently been diagnosed in Burchell's zebras. No wilde-

beest, however, were found to harbour this parasite.

Trichinoscopic and other tests for trichinosis on 222 wildebeest carcases [11, 12] as well as tests for some other infectious diseases all yielded negative results.

The carcases of five wildebeest calves under four months of age were found to be free from any of the above mentioned parasites.

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FACTORS INFLUENCING RABIES OUTBREAKS : THE AGE AND BREEDING CYCLE OF THE YELLOW MONGOOSE, *CYNICTIS PENICILLATA* (G. CUVIER)

I. F. ZUMPT*

SUMMARY

A survey carried out in the Western Transvaal showed that *Cynictis penicillata*, the Yellow Mongoose or Red Meerkat, has a pregnancy period from June to December with a peak in October. The female: male proportion of all animals examined was 1.2 : 1.

Five age groups were established, and the age determination from skulls of meercats derived from rabies cases showed that thirteen of fifteen fell into group III (1—2 years).

It is concluded that the seasonal incidence of rabies with a peak in August is directly correlated with the breeding habits of the Yellow Mongoose.

INTRODUCTION

It is a well known fact that the *Cynictis* type of rabies is seasonally influenced and that according to Snyman[1] and Maré[2] the peak of yearly outbreaks occurs during August (June to September). Both authors conclude that this is due to a decreased food-supply during the dry winter period when the Yellow Mongoose wanders over a great distance in search of food. As the vegetation cover is also at its lowest this animal can be detected easily and has a greater chance of coming into contact with humans and domestic animals. During a survey of the feeding habits of these meerkats[3] it was, however, found that even in severe winter months food was freely available.

Stomach evaluations showed no significant difference in the amount of stomach contents. The conclusions reached by Snyman[1] and Maré[2] must therefore be re-evaluated.

Field observations over the past few years indicate that the peak of rabies outbreaks can be correlated with the breeding cycle as well as with certain age groups of the Yellow Mongoose.

METHODS

Breeding Cycle and Age Determination

From March 1966 to November 1968 a total of 676 Yellow Mongooses were captured in the Western Transvaal by trapping, shooting or digging up entire colonies. *Post-mortem* examinations were performed on all female animals caught by the first two methods, whereas all those dug up were palpated and caged. It was thus possible to record the monthly incidence of pregnancy. In addition fifteen females were subjected to laparotomy to measure uteri and foeti. These animals were kept to establish the gestation period.

As yet no work on the determination of the age of the Yellow Mongoose has been published. Available literature on other species was consulted in respect of eruption of teeth [4-16], growth of the eye lens [13, 17-20], growth of long bones [21-23], skull measurements [13-15], body weights and measurements [16-24] and development of sex organs [13, 22, 24, 25].

It was concluded that the techniques on the progressive growth of the eye lens, the growth of long bones, body weights and measurements and the development of the sex organs would be impracticable in the field as expensive apparatus would be needed. The following criteria were thus selected and evaluated: skull weight (SW), zygomatic width (ZW), intra-orbital constriction (IC), basilar length (BL), eruption and wear of incisor, canine and molar teeth; ossification of naso-maxillary (NM), frontal and fronto-maxillary sutures (FM); completion of the eye orbit and the development of the parietal (P) and inter-parietal (IP) crests.

Sixty-five skulls were used, nine of these were from animals of known age and the rest were collected at random. The animals of known age were born in captivity and fed on various foods closely resembling those consumed naturally.

* Regional Veterianary Investigation Centre, P.O. Box 6, Mafeking.

All skulls of unknown age were evaluated on the above mentioned criteria and placed into an "age line" based on the progressive increase in skull weight, skull measurements, eruption of teeth and ossifications. The skulls of known age were then fitted into this line, each nearest to the skull with corresponding criteria.

Fifteen skulls from animals that had died or suffered from rabies were also aged.

RESULTS

Breeding Cycle

Pregnant meerkats were only found from mid-July to the end of December with a peak during October (see graph II). Mating was observed during August and September. In this period no pregnant females were found suckling another litter. Snyman's [1] statement that the Yellow Mongoose rears two litters per breeding season is not substantiated. The female: male proportion was 1.2 : 1 (SE = 2.38, not statistically significant.

All captured pregnant females operated on or palpated resorbed or aborted their foeti and only those very heavily pregnant gave birth in captivity.

Age Determination

By using the above mentioned criteria all skulls were divided into five groups. The ages of the first three groups could be determined as all skulls of known age fell into these groups.

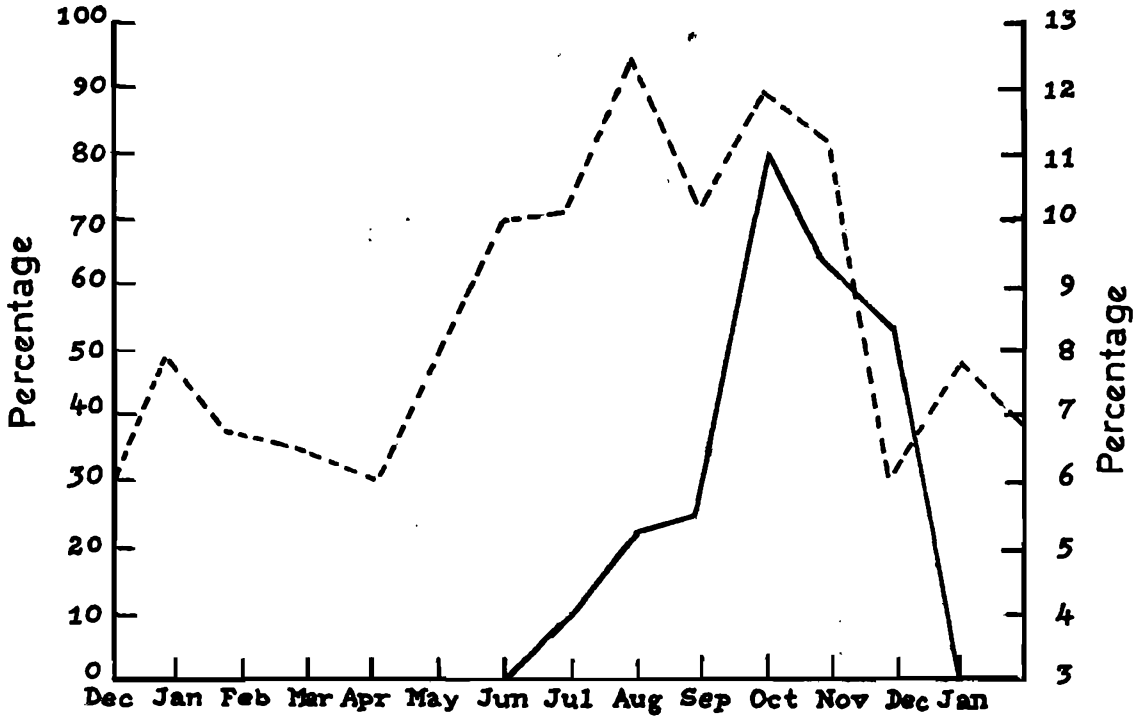
Dention Formula: I 3/3, C 1/1, P 4/4, M 2/2.

Only in the young and sub-adult (groups I and II) a positive correlation exists between the skull weight (sun-dried) and the three measurements.

Each criterion described in this paper has some disadvantages but by combining several criteria, it was found relatively easy to age skulls of the Yellow Mongoose. It is open to speculation how old these animals become in nature but from the distribution of the age groups, it is probable that group V, the oldest animals, were four to five years old although ages of twelve years have been recorded in captivity [26].

