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OVINE JAAGSIEKTE

R. C. TUSTIN*

INTRODUCTION

Mr. Chairman, ladies and gentlemen. In this paper I will review various aspects of our knowledge of jaagsiekte. The information which will be imparted has been gleaned partly from the literature, partly from a country-wide survey which has been held over the past few years with the active support of my colleagues in the field, to whom I would like to extend my appreciation for their hard work and co-operation, and partly from experimental work which has been performed at Onderstepoort.

Several sheep diseases exist in the world which are characterized by the development of slowly progressive pneumonias. Examples of these are *maedi, zwoegerziekte, bouhite, Montana progressive pneumonia* and *jaagsiekte*. The names maedi, bouhite, zwoegerziekte and jaagsiekte all have the same connotation, namely, dyspnoea. The existence of certain similarities in the pathology and/or symptomatology of some of the foregoing diseases has given rise to considerable confusion in the literature as to their exact identity. That certain of them are related, there seems to be no doubt. In this respect Thormer[1], for example, has demonstrated that neutralizing antibodies against the causative virus of maedi are present in the serum of sheep suffering from zwoegerziekte and Montana progressive pneumonia, whereas none existed in the serum of sheep suffering from jaagsiekte. This contribution refers to jaagsiekte as a distinct disease entity.

What, then, is jaagsiekte? Jaagsiekte, or pulmonary adenomatosis, as it is sometimes called, is a specific contagious disease of sheep which is characterized by the slow and progressive development of a primary lung neoplasm, by a long incubation period and by the fact that recoveries, once the typical symptoms of the disease are manifested, do not occur. The aetiology remains enigmatical but is probably a virus. In other words, jaagsiekte is a transmissable neoplasm, just as are avian leukosis, myxomatosis in rabbits, bovine papillomatosis and so on.

HISTORY

It is interesting to consider the history of jaagsiekte and, although I have not delved into the Cape Archives, several early references have been encountered. The disease has been known to farmers in this country for many years and although it is debatable whether all outbreaks and cases referred to were, in fact, jaagsiekte, it is worthy of note that its apparent contagiousness was realized by some of the earlier reporters. An early record of the name jaagsiekte or jagtziekte appears in a letter written in 1825 by Veldtkornet P. Aucamp† in the Rhenoster district of the Cape of Good Hope who reported to some higher authority: "Ik heb u Weledele met deze te rapporten dat zeder de laatste drie maande alhier in Meyn Smaldeel 800 schapen doot is aan vreemde ziekte, die de jaagziekte genaamt wort die scheen aan steekleyk te seyn en niet van door komt." (I hereby wish to inform Your Honour that during the past three months 800 sheep have died in my district of a strange disease called jaagziekte which appears to be contagious and from which recoveries do not occur.)

The disease was apparently familiar to Louis Trigardt, the well-known Voortrekker leader, who recorded in June 1837 in his diary[2], whilst in the Soutpansberg district of the Transvaal and immediately before his trek to Lourenco Marques, that "de Avond heb ik hem een schaap te eeten (gegeven); aan die ouwe Kaffer die de bier gebragt heeft, gaf ik een jaagziekte ooij, hoewel niet onbruikbaar". (In the evening I gave the old native who brought the beer, a sheep to eat.

* Veterinary Research Institute, P.O. Onderstepoort. Paper delivered at 63rd Scientific Congress of the South African Veterinary Medical Association, 10th—13th September, 1968.

† Cape Archieves GR 12/4.

It was a jaagziekte ewe but could still be used (for consumption).) Louis Trigardt had Spanish sheep (Merinos), as well as indigenous sheep in his flocks[3].

Thom[3] in his book on the history of sheep farming in South Africa records that in the early part of the 19th Century jaagsiekte was one of the many diseases which plagued sheep in the Cape. No remedy was known and some farmers even went so far as to destroy all sheep that were affected with it. He quotes one Michiel van Breda who in 1830 was of the opinion that "provided encouragements and rewards be held out to men of science, in order to induce them to devote some portion of their time and talents to the discovery of effective remedies" (translated) some serious diseases like lamsiekte and jaagsiekte did not appear to be insoluble.

The symptomatology and macroscopic pathology were described in great detail in 1891 by Hutcheon[4], a veterinarian employed by the Government of the Cape Colony. He stated that jaagsiekte was more or less prevalent amongst sheep over a large area of the Cape Colony, and he emphasized that there was strong evidence that jaagsiekte was contagious in character and that once it commenced in a flock, it was apt to continue and become intensified. Although he did not know what the cause was and considered it "something special", he advocated immediate slaughter of every animal as soon as it was observed to be affected, not only from an economical viewpoint in order to save the carcase for consumption while still in good condition, but also to endeavour to arrest the further spread of the disease in the flock. Further reference to jaagsiekte was made by Hutcheon in 1892[5], 1893[6], 1905[7] and in an undated publication[8].

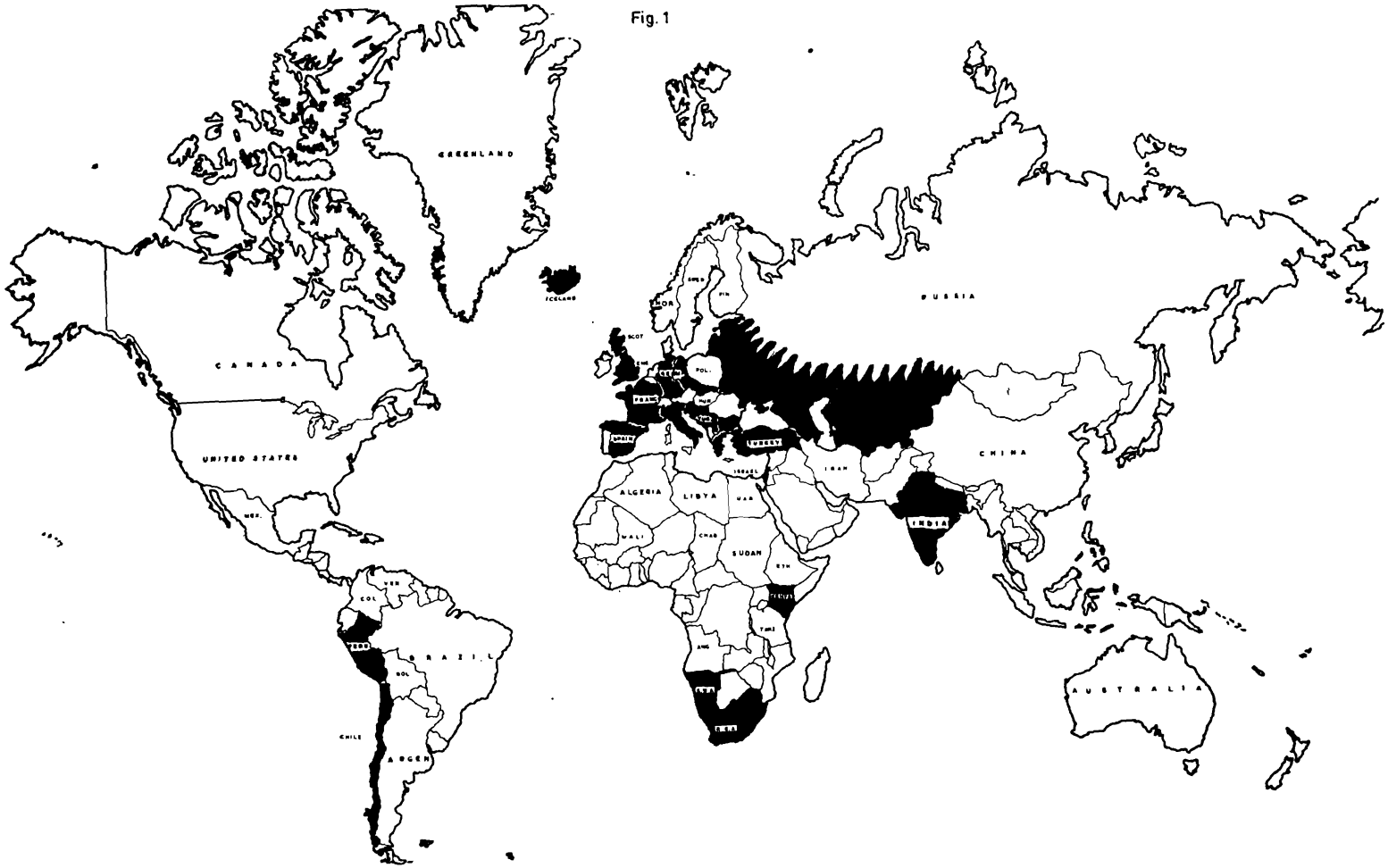
Robertson[9], who was Bacteriologist to the Department of Agriculture, Cape Colony and Hutcheon[10] both reported unsuccessful attemps to transmit the disease by cohabitation of affected and healthy sheep and by inoculation of blood and affected tissues.

Many farmers recorded their experiences with jaagsiekte in the Agricultural Journal of the Cape of Good Hope in the early 1900's. One[11] mentioned that he was very particular in slaughtering every sheep showing signs of jaagsiekte and had kept it up for years, but that it seemed to make no difference in the annual incidence which was about two per cent. Another[12] said that he had known it since about 1850 and was convinced from his own experience that it must have been introduced into this country by sheep imported in the early 1800's, perhaps from Spain. His reason for this statement was that his father had commenced farming with 40 Merino ewes out of a flock originally imported from Spain, and that he always had had a good few cases of jaagsiekte which generally had developed in, and after, the fourth year of age.

In 1915 Mitchell[13] reported that owing to the severe outbreaks appearing in some of the northern districts of the Cape Province in 1911-12, investigation was begun at Onderstepoort with a view to elucidating the cause of jaagsiekte. After a series of transmission attempts using blood, bronchial exudate and suspension of affected lung tussue as inocula, and also by placing healthy sheep in contact with diseased sheep, he considered that he had succeeded in transmitting the disease. However, various aspects of these experiments, amongst which was the very short incubation period reported by Mitchell, led de Kock[14] in 1929 to disclaim that Mitchell had in fact, transmitted the disease. De Kock[15] described a form of ovine pneumonia characterized by an extensive accumulation of round cells chiefly of the lymphoid series, an atypical proliferation of bronchiolar epithelium, i.e. not similar to those proliferations seen in jaagsiekte sheep and chronic catarrhal pneumonia with the presence of giant cell formations in the alveolar exudates. He postulated the probability that in South Africa we might be dealing with two specific lung diseases, viz. this form of pneumonia which has since become known in the literature as "Graaf-Reinet disease" because of the number of cases emanating from the now defunct Experimental Station at Graaf-Reinet, and jaagsiekte. The lesions in "Graaf-Reinet disease" were similar to those described by Mitchell[13]. Duran-Reynals *et al*[16] compared the histopathology of Montana progressive pneumonia with that of "Graaf-Reinet disease" and concluded that the two resembled each other. It is extremely doubtful, however, whether "Graaf-Reinet disease" still exists in South Africa as a distinct disease entity as no definite cases have been encountered for a long time.

In 1929 de Kock[15] propounded the theory that jaagsiekte was a neoplasm and called it papilliform cyst-adenomatosis. He was not, however, the first to consider it to be a

Fig. 1



tumour as Aynaud [17] in France in 1926 had suggested that it might be a carcinoma. De Kock [14, 18] also attempted to determine whether the disease was infectious by exposing healthy sheep to infected animals. Only small numbers of the healthy animals developed lesions which were small, localised and asymptomatic. He appeared to be rather dubious that he had transmitted the disease, however, not only because of his failure to produce what he considered to be the typical disease as seen clinically, but also because he was of the opinion that the lesion was a neoplasm.

Jaagsiekte was first described in England [19] in 1894 (albeit unknowingly—M'Fadyean at the time considering the lesion to be caused by lungworms), in 1899 in Germany [20], in France [17] in 1926 and in various other countries since then. During the 1930's large-scale outbreaks were reported in Iceland [21, 22] where Dungal *et al* [22] were the first to transmit the disease by means of parenteral inoculations of suspensions containing the infectious agent.

DISTRIBUTION

In the map of the world (Fig. 1) are depicted the countries in which jaagsiekte has been diagnosed at least once. It was compiled mainly from reports in the literature and is based in some cases on my interpretation of these reports. the various shadings indicate my impressions of the relative incidence and significance of the disease in the countries concerned. It has occurred in 18 countries: South Africa, Kenya [23-29], Peru [30-31], Chile [34, 35], India [36-41], Israel [42-44], Turkey [45], Iceland [21, 22, 46-50], United Kingdom [19, 51-61], France [17, 62], E. Germany [20, 63, 64], W. Germany [35, 65-67], Bulgaria [68-72], Yugoslavia [73, 74], S.E. Russia [75-79], Greece [80], Italy [81-83] and Spain [84, 85]. In addition, it possibly has occurred in Czechoslovakia [56].

Fig. 2 depicts the position in Europe and the Near East in detail.

Shown in the next map (Fig. 3) is the distribution in South Africa according to the number of farms (per magisterial district) on which the disease has been diagnosed histologically. The compilation of the map is based on figures obtained during the last 44 years from records kept in the Pathology Department at Onderstepoort and each infected flock is reflected only once in the total. Odd districts may have been omitted because our records were incomplete.

The distribution in South West Africa is represented on a similar basis in the next map where at least 41 farms contained infected flocks during this period.

AETIOLOGY

The aetiology of jaagsiekte remains unknown, but is probably a virus. Artificial transmission has been effected by means of inocula consisting of cell-free and bacteria-free material.

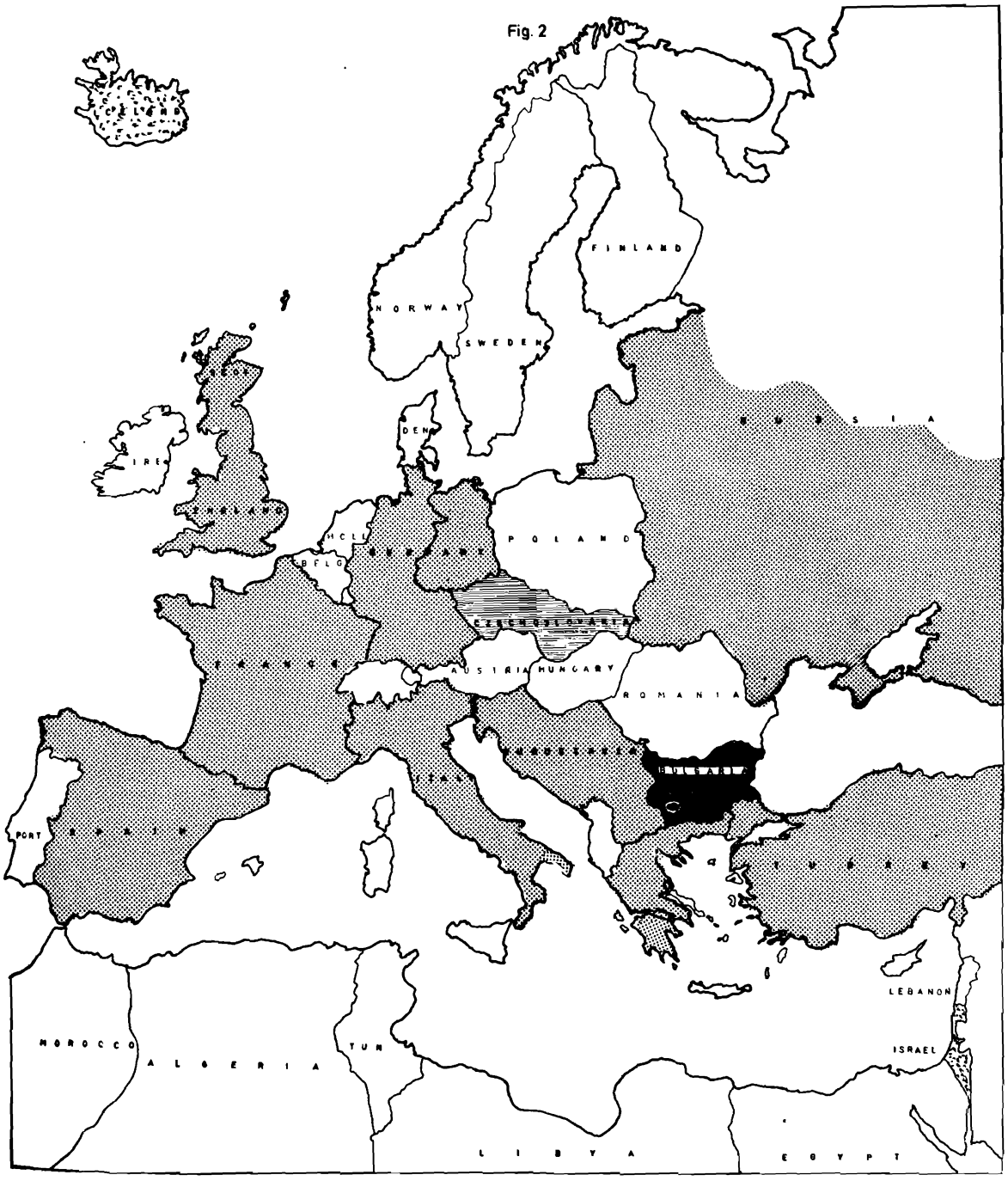
A variety of species of bacteria especially *Pasteurella* spp. and *Corynebacteria* spp. can usually be isolated from extensive lesions. These are, no doubt, opportunists and are responsible for the pneumonic process which invariable accompanies the adenomatous lesion, especially if the latter is large and the animal died from the disease.

In 1963 Mackay *et al* [58] reported that they had isolated pleuropneumonia-like-organisms from lesions and that, using a slide agglutination technique, it had been shown that sera from four clinical cases of the disease agglutinated these antigens, whereas sheep sera taken at random from various sources failed to do so. In another paper, Mackay *et al* [61] concluded that it was unlikely that these organisms have a primary aetiological rôle and that it was more likely that they merely take advantage of the oedematous lesions in which to proliferate. They were, however, present in a high proportion of cases and they might be responsible for some of the other reactions in the lungs eg. peribronchial lymphocytic and the alveolar macrophage response which accompany the adenomatous change.

Todorov *et al* [87] in Bulgaria have isolated a virus from the lesions which is cytopathogenic to human fibroblasts, Detroit-6 cells and other cells grown in tissue culture. Virus neutralising antibodies were found in low titre in the sera of sheep. I must mention that we have been unsuccessful in our attempts on several occasions to produce a similar cytopathogenic phenomenon.