SIGNIFICANCE OF AGE DETERMINATION

This method of age determination was applied to fifteen skulls of known rabies cases.



- - - - - : Mean number of positive cases of rabies in C. Penicillata.
———: Mean number of pregnant C. penicillata.

Table: AGE GROUPS

Age group	Age	Number of skulls of known age	Number of skulls of unknown age	Identification	Classification criteria
I	3 to 9 months	4	7	SW : 6.72 gm* ZW : 32 mm* IC : 12.4 mm* BL : 48.7 mm* Temporary incisor and canine teeth erupting No wear of teeth N-M, F-M sutures open (not ossified) Eye orbit open (not ossified) P and I-P crests not developed Skull with rounded parietal portion	Temporary or erupting teeth Sutures open Orbit open Crests not developed Skull roundish
II	9 to 12 months	2	5	SW : 9.28 gm* ZW : 34.7 mm* IC : 13.4 mm* BL : 54.7 mm* Permanent teeth no wear N-M, F and F-M sutures open Orbit nearly closed P not developed I-P mildly developed Skull oblong	Permanent teeth, no wear Sutures open Orbit nearly closed P not developed I-P mildly developed Skull oblong
III	1—2 years	3	25	SW : 10.48 gm* ZW : 38 mm* IC : 14.5 mm* BL : 58.2 mm* Permanent teeth Lower third incisors and upper molars mild wear Sutures nearly closed (faint) Orbit closed (ossified) P crest mildly developed I-P crest well developed	Permanent teeth Wear on lower I^3 and upper molars Sutures faint Orbit closed P crest mildly developed
IV	unknown	0	13	SW : 12.7 gm* ZW : 39.1 mm* IC : 16.0 mm* BL : 58.6 mm* Permanent teeth Incisors and molars moderately worn Sutures closed Orbit closed Crests well developed	Moderate teeth wear Sutures closed Orbit closed Crests well developed
V	unknown	0	6	SW : 13.76 gm* ZW : 40 mm* IC : 16 mm* BL : 60 mm* All teeth extensively worn Sutures and orbit closed Crests very strongly developed	All teeth extensively worn All sutures and orbit closed Crests strongly developed

*Average figures.

Of thirteen skulls received from Onderstepoort

II fell into group III (1—2 years)
I fell into group I (3—9 months)
and I fell into group V.

Two skulls which originated from positive rabies cases in Mafeking fell into group III (1—2 years).

The eleven group III — skulls were all thought to be from animals 18 to 22 months old.

DISCUSSION AND CONCLUSION

Age determination is limited by the small number of skulls of known age and the fact that feeding in captivity may have an influence on some of the criteria. The merits of the method lie in the combination of several criteria. Furthermore, it can be applied easily and quickly as only one definite breeding period exists per year and age groups are clearly demarcated.

If the pregnancy graph is compared to the graph representing the average number of rabies cases in meerkats per month (five year survey), a positive correlation is obvious (Graph II). Both peaks, namely that of pregnancy and that of the maximum number of monthly rabies cases, are seperated by an interval of approximately two months. From other field and laboratory observations[27] it has been established that the young leave the mother permanently only shortly before she mates again and that these young meerkats do not conceive in their first year of life. When sexually mature and in their second year stress factors[1, 2] such as migration into other areas in search of mates and subsequent fights to establish their own territories[28] as well as the hunt for food in this period make this group more susceptible to rabies. (Thirteen of the fifteen skulls from rabies cases fell into group III). It is therefore concluded that the yearly peak of rabies outbreaks is in direct correlation with the breeding habits of the Yellow Mongoose.

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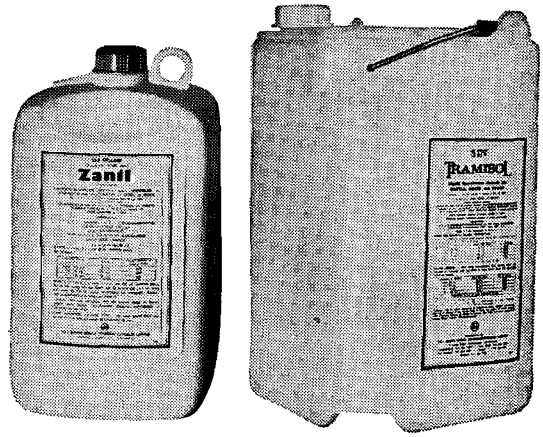

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Uiters doeltreffend teen:

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★ LEWERSLAK in beeste en skape.
★ LEWERLINTWURMS in skape.

Maklik om te gebruik. Geen proefdosering, voorafdosering of uithongering nie. **ZANIL** is veilig, selfs vir koeie wat in die melk is, en vee wat dragtig of in 'n swak kondisie is.

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★ Bruinmaagwurm
★ Knoppieswurm
★ Bankrotwurm
★ Haakwurm
★ Langnek-bankrotwurm
★ Longwurm
★ Grootbekwurm
★ Bees-bankrotwurm (Cooperia spp.)

in beeste, skape en bokke.

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DOSIS: Skape — 5 c.c. per 25 lb.
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BESKIKBAAR IN HOUERS VAN: 26 oz., ½ gell., 2 gell., en die tamaai ekonomiese 5-gelling-**emmer.**

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Pryse is gekwoteer onderworpe aan die gewone handelskorting.

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Jl S. Afr. vet. med. Ass. 40 (3): 325-329 1969

CHANGES IN THE CHLORIDE CONTENT OF THE CERVICAL MUCUS AS A TEST FOR TIME OF OVULATION IN THE EWE

J. M. VAN DER WESTHUYSEN AND C. H. VAN NIEKERK*

SUMMARY

The reliability of a spot test for time of ovulation, based on changes in the chloride concentration of cervical mucus, was compared with actual ovarian observations. The usefulness of the spot test was found to be limited and merely indicated an approximate time of ovulation.

Ovariectomized ewes were employed to compare the chloride spot, vaginal cornification and cervical mucus aborization tests for ovulation after the administration of diethylstilboestrol (DES) or progresterone. The chloride spot test paralleled the vaginal cornification test in response to DES, but the former was more sensitive to progresterone.

INTRODUCTION

A simple test for ovulation in women has been developed, based on changes in the chloride content of cervical mucus[1, 2]. Turnbull, Shutt & Braden[3] subsequently applied this technique to sheep as a test for impending ovulation. The experiment to be reported in this paper was designed to examine the reliability of this test for the determination of the precise time of ovulation in the ewe. In addition, in a second experiment, comparisons were made with other vaginal tests for ovulation.

MATERIALS AND METHODS

Experiment 1 (March-April)

Fourteen Merino ewes were employed as follows: Three received 17 daily intramuscular injections of 10 mg of progesterone (Protormone: Burroughs Wellcome & Co.), five carried intravaginal fluorogestone (FGA) impregnated sponges (Synchro-Mate: G. D. Searle & Co. Ltd.) treated with dihydrostreptomycin (Agarin: Maybaker Pty. Ltd.), for 17 days and the remaining six acted as untreated controls. Four hours after the cessation of the progesterone treatments, all the ewes were teased with active vasectomised rams every six hours until the end of the subsequent oestrous period. Immediately after each teasing, a specimen of cervical mucus from each ewe was applied to a strip of chromatography paper, on which silver chromate had previously been precipitated. The chloride content of the mucus samples was estimated by comparing the resulting colour with the colours produced by standard solutions of sodium chloride ranging from 0.1 to 0.9 per cent[1]. Once there was a fall in the chloride content, which indicated the approximate time of ovulation[3], each animal was laparotomised to determine whether ovulation had, in fact, occurred. If necessary, these laparotomies were repeated at six hourly intervals until the ewe had ovulated.