Size of the infectious agent. The infectious agent passes through the following filters: gradocol membrane with pores 0.9μ in diameter, Chamberland L3, Chamberland

Fig. 2



DISTRIBUTION

OF

JAAGSIEKTE

1968

Fig. 3

DISTRIBUTION OF JAAGSIEKTE

1924 – 1968

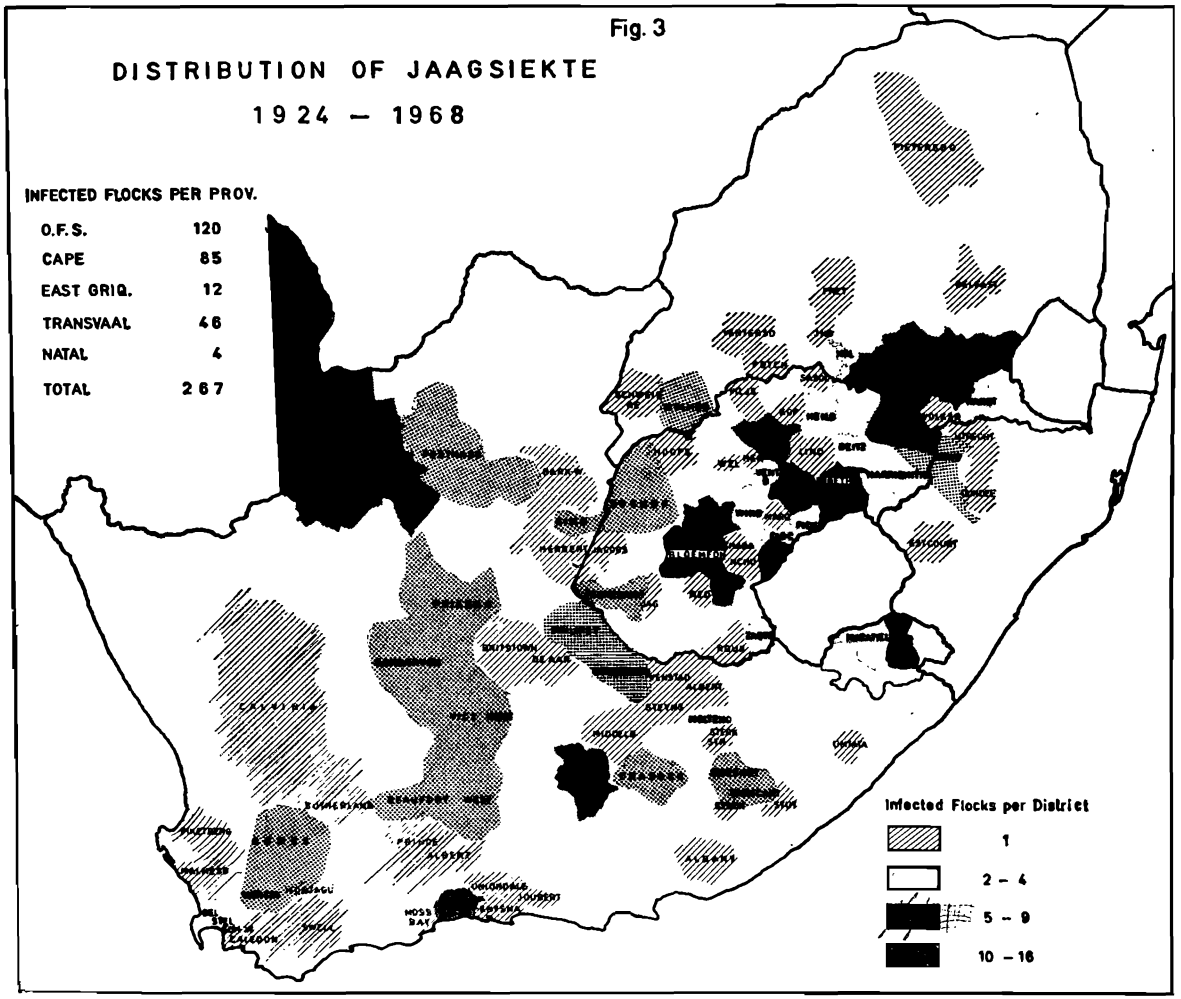
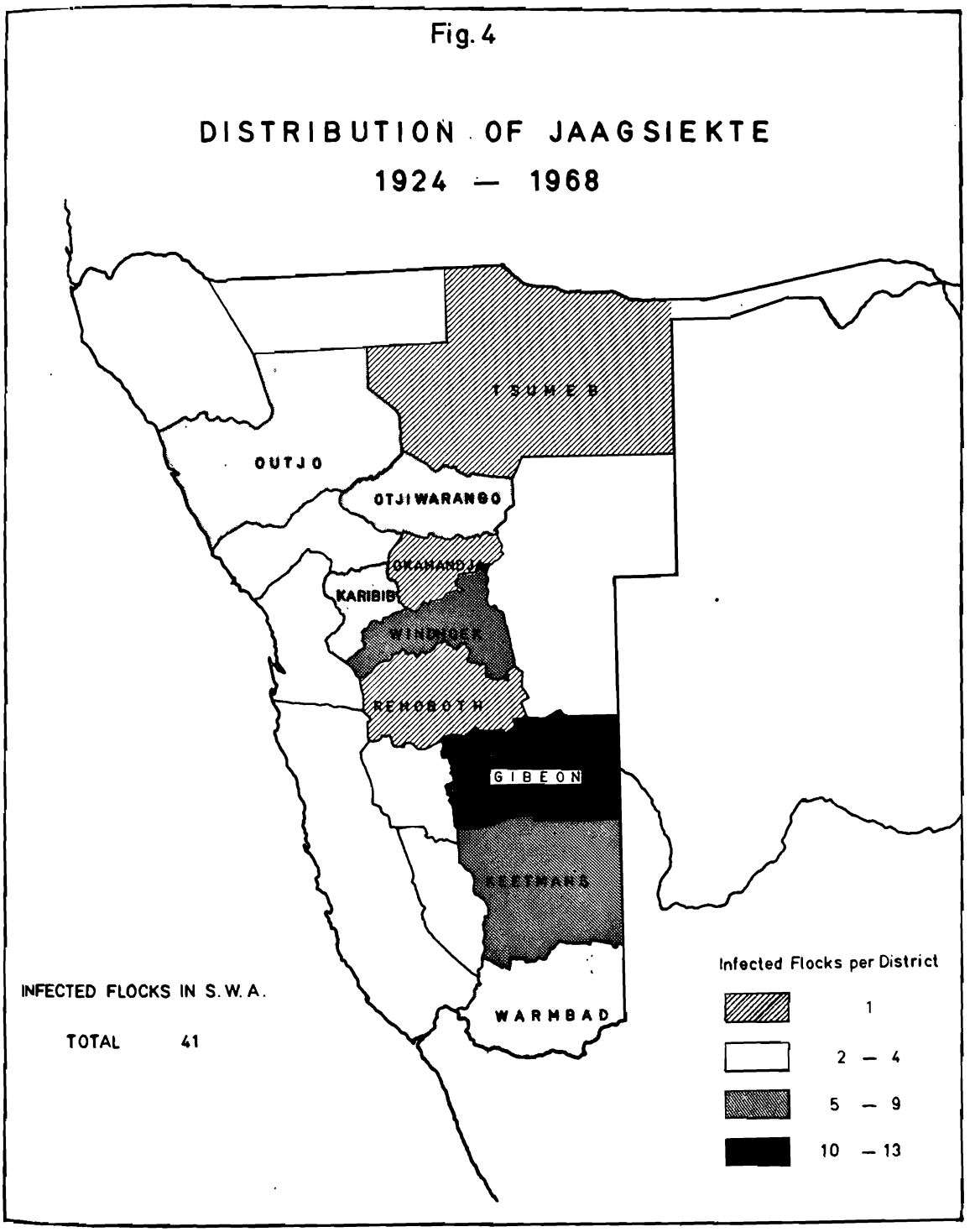


Fig. 4



L2, Seitz EK and Berkefeld N[48]. These are bacterial filters and indicate that the aetiology is not a bacterium. For some reason, possibly because the material used had been stored at -20°C before use and titres of the infectious agent were low, all filtration experiments performed at Onderstepoort in order to determine the size and nature of the causative agent have failed. These have included filtration through Seitz filters and membrane filters with pores 300, 275 and 150 mμ in diameter respectively.

Preservation of the infectious agent. It will remain viable in affected lung tissue when stored at -20°C for at least 52 months[50]. The end-point has not been determined.

It is not known for how long the infectious agent will survive outside the host under natural conditions.

Presence of the infectious agent in the host. It is present in the exhaled respiratory air, bronchial secretion[48] and lung lesions, but probably not in the blood, urine or faeces of affected animals[48].

Resistance to antibiotics. Markson *et al*[60] added streptomycin and penicillin to tissue suspensions of the lesions and subsequently reproduced the disease. At Onderstepoort in tissue culture experiments, small pieces of lesions have been subjected for an hour to immersion in Hank's solution containing 2 mg streptomycin, 1 mg neomycin, 2,000 i.u. penicillin and 20,000 i.u. mycostatin per millilitre; the disease was subsequently reproduced on inoculation of cells from these tissue culture preparations *(vide infra)*.

TRANSMISSION

Artificial Transmission

Transmission of jaagsiekte has been effected in a number of ways.

(1) *By cohabitation of healthy with diseased sheep.* As already mentioned, de Kock in 1929[14, 18] was the first to transmit the disease successfully in this manner. This was repeated by Dungal[21] in Iceland who housed eight sheep with diseased sheep. One of these died after seven months, and lesions were present in six of the others when slaughtered after 10 months.

Dungal[48] also proved in two ways that the exhaled respiratory air of an affected sheep contained the infectious agent. In the first experiment he maintained sheep in an elevated compartment 1.5 yds above the heads of diseased sheep kept in a lower compartment for periods of from four to six months. Faeces, urine and other body excretions were thus excluded as possible sources of infection. Three of the eight lambs used contracted jaagsiekte.

In the second experiment a diseased sheep was made to breathe through a 20 per cent solution of glycerine in normal saline for 30 minutes. Five millilitres of this mixture were injected by the intratracheal route and 2 ml by the intrapulmonary route into each of three lambs. Two of the lambs developed jaagsiekte, one of them showing clinical symptoms four months after inoculation. In a similar experiment, but after filtering the glycerosaline solution through a gradocol membrane with pores 0.9μ in diameter, he produced the disease in one out of four lambs.

(2) *By exposing sheep to droplet infection by means of an aerosol spray.* Markson *et al*[60] exposed a group of six four-month-old lambs to an aerosol spray consisting of a 10 per cent suspension of affected lung tissue in beef infusion broth containing an antibiotic mixture (penicillin and streptomycin). This was sprayed once a week into the air of the loosebox in which they were kept. Isolated lung lesions were present in two of the lambs when killed 380 and 864 days respectively after commencement of the experiment.

Dungal[48] also obtained successful transmission following intranasal spraying of sheep with a filtrate of bronchial secretion from an affected sheep which was obtained by filtration through a Chamberland L3 filter.

(3) *By means of parenteral inoculations of suspensions of diseased lung tissue.* Transmission can be effected relatively easily in this manner. It has been accomplished successfully by the intrapulmonary, intrapleural, intratracheal or subcutaneous routes, or by various combinations of these routes, together with others, such as intranasal[21, 22, 29, 48, 50, 60, 88].

(4) *By means of parenteral inoculations of filtrates.* Successful transmission has been accomplished following inoculation of filtrates obtained by the filtration of suspensions of diseased lung tissue through Seitz EK, Chamberland L2 and L3, and Berkefeld N filters, and through a gradocol membrane with pores 0.9μ in diameter [48].

(5) *By inoculation of the neoplastic cell (and medium) after its growth in tissue culture.* In an experiment at Onderstepoort affected lung tissue was removed from a sheep immediately after slaughter. It was cut up into small pieces and placed for an hour in Hank's solution containing the following antibiotics per millilitre: 2 mg streptomycin, 1 mg neomycin, 2,000 i.u. penicillin and 20,000 i.u. mycostatin. Following trypsinization the cells were grown in roller tubes at 37°C in Hank's medium containing 0.35 per cent sodium bicarbonate, 0.5 per cent lactalbumin, 0.01 per cent yeast and 10 per cent sheep serum. After 10 days' growth and two changes of medium, cells and medium were inoculated intravenously into two day-old lambs (it being considered at the time that, as with some other oncogenic viral diseases, young animals might be more susceptible). One died of jaagsiekte after 249 days, and the other was killed after 253 days; advanced lesions being present in the lungs.

Repetition of this transmission experiment using cells from another sheep and growing them for 21 days with three changes of medium, failed. The 10 lambs used were slaughtered after 190 days.

Attempts by Dungal [48] to transmit the disease by dosing healthy sheep with the faeces from affected sheep, by placing *Melophagus ovinus* from sick sheep onto healthy sheep and by parenteral inoculations of blood, failed. He also kept two sheep in a small compartment. Their heads were placed through padded holes in the wall and a sick sheep was then kept in contact with them. The two sheep did not contract jaagsiekte, thus indicating that natural routes of infection other than the respiratory system and perhaps the alimentary canal probably do no play a rôle.

Natural Transmission

By taking the results of some of the above experiments into consideration, there cannot be any doubt that the main natural mode of transmission of the disease is aerogenous, and that spread of infection is facilitated by close contact.

Whether bodily excretions contain the infectious agent, is not definitely known as the numbers of sheep used by Dungal [48] in the above experiments were too small to allow one to draw definite conclusions. It is doubtful that this as the case.

In an attempt to determine whether intra-uterine infection takes place, 10 pregnant ewes from an infected flock in the Orange Free State were purchased. Immediately after lambing, the ewes were slaughtered and autopsied. The lambs were hand-reared. Four of the ewes were found to be suffering from jaagsiekte. The lambs from these affected ewes, together with one from an unaffected ewe, were kept isolated from other sheep for a period of more than five years. They were of both sexes and were allowed to breed at will. On slaughter, neither the original sheep nor their progeny showed evidence of jaagsiekte; these results thus indicating that prenatal infection is probably not of significance.

EPIZOOTIOLOGY

Any sheep population is vulnerable. An outbreak of the disease may result from the introduction of an affected animal into a clean flock. Dungal *et al* [22] have stated that an interval of from five-and-a-half to eight months elapses after an infected animal is introduced before the appearance of the disease in a clean flock. This was in a highly susceptible population of sheep, and it is probable that in South Africa several years elapse after an infected animal is introduced into a clean flock before the farmer realizes that something is drastically amiss.

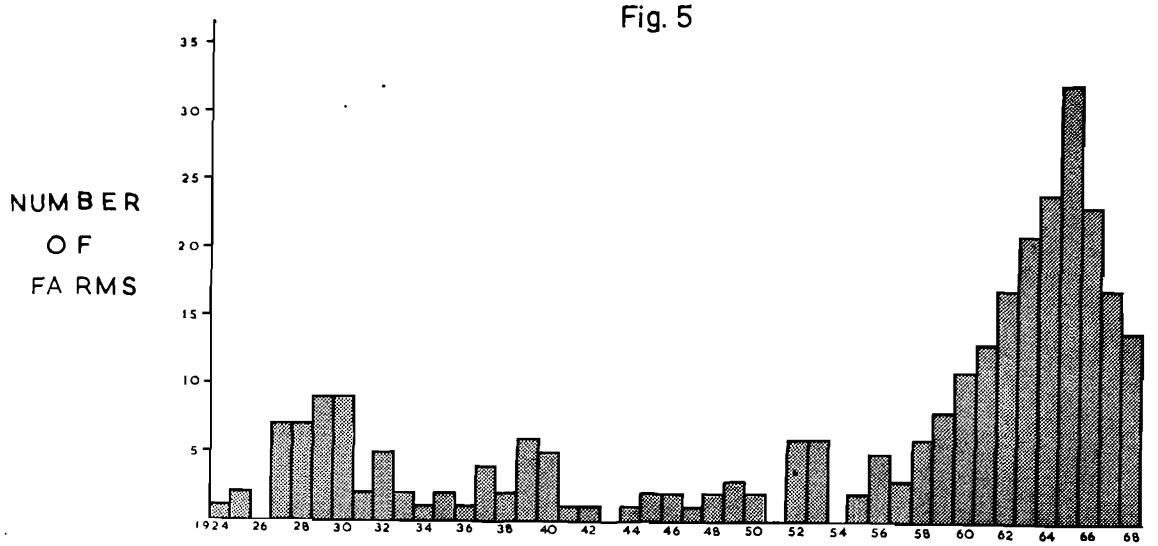
The classic example of an epizootic of jaagsiekte is the experience suffered by the farmers of Iceland [21, 22, 48, 50] during the latter 1930's and 1940's when large scale outbreaks occurred, and the study of the epizootiology of the disease in that country is of considerable interest.

Due to the climate methods of sheep farming in Iceland at that time were, and probably still are conducive to the spread of contagious diseases. During the winters sheep are kept indoors in houses which are apparently badly ventilated. In spring they graze pastures close to the farms and in summer they are driven into the mountain pastures in the interior of the country where they graze in common with other flocks. They remain here for three to four months and then, during autumn, they are gradually driven back to large collecting folds capable of holding up to 4,000 sheep which are situated at the heads of the main valleys. Here the sheep are penned together until they are separated out into flocks belonging to individual owners. They are afterwards driven away to the farms.

The disease is considered to have been introduced into Iceland by the importation of a year-old Karakul ram from Germany during December 1933. This ram was quarantined for a few weeks and was immediately put in use to serve ewes. It was kept throughout the winter in a small compartment with an ewe and her lamb. No symptoms were noticed in this ram during this period, but in the spring when the flock was driven to the mountains it was noticed to be dyspnoeic and in poor condition, and as it did not return the following autumn it was considered that it had probably died from the disease. The ewe died during May 1934; no attention having been paid to the cause of her death, and the lamb was noticed to be dyspnoeic and was killed that autumn. Other sheep had died meanwhile, and from this time onwards losses increased rapidly. Between April and December 1935 the farmer lost 259 out of 475 (54 per cent) of his ewes. The disease spread to other flocks, and by 1938 flocks in approximately one third of the whole country were affected, the incidence being as high as 50 to 60 per cent in the course of one to two years. The epizootic [50] was at its height from 1936 to 1945 but the disease tended to become rarer as the years went by. One wonders if the epizootiology during the 19th Century in South Africa was not similar to that in Iceland, if the early history of the disease is taken into account. Experience in Iceland has also indicated that when jaagsiekte appeared in a flock, the mortality remained the same during the first two years but thereafter decreased rapidly [49].

As far as the position in South Africa is concerned, Fig. 5 indicates the number of farms on which the disease has been diagnosed annually from 1924 to August 1968, the diagnosis having been confirmed histologically at Onderstepoort. Each farm has only

Fig. 5



ANNUAL INCIDENCE OF NEWLY DIAGNOSED FLOCKS. 1924 - 1968

once been incorporated in the year that it was first diagnosed. It must, of course, be realized that this is probably not an exact representation of the true position as the diagnosis has not always been confirmed histologically, and it is appreciated that some regional diagnostic centres have recently been doing their own histological diagnoses.

The high incidence during the 1960's represents the period during which the national survey was held when veterinarians and farmers were made more aware of the disease and were encouraged to submit specimens for histological diagnosis. At the same time I do feel that the number of affected flocks is increasing annually.

This survey on jaagsiekte was commenced in collaboration with Dr. G. L. Muller and Veterinary Field Services in order to determine its distribution in South Africa and South West Africa and its relative economic importance as well as other factors such as epizootiology, incidence, etc. It took the form of a questionnaire which all farmers owning infected flocks that were known to us were requested to complete, if possible with the help of their State Veterinarians or Stock Inspectors. The results will only suffice to give us a representative picture of the position, and as it was apparent that many farmers did not keep records, some of the figures given below are only estimations.

From an epizootiological viewpoint the results of this survey indicate the following:

(1) Table 1 shows the number of completed questionnaires received during the period 1962—August 1968.

Table 1

Orange Free State	=	42
Cape Province	=	19
Transvaal	=	18
South West Africa	=	6
East Griqualand	=	5
Natal	=	2
Total	=	92

(2) Eighty-six of these farms contained 135,365 sheep. The average number of sheep per farm was therefore 1,574 with a range of from 174 to 10,000 sheep per farm.