Experiment II (August)

In this experiment the vaginal response of ovariectomised ewes to treatment with progesterone and diethylstilboestrol (DES) was studied. The chloride "spot" test (described above), cervical mucus arborization test[4], and vaginal cytology test[5] were compared.

Five ovariectomised Merino ewes each received 12 daily injections of 10 mg progesterone in arachis oil, followed by three daily injections of 20 μg DES, and finally a further four daily injections of 10 mg progesterone. All injections were given about 12 noon. The day before the DES injections began, vaginal smears and samples of cervical mucus were taken every 12 hours (6 a.m. and 6 p.m.) and repeated daily for the following seven days. Vaginal smears were fixed and stained with Schorr's stain[3] and the mean degree of cornification of the epithelial cells in the smears was determined from the mean percentages of cornified cells in eight microscopic fields on each slide. Air dried smears of the sam-

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ples of cervical mucus were examined microscopically under low powder and classified as showing strong, medium, weak or negative arborization.

RESULTS

Experiment I

A summary of the results of each ewe is presented in Figures 1 and 2. The mucus samples obtained from ewes receiving progesterone and untreated ewes during dioestrus had a chloride concentration of 0.2 to 0.3 per cent. On the other hand, both the total amount of and chloride concentration of the mucus rose to a mean of 0.8 per cent during a period varying from 8 to 30 hours prior to oestrus and was maintained for a further 20 to 40 hours. However, there was a fall in the chloride concentration before the end of oestrus, but it was found that ovulation was more closely related to the end of oestrus than to the initial drop in cloride level.

Apart from one ewe (Figure 1, no. 6) ovulation usually occurred within 6-24 hours after the first drop in chloride level, but, as can be seen in Figures 1 and 2 the variation was considerable. Ewe no. 6 experienced an unusually short heat period of low intensity and ovulated 42 hours after the first drop in chloride concentration. In two spongetreated ewes which did not show heat (Figure 2, nos. 15 and 18) laparotomies were carried out for the first time 50 hours after a drop in their chloride level. By this time both had ovulated. From the age of their corpora lutea [6] ovu-

FIGURE .1:- Changes in the chloride content of the cervical mucus of normal cyclic ewes.

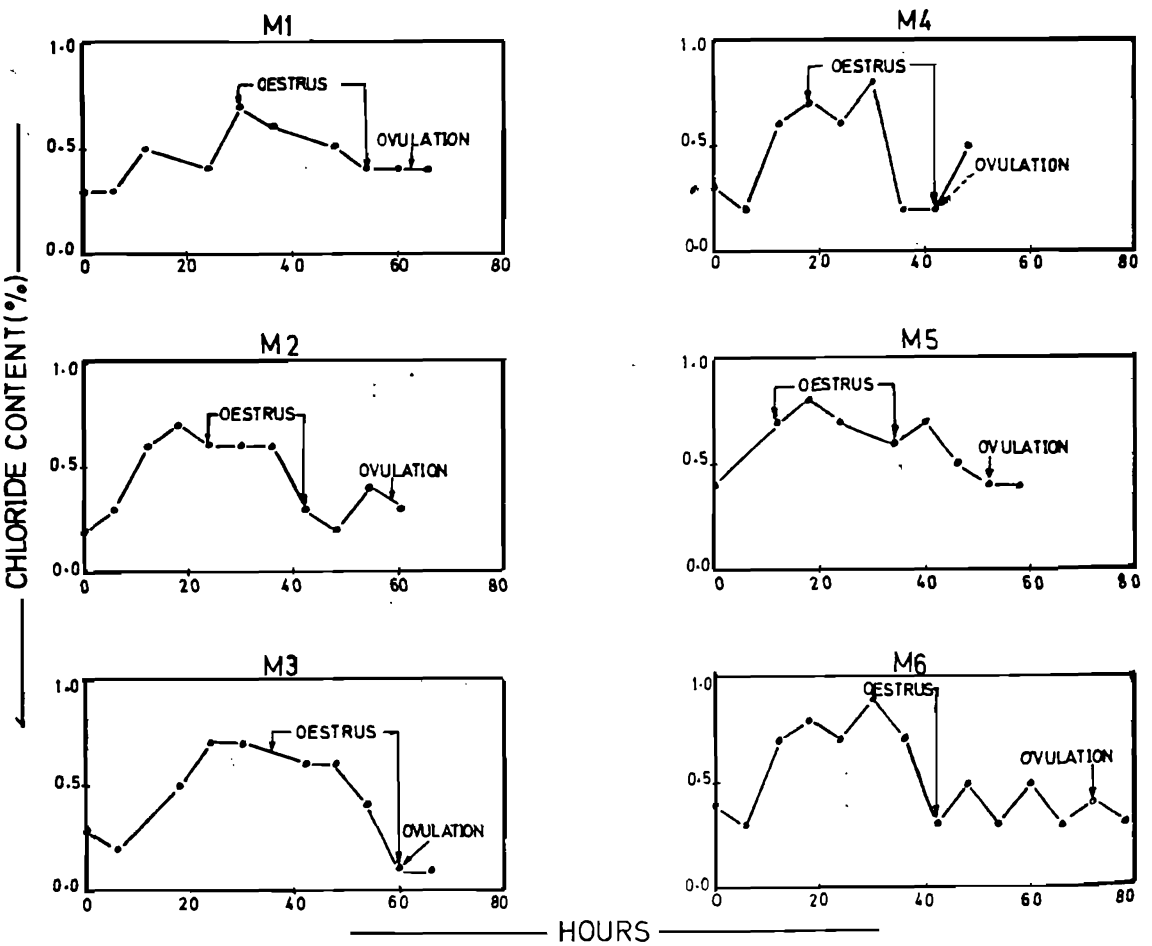
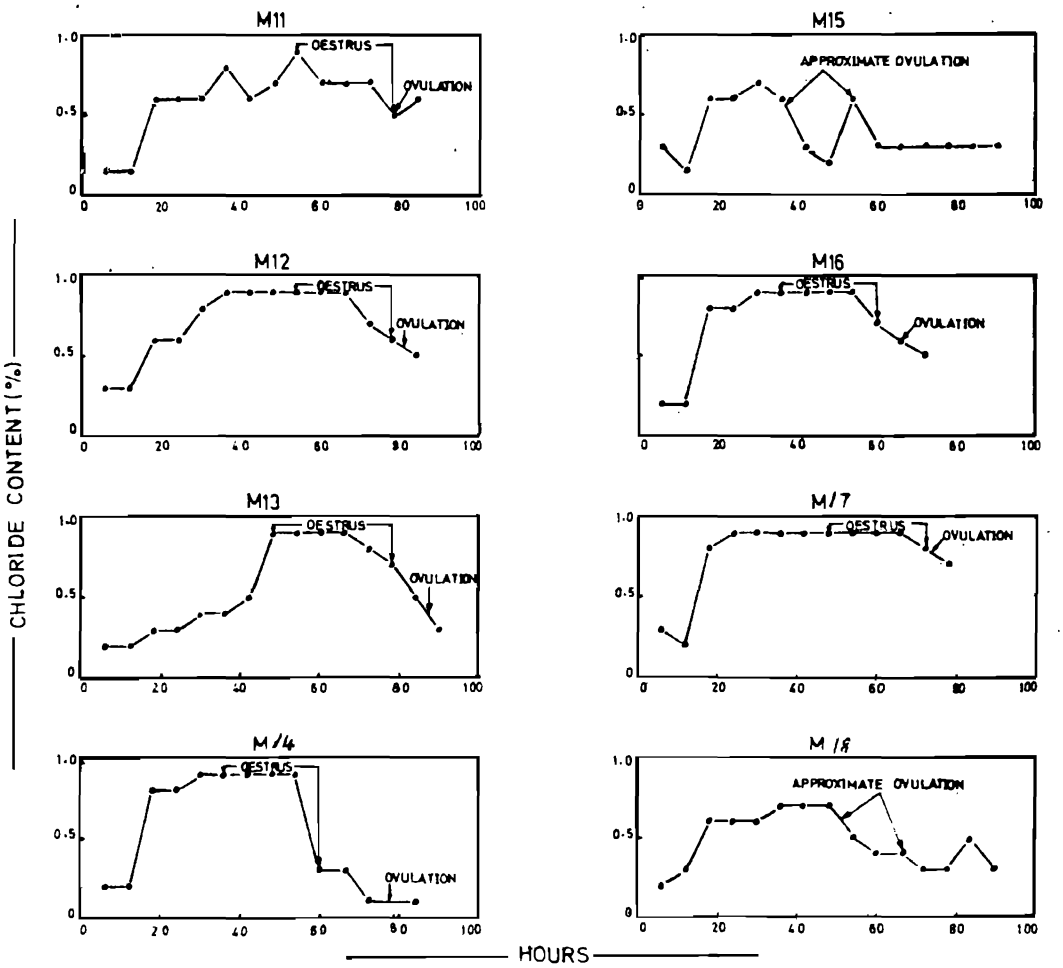