(3) The average duration of infection on 85 farms was five years, with a range of from a few months to 25 years, and the total number of sheep lost on 86 farms, since the first case, was 13,553 with a range of from one within a few months to 1,800 over 13 years.

(4) The average annual incidence of mortality from jaagsiekte according to our calculation from the figures received was 2 per cent, and according to the farmers' estimates 3.6 per cent (range 0.3 to 24 per cent for those flocks where the infection had been present for several years).

(5) No definite trend in the annual incidence could be calculated from the answers received (which in this respect were unsatisfactory).

(6) The breed incidence of sheep on 88 farms is reflected in Table 2. Some farmers owned more than one breed.

Table 2

Merino	64
German Merino	6
Dohne Merino	4
Merino crosses	9
Baster	5
Dorper	6
Karakul	8
Total	102

This table merely indicates that all these breeds are susceptible. The Merino is, of course, by far the most popular breed in South Africa. The number of farmers owning more than one breed was too small to draw definite conclusions as to increased susceptibility of any one particular breed.

(7) Table 3 reflects the sex incidence on 60 farms (excluding those who farmed only with ewes or wethers and those who did not know). Farmers were asked to inform us in which sex the disease were more prevalent.

Table 3

Wethers	13	(21.6%)
Ewes	14	(23.3%)
Even	30	(50%)
Rams	3	(5%)

We can conclude from this that sex plays no rôle in susceptibility to the disease.

(8) Incidence in rams. Of the 86 farmers who answered the question as to whether rams had died of the disease on their farms, 25 replied in the affirmative. These farmers had lost a total of 177 rams since the infection was introduced i.e. an average of seven per farm. On three farms the disease had occurred only in rams.

(9) In an attempt to determine the source of infection, the farmers were asked whether they maintained open or closed flocks and how, in their opinions, the infection originated. Table 4 reflects the replies of those who answered.

Table 4

(a) System of Farming		
Closed flock	26	(31%)
Open flock	40	(47%)
Closed (except for rams)	19	(22%)
Total	85	
(b) Origin of Infection		
Purchased sheep	22	
Purchased rams	3	
Don't know	38	
Definitely no introduction	3	
Total	66	

(10) In an attempt to determine whether the incidence of infection could be correlated with the kraaling of sheep at night replies to the appropriate questions and the results obtained are presented in Table 5.

Table 5: ANNUAL INCIDENCE CORRELATED WITH KRAALING

	No. of Farms	Av. Incidence
No kraaling	29	3.9%
Kraaled	42	3.7%
Sometimes	7	1.7%
	78	3.1%

The result of this is unexpected—one would have thought that kraaling would have increased the incidence of the disease. If one observes the habit of a sheep in a flock on a hot day, however, it will be noticed that, in the absence of shade, it will place its head beneath the belly of its neighbour in order to gain some respite from the sun. This will, of courses enhance spread of infection.

(11) The veld type and average annual rainfall of 85 of the farms are shown in Table 6.

Table 6

(a) Veld Type	
Grassveld (sweet and sour)	71
Karoo	9
Bushveld	3
Mountain shrub	1
Semi-desert	1

(b) Rainfall

Rainfall	No. of Farms
0— 9 inches	5
10—19 ,,	12
20—29 ,,	42
30—40 ,,	26

(Range 3 to 40 inches)

These figures merely indicate that the disease occurs in sheep maintained under a wide range of climatic conditions and on a variety of veld types.

(12) The results concerning the seasonal incidence of the disease in individual flocks are given in Table 7. These are farmers' impressions only; they were requested to inform us in which season of the year the disease was most prevalent. Although only 64 farmers replied, it will be noted that the total amounts to 68—some farmers said eg. spring and summer and both were thus incorporated in the table.

Table 7: SEASONAL INCIDENCE

Spring	5	(7.8%)
Summer	13	(20.3%)
Autumn	7	(10.9%)
Winter	22	(34.4%)
Even	21	(32.8%)
Total	68	(106.2%)

As can be deduced from this table, a higher percentage of farmers replied that the incidence of the disease was greater in winter. It is possible that secondary bacterial invasion of the lung lesions due to the climatic conditions plays a more significant rôle in winter and that the course of the disease is shorter, thus giving a false impression

of a greater prevalence at this time of the year. According to Dungal *et al*[22] a sudden drop in environmental tempeture is always followed by an increase in the death rate.

In November 1965 we acquired a flock of 184 Dorper sheep, amongst which cases of jaagsiekte had occurred, with the object of maintaining them under natural conditions in order to study the epizootiology and other aspects of the disease, in addition to having affected animals available at any time for transmission and other experimental work. This flock comprised 59 six-month-old lambs, 120 ewes (most of them pregnant) and five rams. Soon after acquisition the ewes commenced lambing. Unfortunately only a limited number of these animals could be retained for a protracted period and so 140 sheep (39 six-month-old lambs, 62 ewes and 39 lambs one to three weeks old) were slaughtered. In addition 19 of the young lambs died and were autopsied. Jaagsiekte lesions in various stages of development were present in the lungs of nine adult ewes. None were present in the very young or six-month-old lambs.

The sheep were kept isolated as a flock on our farm at Kaalplaas and were kraaled at night. They were allowed to breed at certain times of the year and, during the experiment, numerous lambs were born. Sheep were slaughtered periodically in order to reduce their numbers. The experiment terminated after two and a half years when all but eight of the animals were slaughtered and autopsied. Table 8 reflects the number of cases of jaagsiekte which were detected.

It is interesting to note that none of the animals born at Onderstepoort contracted the disease. I cannot find a suitable explanation for this phenomenon, but can only assume that the brief two-and-a-half year span of the experiment was not sufficiently long to have allowed infection to take place and develop; one should learn not to be unduly hasty when studying this particular disease. The results of this experiment also indicate that apparently no intra-uterine infection occurs under natural conditions.

PATHOGENICITY

Of the domestic animals only sheep, and probably all breeds of sheep are susceptible to jaagsiekte. Some breeds and families within breeds are, however, more resistant than others.

Table 8

No., age and sex of sheep	Length of time in experiment	No. dead during experiment		No. examined at slaughter		Percentage of JS cases
		of intercurrent disease—JS* negative	of JS	JS negative	JS positive (no symptoms)	
58 original adult ewes	2½ years	16	5	33	4	15.5
5 original adult rams	2½ years	0	0	5	0	0
20 ewes (6 months old at commencement)	2½ years	2	0	16	2	10
19 ewes (born shortly after commencement)	2½ years	3	0	16	0	0
16 wethers (born shortly after commencement)	14–15 months	2	0	14	0	0
31 lambs (born during experiment)	4–6 months	3	0	28	0	0
16 lambs (born during experiment)	7–8 months	1	0	15	0	0
63 lambs (born during experiment)	9–12 months	5	0	58	0	0
14 sheep (born during experiment)	20–22 months	1	0	13	0	0

*JS = Jaagsiekte

De Kock [18] in 1958, commenting on experiments carried out during the 1930's, suggested that susceptibility to jaagsiekte might be an inherited factor. He considered that the ram plays a major part with respect to an inherited predisposition.

Inherited predisposition was shown beyond doubt in Iceland [22] where the Gottorp breed of sheep was the most susceptible. Unfortunately it also proved to be the most popular and some farmers lost as many as 90 per cent of their sheep of this breed. The Adalbol breed on the other hand proved to be conspicuously resistant; on some severely affected farms only 10 per cent of this breed succumbed. In addition, offspring of different rams varied greatly in their susceptibility to the disease. On recognition of these genetic differences in susceptibility farmers were urged to breed from resistant families and breeds. As mentioned previously resistance in affected flocks increased in proportion to the length of time that they had been in contact with the infection.

Hutt [89] states that in parts of the world where jaagsiekte has been known for a long time, natural selection has presumably produced stocks which are genetically highly resistant, and that the high susceptibility of the Icelandic sheep could be attributed to the fact that they had not previously been exposed to jaagsiekte. He also considered it probable that, as with most diseases, susceptibility to jaagsiekte was influenced greatly by environment. Improved conditions of husbandry in Iceland together with continued selection (for resistance) had greaty reduced the losses.

This resistance to infection is borne out by the results obtained in experimental work in this country and in some others where the disease has been present for many years. The percentage of "takes" is relatively small *(vide infra)*.

There have been several reports of jaagsiekte occurring in goats [4, 32, 39, 41, 79, 90]. Dungal [48] reported from Iceland, however, that it had not been seen in goats or cattle on the farms where they were kept in close contact with sick sheep. These reports of the disease in goats are, in my opinion, erroneous and arise from the apparent inability of histopathologists and others to differentiate between epithelialization of the alveolar lining cells and adenomatosis.

Numerous attempts by Dungal *et al* [22] to produce the typical disease in laboratory animals were unsuccessful. Zilber *et al* [91] in Russia, however, reported the development after four to six months of multiple cysts filled with serous fluid in lymph nodes of mice following subcutaneous inoculation of a Seitz-filtered filtrate of a suspension of adenomatous lung.