FIGURE 2:- Changes in the chloride content of the cervical mucus of ewes treated with progesterone injections (M11-13) and FGA sponges (M14-18). Last injection was given at 0 hours, while sponges were also removed at that time.

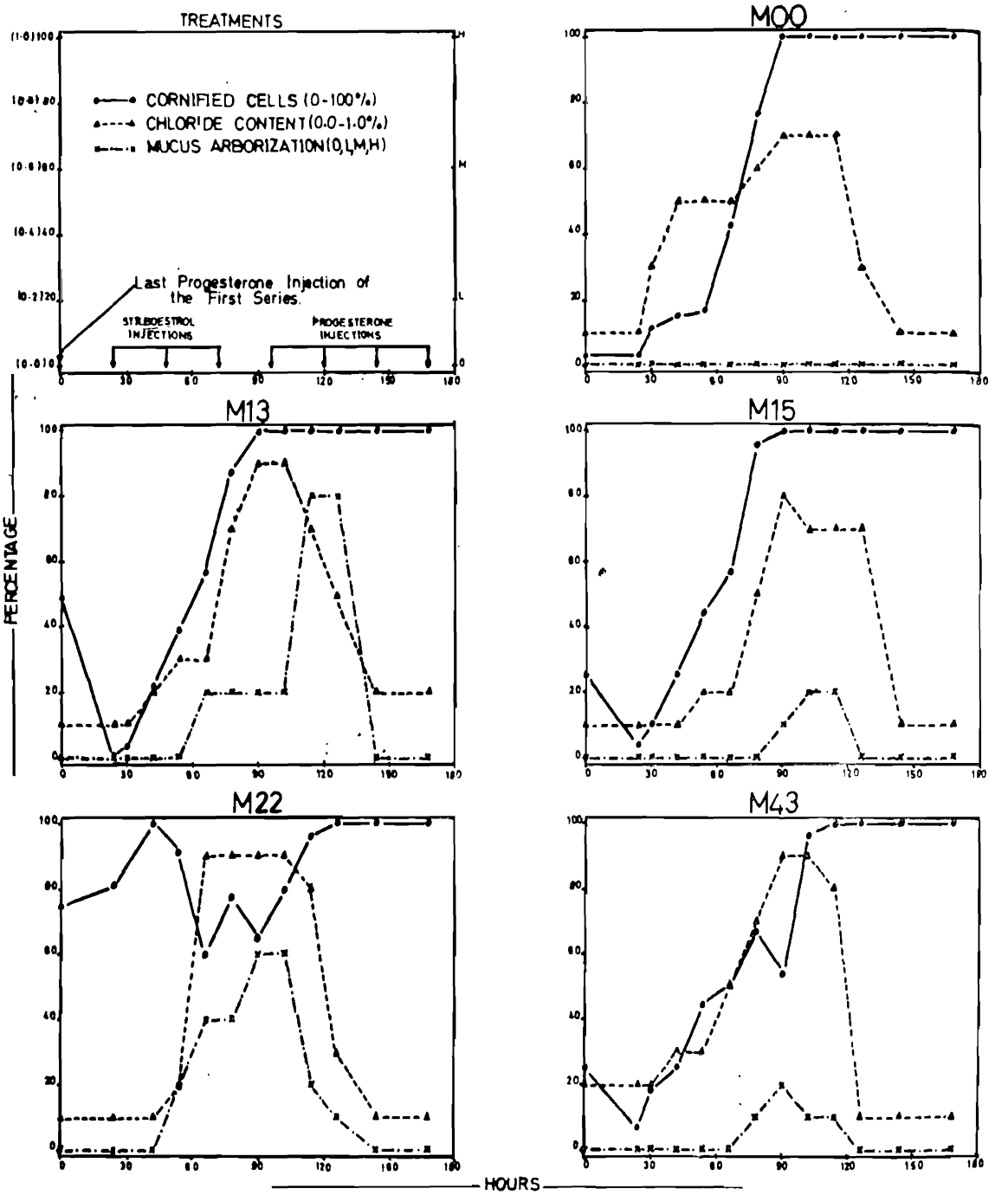


lated seemed to have occurred during the same period after the drop in chloride level, as for the ewes which showed heat.

Experiment II

The results of this experiment are presented in Figure3. A high degree of similarity in response to DES treatment occurred in all sheep between the chloride spot test, vaginal cornification and cervical mucus arborization tests (except M22, which had pyometra). Within 6-8 hours after the first DES injection, there was an increase in the vaginal cornification, folowed by a rise in the chloride concentration of the cervical mucus. Both the chloride content of the mucus and the degree of vaginal cornification reached their maximum approximately 66 hours after the initial DES injection. Within 18-48 hours after the

FIGURE 3:- The chloride content and arborization of the cervical mucus and the vaginal cornification of ovariectomised ewes in response to stilboestrol and progesterone administration.



commencement of the second series of progesterone injections, the chloride content of the cervical mucus dropped sharply, while within 72 hours no response in the vaginal cornification occurred.

DISCUSSION

In accordance with McSweeney & Sbarra [1, 2] and Turnbull *et al.* [3] the increase in the chloride content of the cervical mucus was found to be associated with follicular maturation and thus presumeably with a rise in oestrogen secretion, while a decrease in chloride content is attributed to an increase in progesterone secretion. This rise in the chloride content, approximately 24 hours before oestrus, was always accompanied by a change in the quantity and viscosity of the cervical secretions before and during oestrus [7, 8]. Although ovulation invariably took place about 15 hours after a drop in the chloride content of the cervical mucus, there was too much variation between ewes for a reliable determination of the exact time of ovulation by this test alone. (Figures 1 & 2). No close relationship could be found with either the rise or drop in chloride content and ovulation. Nevertheless, in two ewes which showed no behavioural oestrus, a "normal" chloride concentration pattern occurred, while ovulation seemed to have taken place at the normal time relative to a drop in the chloride content of the cervical mucus.