SYMPTOMS

The duration of the natural incubation period is not definitely known. Experimentally, from our own experiences here and elsewhere, the incubation period varies from months to years. Tables 9 and 10 reflect some of our results and those of several over-

Table 9: INCUBATION PERIOD WHEN SHEEP PLACED IN CONTACT AND/OR SPRAYED WITH AEROSOL

Experiment	Interval after first contact (days)		
	Frank case or died of jaagsiekte	Slaughtered. Lesions present. (No symptoms)	No. of successful transmissions in experiment
Dungal[48]	122–183(3)		3/8
de Kock[18]		700	1/27
Onderstepoort (unpublished data)		454	1/7
Markson et al[60] (Weekly spray)		380–864(2)	2/6
Markson et al[60] (In contact + weekly spray for 2 years and 15 weeks)		1034	1/6
	Range 122–183	Range 380–1034	8/54 (15%)

Table 10: INCUBATION PERIOD FOLLOWING PARENTERAL ADMINISTRATION

Experiment	Interval after last inoculation (days)		No. of successful transmissions in experiment
	Frank case or died of jaagsiekte	Sluaghtered. Lesions present. (No symptoms)	
Dungal et al[22]	230		1/3
Sigurdsson[50]		427(2)	2/4
Onderstepoort (unpublished data)	247–427(5)	213–468(2)	7/37
Markson et al[60]	577	729–752(3)	4/11
Enchev[98]	348–879(3)	230–515(2)	5/16
	Range 230–879	Range 213–752	19/71(27%)

seas research workers in this respect. What is of great significance here is the fact that small lesions may be present a long time after artificial infection, and one must consider the possibility that these lesions are not necessarily progressive but may be static *(vide infra)*.

Sheep of all ages are susceptible, but because of the long incubation period the disease is very rarely seen in its clinical form in lambs under the age of seven months[21]. Thus, if an outbreak of pneumonia in lambs under this age is investigated, one can be certain that it is not jaagsiekte. The experiments cited above have shown that prenatal infection probably does not occur although this cannot be regarded as conclusive evidence because of the relatively small numbers of sheep used. In Table 11 the results obtained from the survey as regards the average age of affected animals are represented.

Table 11: AGE OF AFFECTED ANIMALS

72 farmers replied:—	
1½ to 3 years	56
Older than above	12
Younger than above	2
All ages	2

(Range: Few weeks to very old)

One of the farmers replied that he had observed the disease in lambs a few weeks of age; it is extremely doubtful that this observation is correct.

Symptoms are manifested when lung lesions are relatively advanced. The onset is insidious and the first sign is that the respiration rate is more rapid than normal after an affected animal has been driven. At this stage the animal may be in good condition but later loses weight rapidly. As the disease progresses affected animals lag behind the flock when driven. Marked respiratory distress is evident on exercise, the respiratory movements being short and jerky. Dyspnoea becomes progressive. Moist râles will be heard on auscultation of the chest and sometimes without the aid of a stethoscope. Spasmodic bouts of coughing occur. There is a great increase in the amount of secretion from the lungs, and if the animal is up-ended with the head down, this fluid streams out of the nostrils. This is regarded as a pathognomonic symptom of jaagsiekte[13, 21]. There is, initially, no fever, and in the later stages appetite is impaired.

The duration of the disease in its clinical form varies considerably. The sheep may survive a few weeks or months, and cases are on record where affected animals have survived for longer than a year[21]. This is especially the case if sick sheep are placed in small pens and well cared for. Under field conditions death usually occurs much earlier. According to results obtained from the national survey, the average duration of symptoms was two months, with a range of from a few days to six months.

In the majority of cases a terminal secondary pneumonia due to bacterial invasion of the lungs eventually supervenes, and at this stage a fever may be present.

PATHOLOGY

Macroscopic Pathology

In an advanced case of the disease the following lesions will be observed:—

The lungs do not collapse when the chest is opened; they appear to fill the thoracic cavity. A chronic adhesive pleuritis mainly due to secondary bacterial infection and some fluid in the thorax are present. On removal of the lungs from the carcase, it is noticed that they are three or more times their normal weight. Both lungs are usually involved but not necessarily to the same degree. The apical, cardiac and the anterior part of the diaphragmatic lobes are perhaps particularly affected, but any part of the lung may be involved, and patches and nodules of diseased tissue of various sizes are scattered in the more normal lung tissue. The lesions have a solid tumour-like appearance, are greyish white and have a tough consistency due to fibroplasia which in some cases is quite extensive. The cut surface is moist.

From observation of these lesions it is obvious that the primary lesion or lesions grow by expansion. Intrapulmonary metastasis, or spread of infection, probably occurs primarily aerogenously but lymphogenous dissemination may also play a rôle. Each new locus is seen macroscopically as a small greyish-white, semi-transparent nodule at first barely visible to the naked eye. This grows expansively and coalesces with neighbouring nodules until a large part of the lobe is affected. Pneumonia and abscesses due to secondary bacterial invasion are frequently seen.

Aynaud in France [17] was the first person to describe metastasis to regional lymph nodes. It has subsequently been noted in a number of countries [29, 31, 32, 59, 71, 72, 84, 92, 93] and we have seen it in several sheep in this country. Martincic and Cvjetanovic [73] have reported metastasis to mediastinal and mesenteric lymph nodes, while Enchev [70] has reported it in other parenchymatous organs. Nobel, Neumann and Klopfer [44], too, have recently recorded three cases of extrathoracic metastasis, one of which involved the mesenteric peritoneum, one a psoas muscle and the other the subcutaneous tissue and musculature of the right retrofemoral region. I have observed two cases of jaagsiekte in which extensive spread of the neoplasm to the pleura had occurred.

Of particular interest to me are those apparently healthy sheep which, when slaughtered, reveal the presence of one, or perhaps more, isolated lesions; these occurring in virtually any situation in the lungs. Some of the lesions are associated with fairly extensive fibroplasia and appear old. The question arises as to whether these are progressive or are perhaps "static" lesions, i.e. has the host by some immunological response come to terms with the infection and so limited its spread? Are these animals able to transmit infection? If this is indeed so, then these "carrier" animals constitute a great hazard.

Histopathology

The initial lesion is a change of the normal flat epithelium lining the alveoli to a cuboidal type of epithelial cell. This occurs, initially, in possibly one or a few cells of an alveolus. They then proliferate and eventually form a papilliform mass supported by a connective tissue framework within the alveolus. It appears that this lesion then grows by expansion and also coalesces with neighbouring lesions to form eventually a large macroscopically visible mass. In some lesions similar, but usually not as extensive, papilliform proliferations occur in the bronchiolar epithelium.

Alveolar macrophage response is a feature of the histopathology seen in some relatively advanced lesions, but it seems doubtful that these proliferate in response to the primary infection as they are usually not present, or are not present in conspicious numbers, in early lesions. In some cases, too, conspicuous hyperplasia of lymphoid nodules occur.

Simultaneously with the epithelial proliferation there is a progressive fibroplasia of interstitial tissues. This fibroplasia in old lesions may be very prominent. I have seen cases where a fibroblastic layer a quarter of an inch or more in thickness has been present beneath the pleura.

The metastatic lesions in the regional lymph nodes and elsewhere resemble the primary lesion.

Varying degrees of purulent or fibrinous inflammatory response is invariably seen in extensive lesions, and the presence of inclusions in histiocytes has been reported by Enchev [94].

DIAGNOSIS

Clinical

The clinical diagnosis of jaagsiekte cannot be made with certainty as it cannot be differentiated from other forms of pneumonia. Copious mucous exudate issuing from the nose when the sheep is up-ended is, however, a useful diagnostic sign when present [13, 22]. X-ray examination as an aid to diagnosis is of doubtful value. Van der Walt [95] attempted this with the assistance of experienced medical radiologists on an affected Karakul ram, but the results proved unsatisfactory.

Histological

The diagnosis of jaagsiekte, when this disease is suspected in a flock, should, at least once, be performed histopathologically. The histopathologist responsible for the diagnosis should be experienced in lung conditions and should be able to differentiate between adenomatosis and epithelialization of the alveolar lining cells. It should be remembered that not all cases of pneumonia in a flock need, of necessity, be jaagsiekte after the diagnosis has been confirmed.

Serological

Enchev *et al* [96] have reported the development of a complement fixation test for the diagnosis of jaagsiekte. Antigens were obtained by extracting adenomatous lesions with normal saline or ethyl alcohol. Their results were as follows:—
Positive reactions in:

(a) 145 of 172 sheep (84%) with histologically confirmed adenomatosis;

(b) 63 of 1,867 (3%) apparently healthy sheep from affected flocks;

(c) 21 of 3,888 sheep (0.5%) from unaffected flocks; and

(d) 17% of sheep with generalized echinococcosis.

Seven sheep with early adenomatosis gave negative results.

DIFFERENTIAL DIAGNOSIS

It is, on occasion, difficult to differentiate jaagsiekte macroscopically from enzootic pneumonia (pasteurellosis) and pneumonia due to other bacterial causes.

In countries where these diseases occur, progressive pneumonia, zwoegerziekte, maedi, bouhite and pneumonia due to lungworm infestation should also be excluded.

PROGNOSIS

Once symptoms of the disease are manifested recovery does not occur. However, it is not known for how long animals with "static" localized lung lesions, which are impossible to diagnose clinically, may survive. It is possible that this period may span several years.

TREATMENT

No effective form of treatment is known.

PROPHYLAXIS

The only method of preventing the occurrence of jaagsiekte is by the maintenance of a completely closed flock. No sheep whatsoever should be introduced into a clean flock as any introduced sheep constitutes a potential danger. Due to the long incubation period which may span a period of years in some cases, quarantining of newly-introduced sheep is not practical.

Once infection is manifested in a flock, one of the following methods may be attempted to control it:—

(1) *Immediate slaughter of all suspected cases as soon as they are detected.* This procedure may reduce the incidence but will not eliminate the disease.

(2) *Maintenance of a two-flock system.* In this system an infected flock is reduced in numbers as quickly as possible by sale to a butcher. At the same time a clean flock is built up by purchase of clean breeding stock from a reputable breeder. The two flocks must be kept strictly isolated from each other, and possible means of infection other than by cohabitation and propinquity e.g. by dosing guns, drinking troughs, etc. must be prevented.

(3) *Slaughter-out system.* The whole flock is sold to a butcher and new stock purchased from a reputable breeder. It is not known for how long premises, once they have contained sheep suffering from jaagsiekte, will remain infective. In my opinion due to the low infectivity rate experienced in affected flocks in this country and the method of infection, this is probably not very long; a period of six weeks in summer and three months in winter would probably suffice, if water and feed troughs are disinfected, and the use of sheds and kraals which previously housed infected animals is avoided for a somewhat longer period.