Due to the fact that the vaginal glandular and cytological reactions depend on changes in the secretion of ovarian hormones, comparisons were made between the vaginal tests after the administration of ovarian hormones to ovariectomised ewes (Experiment II). A high degree of similarity occurred between the chloride spot test, vaginal cornification and cervical mucus arborization tests in response to diethylstilboestrol (DES) injections. The DES treatment paralleled the reactions of the intact ewe prior to oestrus, as there was an increase in vaginal cornification, as well as an increase in cervical mucous arborization,, chloride content and volume. On the other hand, the sudden decrease in the chloride concentration of the cervical mucus near the end of oestrus (Experiment I), and after the second series of progesterone injections (Experiment II), indicates a rise in blood progesterone. McSweeney & Sbarra[1] state that a postovulatory decrease in the chloride content of the cervical mucus in women and a concomittant elevation in basal body temperature are indicative of progresterone production. The preovulatory decrease in the chloride content in Experiment I thus shows that some progresterone is produced before ovulation occurs. It is of interest that the vaginal cornification test did not react to the second series of progesterone injections in Experiment II. This slow reaction of the vaginal cornification to progesterone may explain the lag of this test behind ovarian changes [9, 10].

It is concluded that although this chloride spot test provides a simple, less cumbersome test for ovulation than the existing vaginal tests, its use is limited to the prediction of the onset of oestrus and to a rather approximate estimation of the time of ovulation.

ACKNOWLEDGEMENTS

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BRUCELLOSIS IN THE KRUGER NATIONAL PARK

V. DE VOS AND C. A. W. J. VAN NIEKERK*

SUMMARY

Brucella serum agglutination tests were carried out on 642 free-living wild animals in the Kruger National Park. These included 17 different mammalian species.

Although reactors were found among buffalo, hippopotamus, impala and waterbuck, it is concluded that the disease has an almost negligible effect upon these populations.

Negative reactions among roan antelope rules out brucellosis as a contributing factor towards the animals apparent inability to increase its numbers beyond the present vulnerable position in the Park.

INTRODUCTION

A wide host-range and adaptability to new host species are characteristic of *Brucella* organisms. Natural and artificial infections have been reported from a large number of species, mostly mammals but also birds and arthropods [1,5]. Most infections found in wild animals appear to have been derived from domestic animals and tend to disappear after the elimination of the infection from the reservoir but in some cases they may persist independently for a long time in the wild host, e.g. *Brucella suis* infection in *Lepus europaeus*, *Brucella melitensis* in *Saiga tatarica* and *Brucella abortus* in *Bison bison* [6].

Recently foci of infection, which appear to exist independently of infections in domestic animals or man, have been found in wild animal populations in Africa. The first indication of the existence of brucellosis in wild animals in southern Africa was provided by Guilbride and co-workers [7] when eight out of 144 serum samples from hippopotami gave positive agglutination tests for brucellosis. Results obtained by Rollinson [8] suggest that brucellosis may be of significance among game in Uganda. Heisch, Cooke, Harvey and de Souza [9] indicated the existence of brucellosis in East Africa.

Renoux [1] stressed the fact that further studies on the ecology of the infection in various types of wild animals are needed for the control of the disease in domestic animals and man.

MATERIALS AND METHODS

Cropping procedures in the Kruger National Park are now providing opportunities for further studies on the incidence and ecology of brucellosis among various wild animal populations living under essentially natural conditions. Serum samples were taken immediately after the animals were killed or immobilized, and agglutination tests were performed according to the standard technique for cattle as laid down by the Joint FAO/WHO Expert Committee on Brucellosis [6].

In the cropping schemes use was made of an essentially randomized sampling method in order to obtain an acceptable respresentation of the various sex and age groups of the hippopotamus *(Hippopotamus amphibius)*, Buffalo *(Syncerus caffer)*, blue wildebeest *(Connochaetes taurinus)*, impala *(Aepyceros melampus)* and elephant *(Loxodonta africana)* populations. The roan antelope *(Hip-*

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Table 1: THE RESULTS OF **BRUCELLA** AGGLUTINATION TESTS ON SERA OF VARIOUS GAME SPECIES

SPECIES	Estimated population*	No. tested	No. positive	No. suspicious
Hippopotamus (**Hippopotamus amphibius**)	2157	51	6	1
Cape buffalo (**Syncerus caffer**)	15808	253	28	8
Roan antelope (**Hippotragus equinus**)	263	9	0	0
Impala (**Aepyceros melampus**)	132500	120	1	0
Blue wildebeest (**Connochates taurinus**)	15300	105	0	0
Elephant (**Loxodonta africana**)	7701	76	0	0
Kudu (**Tragelaphus strepsiceros**)	5090	1	0	0
Sable antelope (**Hippotragus niger**)	950	1	0	0
Tsesseby (**Damaliscus lunatus**)	608	2	0	0
Waterbuck (**Kobus ellipsiprymnus**)	3155	1	1	0
Giraffe (**Giraffa camelopardalis**)	2765	2	0	0
Zebra (**Equus burchelli**)	16995	5	0	0
Vervet monkey (**Cercopithecus aethiops**)	—	1	0	0
Chacma baboon (**Papio ursinus**)	—	8	0	0
Lion (**Panthera leo**)	—	4	0	0
Spotted hyaena (**Crocuta crocuta**)	—	2	0	0
Rhinoceros (**Ceratotherium simum**)	—	1	0	0

*From data provided by Pienaar [10, 11, 12, 13].

potragus equinus) were captured (drug immobilized) and placed in a specially constructed study area with the object of launching an intensive investigation into the bioecology and reasons for the rarity of this species [10]. Serum samples from other species were essentially incidental collections, viz. animals slaughtered for human consumption, animals used for experimental immobilization procedures and sick or otherwise physically defective animals destroyed for humane reasons.

RESULTS AND DISCUSSION

The results of the *Brucella* serum agglutination tests are presented in Tables I and 2.

Hippopotami

The samples from this species were obtained during culling operations from May to August 1964 when 104 animals were killed in the Letaba area. The results indicate a fairly high proportion of reactors (13.7%), but of the 36 adult females killed, 20 were found to be lactating and two were pregnant and no clinical signs of abortions could be found. These findings pointed to a high fecundity despite severe drought during the previous two years. Annual counts also indicate a steady rise in the hippopotamus population. Therefore it appears that the presence of the infection does not significantly affect the reproduction of this species.