Adenomatosis was eradicated from Iceland by means of a successful slaughter-out campaign which was also aimed at the control of maedi. Three hundred thousand sheep in infected areas were destroyed from 1944 to 1951, and no cases have been seen since 1952[50]. The sheep population in Iceland in 1939 was 700,000[47].

INMUNIZATION

In a carefully controlled experiment at Onderstepoort attempts to immunize sheep with a formalized vaccine prepared from the lesions of an affected animal were not successful. Of the 47 sheep used in this experiment, 10 were unvaccinated, unchallenged controls and were kept isolated from the others, 10 were vaccinated but not challenged, 10 were vaccinated and challenged, 10 were challenged but not vaccinated and seven were in-contact controls. The immunized sheep were vaccinated on four occasions during a period of 97 days (Day 1, 25, 69 and 97). The challenged sheep were inoculated parenterally with infective material on six occasions during a period of 64 days (Day 77, 84, 106, 111, 119 and 141). The only sheep to develop jaagsiekte were two in the vaccinated and challenged group (they died of the disease 321 and 422 days respectively after the first challenge) and one in the in-contact control group which revealed the presence of early lung lesions when all surviving animals were slaughtered 551 days after commencement of the experiment.

One may conclude from this experiment:

(1) That the vaccine was immunogenically inert and failed to protect the sheep; or

(2) that the challenge doses of the causative agent were too large and overcame what immunity was present, it being difficult to simulate natural exposure by the means employed under experimetal conditions; or

(3) that the two sheep which developed jaagsiekte did not respond immunogenically due to inherent factors; or

(4) that other factors such as the interval between vaccination and challenge played a rôle.

In the literature there has been reference to the prophylactic use of vaccination in three countries. Shirlaw[25] and Anon.[23] in Kenya reported spectacular results using a formalized affected-lung-tissue vaccine against Laikipia lung disease. Use of this vaccine reduced the incidence of this disease from 30 per cent to one per cent on some farms. There does, however, appear to be some doubt as to the exact nature of Laikipia lung disease[60]; it probably comprises several distinct disease entities, amongst which is jaagsiekte.

Cuba-Caparó[97] in Peru has considered that formalin treated suspensions of diseased lung emulsion are effective as vaccines, and Moráillon *et al*[62] from France have reported that a prophylactic programme, which consisted of the immediate elimination of diseased animals and twice yearly vaccination, proved efficacious in controlling the disease. Out of 120 flocks, 42 were rendered healthy within two years by the use of these methods. I have unfortunately been unable, to date, to obtain details as to the method of manufacture of this vaccine.

CONCLUSIONS

I have attempted, Mr. Chairman, to review the present state of our knowledge regarding jaagsiekte. There can be no doubt that jaagsiekte is a distinct disease entity, that it is an infectious disease and that, while known in most overseas countries as pulmonary adenomatosis, a more correct appellation would be pulmonary carcinomatosis, if one considers that metastasis is a sign of malignancy. It is for these reasons, in addition to the fact that presumably non-infectious forms of pulmonary adenomatosis occur in man and a variety of animal species other than sheep, that I have preferred the use of the original name of the disease, which is jaagsiekte.

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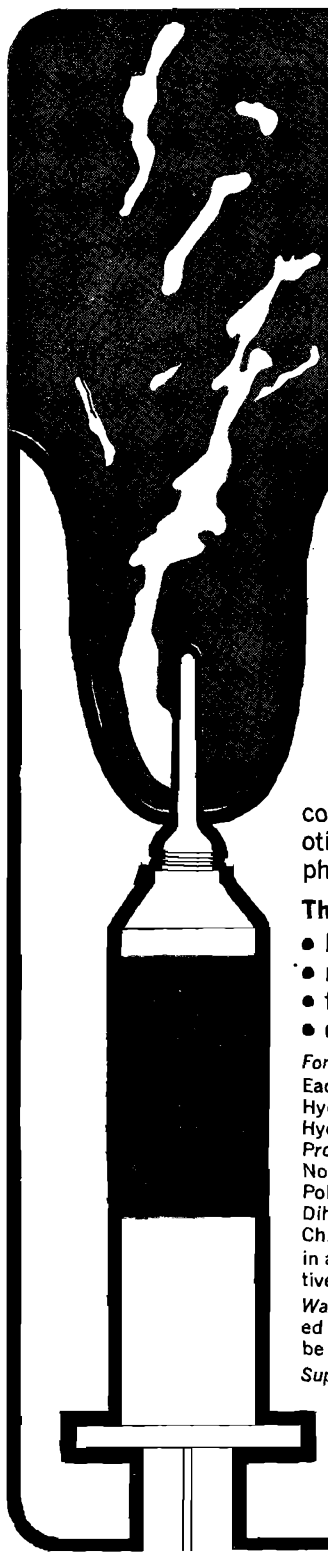

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THE CYCLE OF INFECTION WITH LEUKOSIS VIRUSES

H. GRAHAM PURCHASE*

INTRODUCTION

The original method for the detection of lymphoid leukosis (LL) viruses employed the inoculation of day old chicks with sample material, and in order to obtain a satisfactory response an experimental period of 200 to 270 days was necessary [1]. The time required for quantitative assay was shortened to 63 days by using the less sensitive erythroblastosis response [2] and to 43 days when embryos were inoculated intravenously [3]. The most commonly used quantitative and qualitative assay for LL viruses is the resistance inducing factor (RIF) test introduced by Rubin in 1960 [4]. This test relies on the fact that cell cultures that have been previously infected with noncytopathic avian leukosis viruses are resistant to infection with Rous sarcoma virus (RSV). RSV produces foci of neoplastic transformed cells in susceptible chick embryo fibroblast (CEF) monolayers [5]. The RSV and LL viruses must be related (i.e., they must belong to the same subgroup of the avian leukosis viruses of which there are two important subgroups A and B) or interference does not occur.

More recently a defective strain of RSV has been used to transform chick embryo fibroblasts to tumour cells. These transformed cells do not produce infectious virus since the virus genome contained within them is defective [6, 7]. When such cells are superinfected with a leukosis virus, the RSV genome is complemented and infectious RSV is produced together with the leukosis virus [8]. The presence of the RSV can be demonstrated easily and in a short time since it produces foci in susceptible CEF, pocks on chorioallantoic membranes and tumours in chicks. This is the basis of the nonproducer cells (NP) test which may prove to be more sensitive than the RIF test (Rispens, B. M.; unpublished, 1967).

Antibody produced in hamsters will fix complement with avian leukosis virus antigens [9]. However, the complement fixation test suffers from problems with nonspecific positive results.

The presence of antibody in the serum of birds is detected by mixing the serum with a known virus, incubating for a period of time, and assaying for residual virus by any of the above methods [10, 11].

THE CYCLE OF INFECTION

The discussion which follows will consider the presence of virus or antibody demonstrated by the above means in two generations of chickens, namely, the parent and the offspring. The parental flock can be divided into four groups on the basis of whether the birds have antibody (A+) or not (A−), and whether virus can be detected in the serum, plasma, or embryos from such an animal (V+) or not (V−). These groups are illustrated at the top of Figure 1.

In most parental flocks the A+ V− group is the largest and comprises about 75% of the birds. Antibody in these parents is passed on to the progeny through the yolk [11]. This maternal antibody usually lasts until the chick is three to four weeks old; the duration is proportional to the titer of antibody in the serum of the dam [12]. It is this antibody which protects the chicks from infection during the early days of life. Eventually the chicks lose their maternal antibody and become infected from their environment or from penmates. There then follows a transient viraemia which in turn is replaced by circulating acquired antibody. Antibody can be demonstrated for the rest of the life of the bird.

Some parents are viraemic and do not have circulating antibodies to the homologous virus in their serum (A− V+). Dams in this category always shed virus in their eggs. Large amounts of virus particles can be demonstrated in most organs of the embryo

*Poultry Research Branch, Animal Husbandry Research Division, ARS, U.S.D.A., Regional Poultry Research Laboratory, East Lansing, Michigan, 48823, U.S.A.