Buffalo

Six of the 28 positive samples from buffaloes came from 100 samples taken from animals killed in the Crocodile bridge area. Of the 38 adult females in this group 25 were found to be either pregnant or lactating. Differential counts made on 2,587 buffaloes over a period of six months showed that 36.4% of the cows had calves running with them. In

Table 2: **BRUCELLA** AGGLUTININ CONTENT IN SERA OF FOUR ANIMAL SPECIES

Animal	Location	Identification No.	Agglutinin content IU/ml*	Interpretation
Hippopotamus	Letaba river	L.2	320	Pos.
		L.6	640	Pos.
		L.11	160	Pos.
		L.12	160	Pos.
		L.23	320	Pos.
		L.31	40	Susp.
		L.48	320	Pos.
Buffalo	Crocodile Bridge	C.5	320	Pos.
		C.8	320	Pos.
		C.27	160	Pos.
		C.34	40	Susp.
		C.34(b)	80	Pos.
		C.42	320	Pos.
		C.44	40	Susp.
		C.57	160	Pos.
		C.71	40	Susp.
		I.4	160	Pos.
		I.4	160	Pos.
		I.14	2560	Pos.
		I.23	320	Pos.
	Skukuza	Sk.1	320	Pos.
	Shingwidzi	Sh.1	160	Pos.
	Mlondozi	T.6	160	Pos.
		T.8	40	Susp.
		T.36	1280	Pos.
		T.37	1280	Pos.
		T.38	80	Pos.
		T.63	40	Susp.
		T.65	100	Pos.
		T.122	80	Pos.
		T.123	80	Pos.
		T.135	80	Pos.
		T.114	40	Susp.
Impala	Skukuza	Sk.119	160	Pos.
Waterbuck	Sand River	S.1	640	Pos.

*The agglutinin levels comply with the international standard as set down by the WHO Expert Committee on Biological Standardization (1954).

view of an inevitable high post natal mortality, there does not appear to be any serious disturbance in reproduction among this species.

One of the positive reactors was a week old calf whose mother gave a suspicious reaction (40 IU/ml.) The calf's antibodies can be assumed to have been passively acquired. Among the 36 reactors, six showed hygroma of the knee joint, one bilateral orchitis and epididymitis and one purulent endometritis. Figure I illustrates a hygromatous lesion from

buffalo no. C. 42. The hygroma fluid as well as the serum gave a positive agglutination test in all six cases, but attempts to cultivate organisms from these and the other lesions mentioned were unsuccessful. Nevertheless these lesions are known to be frequently associated with brucellosis in cattle [5, 15].

According to Stableforth [2] and van der Hoeden [5], *Brucella* organisms can remain viable for long periods in water. It is therefore interesting to note that the two species among which the most reactors were found are both water loving, i.e. hippopotami and buffaloes. The latter frequently graze reeds along river banks and enter the water to drink and wallow. The one serum sample from a waterbuck, a reputable waterloving species, also contained *Brucella* antibodies.

It is also significant that no sign of the disease could be found among the nine roan antelope which were tested. The absence of the infection rules out brucellosis as a cause for their apparent inability to multiply.

ACKNOWLEDGEMENTS

Thanks are due to the National Parks Board for the opportunity and facilities provided for this study to be undertaken, and to the Chief of Veterinary Services for permission to publish this paper.

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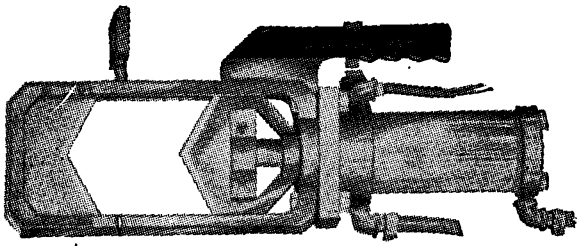
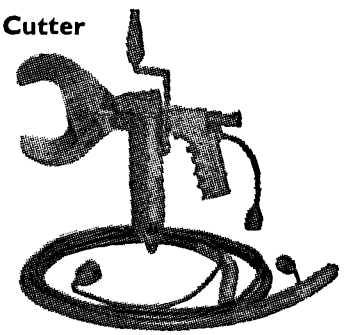


Horn Cutter



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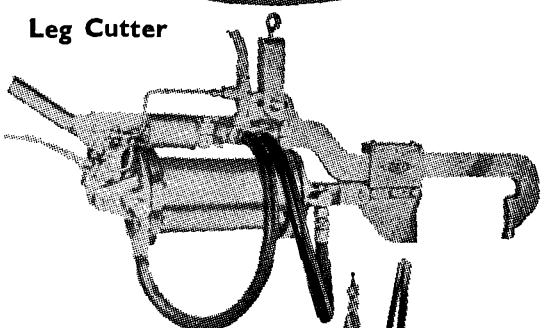
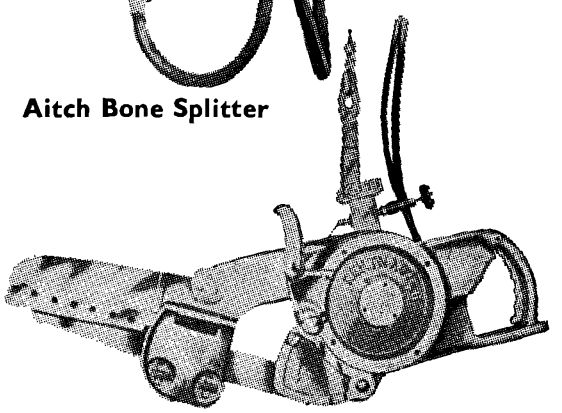
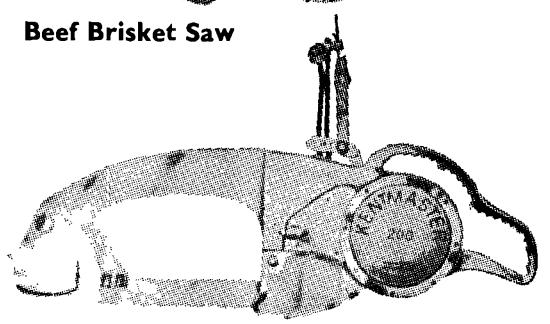


Aitch Bone Splitter



Beef Brisket Saw



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TWO INSTANCES OF BOVINE AGAMMAGLOBULINAEMIA AS REVEALED BY IMMUNOELECTROPHORESIS

I. S. WARD-COX*

Sera from five heifers that showed no detectable antibody response to vaccination with strain 19 *Brucella abortus* vaccine were submitted to this laboratory for immunoelectrophoresis.

Variations of the technique of Scheidegger [1] and Grabar and Williams [2] were employed in the basic procedure. Grabar and Burtin [3] cited an example in which borate buffer, pH 8.6, was reputed to have given greater clarity and separation of the beta and gamma globulin arcs. Based on this assertion a discontinuous buffer system was set up, using a Veronal buffer, pH 7.6, for the gels and a lithium hydroxide-boric acid mixture, pH 8.6, as an electrode buffer. The running time was reduced to 1½ hours and a broad spectrum anti-bovine antiserum was used, specifically prepared to reduce the anti-albumin and anti-α body activity.

The photograph shows the results of the investigation, one slide having been employed for each animal. The following conclusions can be drawn from the patterns obtained:

Slide 7: A complete lack of γ-globulin is evident with a trace of γ-globulin precursor as previously mentioned by Ward-Cox [4]. There is also no sign of β_2-globulin.

Slide 8: As in Slide 7 there is no mature γ-globulin, but a strong γ-globulin precursor. The β_2-globulins are still absent.

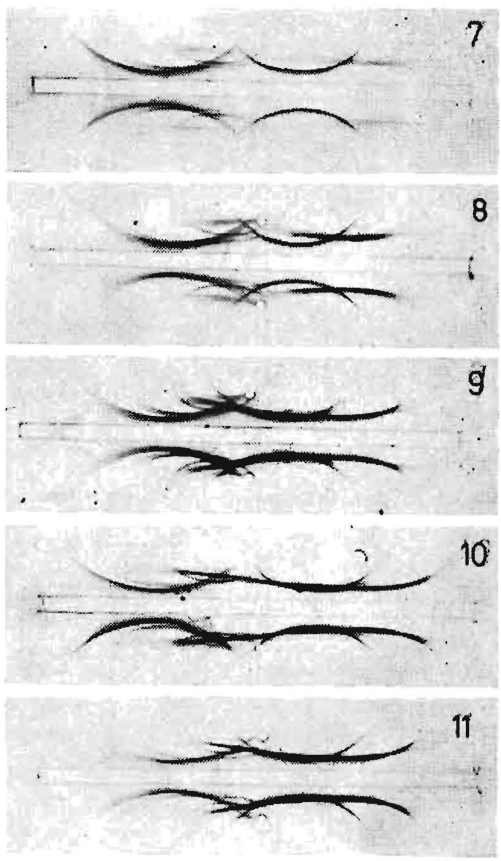
Slide 9: Both γ- and β_2-globulins are evident and strongly developed.

Slide 10: This is similar to slide 9 but the γ-globulin shows a broader activity, together with two β_2-globulin arcs.

Slide 11: Again a similar pattern to those of Slides 9 and 10, but with an absence of β_2-globulin.

The overall impression is gained that the first two animals show a lack of mature γ-globulin and β_2-globulin, explaining the lack of an immune response to vaccination although this does not appear to be the reason in the last two animals.

The assistance and criticism of Prof. D. R. Osterhoff, Chief of the Dept. Zootechnology, is hereby greatly appreciated.



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DIE „NOMINA ANATOMICA VETERINARIA."

In Oktober 1968 is 'n beskeie boekie van 146 bladsye, in ewe beskeie liggrys omslag met slegs die titel „Nomina Anatomica Veterinaria" daarop, deur die Internasionale Komitee oor Veeartsenykundige Anatomienomenklatuur gepubliseer. Hiermee het die oudste biologiese dissipline nou eers mondigheid op veeartsenykundige gebied bereik.

Die groeipyne, die lofwaardige pogings van die verlede, wat telkens net tot die amper voltrokke stadium gevorder het, word in die inleiding tot hierdie werk uiteengesit. Die sneller tot die jongste en suksesvolle poging was die ontevredenheid van die veeartsenykundige afgevaardigdes na die Sesde Internasionale Anatomiese Kongres met die aldaar aanvaarde Nomina Anatomica in 1955 te Parys. Onder aansporing van professor C. Bressou het hulle besluit om 'n Internasionale Vereniging van Veterinêre Anatome te stig, met eerste doelwit die opstel van 'n veeartsenykundige anatomienomenklatuur. Die eerste vergadering van hierdie vereniging in 1957 het eintlik met die aanvoorwerk onder voorsitterskap van professor J. Schreiber begin, en reeds by die 1961-vergadering van die nou herdoopte Wêreldvereniging van Veterinêre Anatome kon 'n deel van die terminologie bespreek en aanvaar word. Die finale lys is in 1967 aanvaar. Dus binne bestek van elf jaar is die eerste taak volvoer. Die „Anatomia Avium" en Histologie- en Embriologieterme is nog in voorbereiding.

Dit ly geen twyfel dat die internasionale komitee oor nomenklatuur en sy elf onderkomitees puik werk gelewer het nie. Die subkomitee vir die senustelsel is versterk deur 'n groot groep konsultante op die gebied van die vergelykende neuro-anatomie. Diegene wat vierkantig in die vakgebied staan sal maar al te goed besef hoeveel kloutjies by hoeveel oortjies gebring moes word. Die komitees verdien alle eer vir hulle werk, wat steeds getuig van besondere verantwoordelikheidsin, deeglikheid en nugtere besinning. Dit is 'n uiters geslaagde poging om die uiteenlopendheid van menslike, veeartsenykundige en vergelykend-anatomiese (in die sin van dierkundige) terme, asook die onderskeie nasionale terminologieë, onder een dak te bring. Veral verblydend is die logiese uitstryk van die dikwels uiters onlogiese verskille van benamings by die verskillende huisdierspesies, wat op sy beurt te wyte was aan te enge spesiesbeheptheid by navorsing en onderwys. Die resultaat is beslis nie 'n betekenislose stel kompromieë nie. Dit moes fyn werk gekos het en 'n mens sou wat gegee het om sommige van die deliberasies aan te hoor. Hier en daar is 'n skaakmat uit die weg geruim deur aanvaarding van alternatiewe terme, maar dit gaan meesal om die spellingvorm. Deurgaans is gestreef na konsekwente en logiese handhawing van fundamentele beginsels, na 'n minimale divergensie van die Nomina Anatomica, na eenvoud, ondubbelsinnigheid en na uitskakeling van verwarrende terme. Dit is ook duidelik dat die komitees toegang gehad het tot gegewens wat nog nie algemene besit word nie.

Aan die ander kant is dit voor-die-handliggend dat ons 'n hele klomp geliefde terme sal moet laat vaar, nie sonder 'n tikkie nostalgie wat die meer kleurrykes betref nie, en dat die nuwe vorm en/of betekenis van party ou name nie sonder 'n intellektuele gekrap van ratte uiteindelik in die praktyk aanvaar sal word nie. Tenspyte van die 555 verduidelikende aantekenings, bly daar nog 'n aantal terme wat die gewone man sal laat wonder wat die opstellers nou presies in gedagte gehad het.

Bestaande anatomieboeke op veeartsenykundige gebied, op die jongste uitsonderings na, is natuurlik nou uit die oue doos sover dit terminologie aanbetref. Butterfield en May (1966) het in hul „Muscles of the Ox" die nuwe terminologie t.o.v. spiere, wat reeds in 1963 in afgerolde vorm beskikbaar was, by wyse van 'n aanhangsel aangebring. Rooney, Sack en Habel (1967) volg die nuwe terminologie in hul „Guide to the Dissection of the Horse" en so ook Barone in sy omvangryke werk: „Anatomie Comparée des Mammifères Domestiques", waarvan die eerste twee dele, „Ostéologie" en „Arthrologie et Myologie" reeds verskyn het.

Op alle kollega's word 'n dringende be-

roep gedoen om die Nomina Anatomica Veterinaria in hul publikasies en in hul onderwys te volg. Selfs al gebruik hulle eietalige terme, behoort daardie terme 'n direkte weerspieëling van die latynse vorms in die NAV te wees, en behoort die neergelegde beginsels van die NAV gevolg te word. Dit beteken natuurlik dat ons ons anatomie van vooraf sal moet leer, of altans alle terme sal moet kontroleer of hulle nog gangbaar is. In hierdie opsig geld die goeie nederlandse segswyse: „Uithuilen en weer beginnen." Dit sal nodig wees as ons van hierdie mylpaal 'n verdiende en werklik betekenisvolle gebeurtenis in die geskiedenis van die veeartsenykunde wil maak. Alle voorgestelde wysigings moet met behoorlike dokumentasie aan die sekretaris van die nomenklatuurkomitee: J. Frewein, Tierärztliche Hochschule, Linke Bahngasse 11, A-1030 Wien, Oostenryk, voorgelê word.

Die Nomina Anatomica Veterinaria is verkrybaar van enigeen van die ondergenoemde drie distribusiepunte, teen betaling van f 18.00 (5 Dollar).

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H. P. A. de B.

In Memoriam

BASIL ARTHUR MATSON

The untimely death of Basil Matson on the 20th May, 1969, at the age of only 34 years has deprived Southern Africa of one of its most promising veterinary scientists.

Basil was born in Lusaka, Northern Rhodesia, and received his schooling at St. Andrews College, Grahamstown matriculating in 1952. He obtained the B.V.Sc. degree at the Faculty of Veterinary Science, University of Pretoria in 1957. Before accepting an appointment as Veterinary Officer in Nyasaland in 1958, he spent several weeks at the Veterinary Research Institute, Onderstepoort, stu-

dying trypanosomiasis and tsetse flies in preparation for his new vocation. Thus the foundation was laid for his consuming interest in arthropod-borne protozal diseases.

His investigations on trypanosomiasis in the Shire Valley, Nyasaland and the control measures he subsequently instituted, in due course resulted in a virtual doubling of the cattle population in that area, an achievement for which he is highly esteemed. After two years of field work he was placed in charge of the Veterinary Research Laboratory at Blantyre, thus gaining wide experience in tropical diseases of animals in general.

In 1960 he was elected a Beit Fellow and awarded a Commonwealth Scholarship which enabled him to obtain the Academic Postgraduate Diploma in Applied Parasitology and Entomology at the London School of Hygiene and Tropical Medicine in 1961. His dissertation was on the ticks of the Shire Valley. Thereafter Basil enrolled at the University of Cambridge where he studied in the Department of Animal Pathology, obtaining the Ph.D. degree in October, 1964. His thesis was entitled "Tick transmission in *Babesia divergens* and immunity in *B. rodhaini*".

By this time Basil was itching to get to grips with Africa's many challenging parasitic diseases once again. As Senior Veterinary Research Officer at the Veterinary Research Laboratory, Salisbury, Rhodesia, he set out with characteristic enthusiasm and diligence to study tick-borne diseases. He devoted most of his attention to sorting out the problem of theileriosis as it presents itself in Rhodesia. His investigations led to several publications and a thesis entitled "Theileriosis in Rhodesia" for which he was made a F.R.C.V.S. in November 1968. In the meantime he had been appointed Senior Lecturer in Biological Sciences at the University College of Rhodesia, in which capacity he served until his death.

Basil was not only a dedicated, purposeful scientist often driving himself mercilessly, but was always brimful of enthusiasm and new ideas. He had a flair for delivering a stimulating address, as everybody who heard him speak at S.A.V.M.A. congresses will testify. The fruits of his scientific endeavours were reported in some 20 articles, several still being in press.

Above all, Basil will be remembered as a courteous, considerate and warm-hearted person, with a very positive approach to life. He will be sorely missed by his many friends.

To his family, particularly his wife June and their three children, we extend our heartfelt sympathy.

FEATURE PAGE

BOVINE MASTITIS DUE TO **CLOSTRIDIUM PERFRINGENS** TYPE A AND **BACILLUS CEREUS**

W. H. Giesecke, R. C. Tustin, F. S. Malan and G. H. de Waal

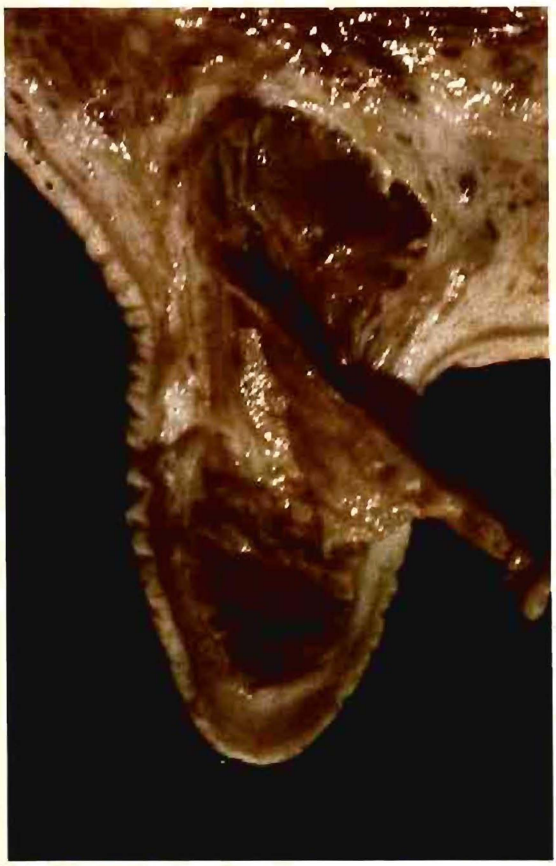

Fig. 1

Shortly after parturition the secretion of a hindquarter of a Friesland cow was watery and flocculous, becoming serohaemorrhagic within 48 hours despite intensive intramammary and parental antibiotic therapy. Clinically the teat was enlarged, turgid and slightly sensitive — extensive perinatal oedema prevented detailed manual examination of the udder. A poor prognosis prompted slaughter.

Necropsy revealed a hard swollen quarter and a stiff, thickened teat, severe subcutaneous oedema and some lymphatic thrombosis. The affected mammary tissue was sharply differentiated from the adjoining normal quarter, diffusely red with discernible gas bubbles and crepitation on palpation, and emitting a foul odour. Some bloodvessels were thrombosed and a yellow fibrinous pseudomembrane lined the gland and teat cisterns and some lactiferous ducts. (Fig. 1 and 2). Several large areas of necrosis occurred in the **Ln. inguinalis superficialis.**

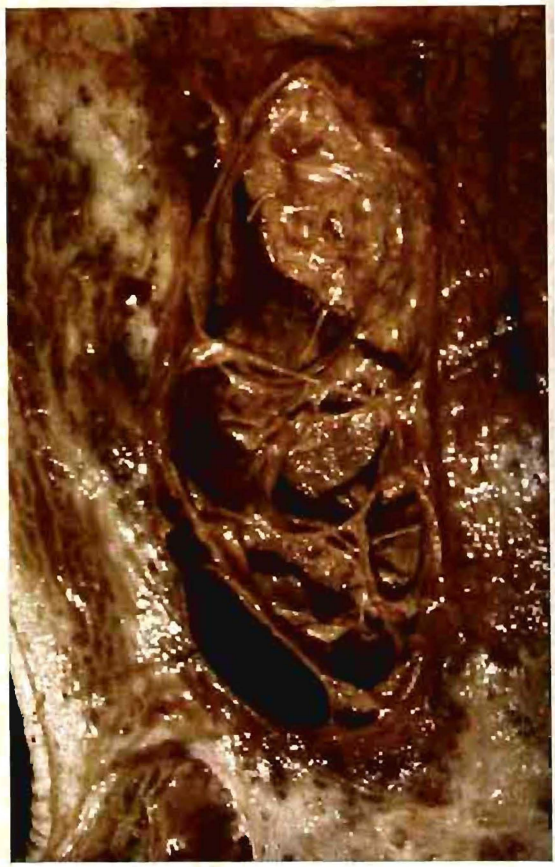

Fig. 2

Histologically the main lesions were fibrinopurulent galactophoritis, some thrombosis of bloodvessels and lymphatics, lymphangiectasis, scattered petechiae, inflammatory oedema of interstitium and subcutis, extensive focal necrosis of mammary tissue (probably infarcted) surrounded by a dense zone of leucocytes, and severe leukocytic infiltration and cellular necrosis elsewhere.

Aerobic culture of secretion drawn aseptically before slaughter revealed numerous **B. cereus** in pure culture, whereas bacteriological examination of mammary tissue disclosed **Cl. perfringens** type A in large and **B. cereus** in lesser numbers. Gram-positive rods were also seen histologically.

Cl. perfringens type A causes classical gas gangrene in man and some strains are responsible for food poisoning.

(Photography: A. de Bruyn)