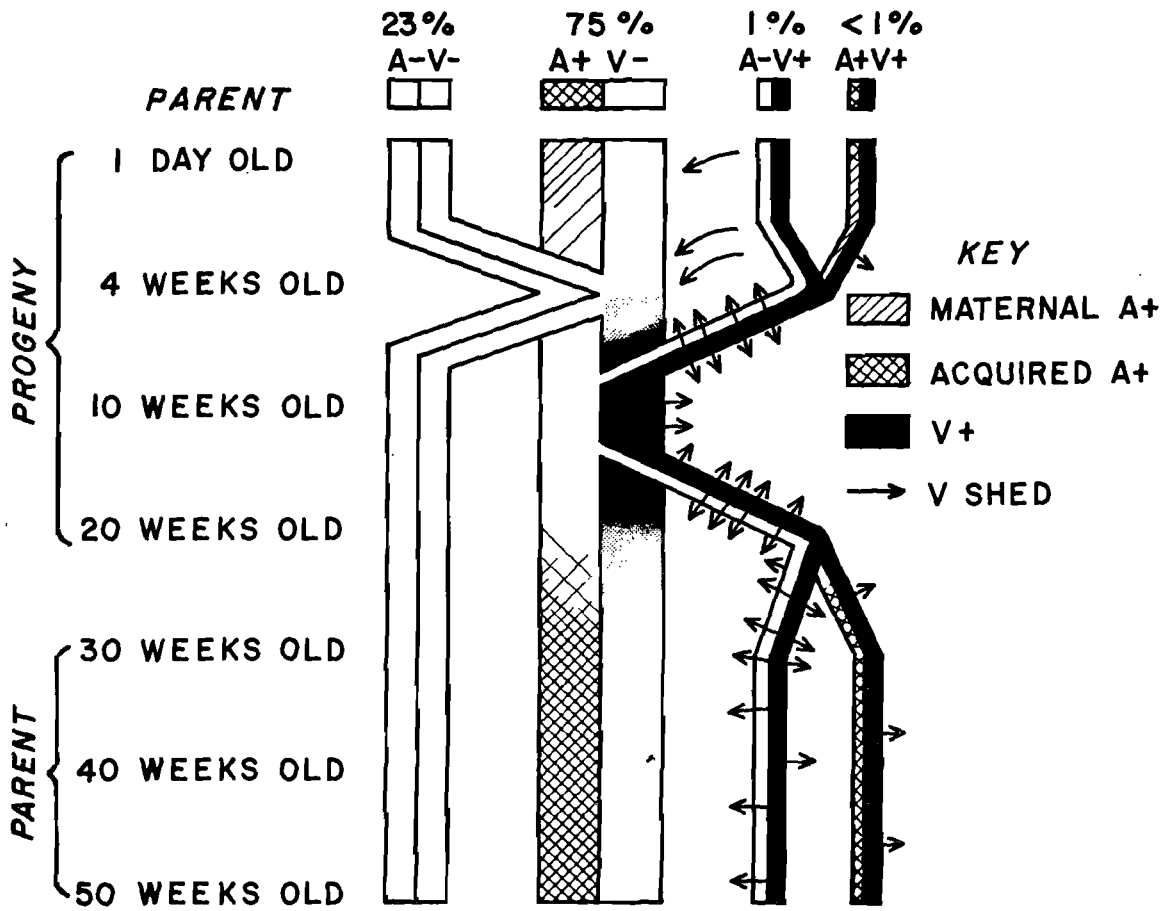

FIGURE 1—The cycle of infection with avian leukosis viruses.

both by virological and electron microscopical techniques; however, virus budding can only be observed in the pancreas [13, 14]. Large numbers of virus particles can also be seen in the lumen of the pancreatic ducts and this virus finds its way into the intestinal tract and is excreted in the meconium. Virus can be demonstrated in the saliva and faeces of infected birds for prolonged periods [15, 11]. This virus forms the source of infection for antibody-free, susceptible birds in the flock. A few of the viraemic birds may acquire antibody although the majority are tolerant to the virus infection, and do not develop antibody, hence the virus is never eliminated from the body. The rate of excretion of the virus decreases with age.

Some parents shed virus in their eggs intermittently. Circulating virus cannot be demonstrated in these birds and most of them have circulating acquired antibody (A+ V+).

The male plays only a minor role in the epizootiology of LL, acting solely as a carrier of virus and source of contact infection. There is no congenital spread from the male.

In the remaining birds, neither virus nor antibody can be detected (A− V−). There are several explanations for this. One is that the techniques used are not sensitive enough to detect very low amounts of either virus or antibody. Another is that these birds may be genetically resistant to one or more subgroups of avian leukosis virus and may never have been infected. It is possible that some birds, even though they are susceptible, by chance never acquire an infection and so never develop antibody; however, this seems unlikely. Another possibility is that some of these birds may have been infected with an avian leukosis virus of a different subgroup or one which is antigenically only distantly related to the one used for testing.

Antibodies produced against this virus may protect against most of the viruses in the flock and yet not be detectable by the laboratory test employed.

Subgroup A LL viruses are ubiquitous. Information on subgroup B viruses is lacking though they seem to be less prevalent. No commercial flock that has been tested has been found to be completely free of LL viruses. However, there is considerable variation in the number of birds with virus or antibody in different flocks [16] and there may be a considerable variation at different times in the same flock [17]. Birds from experimental flocks from which LL viruses have been eliminated do not die of LL and do not have antibody.

ONCOGENESIS RESULTING FROM LYMPHOID LEUKOSIS VIRUS INFECTION

A considerable age resistance to the development of tumours induced by LL viruses has been demonsrtated. By 21 days of age, birds are at least five times more resistant than at one day of age [18]. Bearing this in mind ,it is not surprising that Rubin [11] found that birds with a congenital infection were seven times more likely to succumb from LL than those that acquired the virus infection later in their lives. Nevertheless, some of the progeny of birds that were not congenitally infected died of LL. It is probable that in these birds the maternal antibody was lost early and that they then became infected at an early age when they were still susceptible to the development of tumours or that they were infected early with a virus which was not neutralized by the maternal antibody. Early loss of antibody could be due either to a low titer of antibody in the dam or to some poorly understood mechanisms, some of which may be nonspecific, which cause the antibody to be "catabolized" quickly. Since congenitally infected birds constitute only a very small proportion of most flocks (less than 1%), it can be concluded that the greatest number of birds that die from LL are likely to be those that have an acquired infection. Serum taken from birds just before they succumb to LL often has a high titer of antibody. Thus, the presence or absence of antibody is no indication of whether a bird will die of LL.

In summary, then, it can be said that most of the birds that die in flocks that are experiencing an outbreak of LL are birds that have acquired an infection early. Anything that is liable to cause an infection of a large number of young birds in a flock is liable to result in heavy losses from LL later on. Virus infection and LL tumour development are not synonymous.

ONCOGENESIS IN THE INDIVIDUAL BIRD

The bursa of Fabricius, the hindgut lymphoid organ which is responsible for humoural immunity, has been shown to be the target organ in LL. Removal of this organ from birds up to 20 weeks of age greatly reduces or eliminates the incidence of LL in experimentally infected birds [19, 20]. Recent sequential histological observations have demonstrated the presence of enlarged follicles whose cells have many of the properties of neoplastic cells as early as 8 weeks of age [21]. However, LL does not occur before birds are about 15 weeks of age and many attempts to induce early tumours have failed. It appears that the bursa cells themselves transform under the influence of the leukosis virus and that, when the bursa involutes around the time of sexual maturity, they metastasize producing the typical LL lesions in the liver, spleen, and other visceral organs.

Many of the birds with the enlarged neoplastic follicles do not die from LL within a reasonable experimental period afterwards (Payne, L. N.; Dent, P. B.; Cooper, M. D.; Burmester, B. R.; unpublished, 1967). This leads one to the conclusion that many of these follicles must regress and in actual fact never form LL tumours.

It has already been mentioned that the presence of antibody to virus is only an indication of virus infection and has no relationship to the development of the tumours. A different antigen eg. a neo-antigen unrelated to the virus antigen may be present in the tumour. This has been demonstrated in tumours induced by the Papova group of viruses [22]. Tekeli and Olson [23], using the fluorescent antibody test, have probably demonstrated the presence of a tumour antigen in LL tumours that contain little or no virus. These tumour antigens or neo-antigens only appear when the cells become "transformed" which in LL occurs at about 8 to 10 weeks of age. At this stage, the birds are immunologically competent and it is not surprising that the natural policing system of the body eliminates many of them. However, in some

instances, immune elimination does not result and a tumour develops.

It is interesting to speculate on the role of immunologic enhancement in the development of LL tumours. A description of a hypothetical model follows. In the early stages, the neo-antigen containing transformed cells are protected within the bursa follicle and no neo-antigens reach immunologically competent cells. However, during involution, the bursa cells actively "peripheralize" and the transformed cells enter the circulation with the unaffected bursa cells (this has not been demonstrated yet). Since the transformed cells and immunologically competent bursa cells originate from the same site, they are more likely to encounter one another than are transformed cells and thymus cells. The result is antigenic stimulation of the competent immune system and the rapid production of antibody. The presence of circulating antibody against antigens in or on grafted cells or transplanted tumour cells has been shown to be necessary for immunologic enhancement[24]. The antibody attaches to the foreign antigen on the surface of the transformed cells protecting them from the lymphocytes which in normal situations are responsible for graft rejection. These thymic lymphocytes may not have encountered the neo-antigen prior to this time and may not be sensitized since the antigen is contained within the bursa. According to this model, a combination of peripheralization of the bursa-dependent lymphoid system, simultaneous humoral antibody production, and the resultant immunologic enhancement are responsible for the development of LL tumours. In some cases transformed cells with neo-antigens, on liberation from the bursa, encounter lymphocytes from the thymus-dependent system first, sensitizing them and resulting in immune elimination.

It can be seen that any disease or external factor which effects the immunological system of the bird at this age may have a drastic effect on the subsequent incidence of LL. Thus, factors, infectious or otherwise, which cause an early rapid involution of the bursa may induce lesions of LL in young birds and may also contribute to the syndrome known in the field as "acute leukosis".

GENETIC RESISTANCE

Cellular resistance to LL viruses has been demonstrated in experimental inbred flocks and in field flocks[25, 26, 27, 28]. This resistance to virus infection is of a very high order (at least 1000-fold) and can be demonstrated by bird or embryo inoculation, or in cell culture. The resistance is governed by autosomal genes and susceptibility is dominant. There are two different pairs of alleles responsible for the genetic resistance to the two subgroups of avian leukosis viruses which have been studied in detail.

If these genes are in fact so strong and are present in commercial flocks, one would expect that breeding for viability and breeding from families with a low incidence of LL would increase the incidence of these genes in the flocks.

Most flocks are highly susceptible, especially to subgroup A viruses. One explanation is that selection pressure in favour of maternal antibody protection and against genetic resistance is stronger than the pressure in the opposite direction[29]. A description of a model illustrating this and represented by Figure 2 follows. When a homozygous susceptible female (RsRs) is mated to a homozygous resistant male (rsrs) the progeny are all susceptible (rsRs). However, because the female is susceptible to virus infection, it usually acquires the infection, overcomes it and develops antibody. This antibody is passed on to the genetically susceptible (Rsrs) progeny proctecting them from early infection and the subsequent development of disease. In the reverse situation, when a homozygous susceptible male (RsRs) is mated to a homozygous resistant female (rsrs) the progeny are still all genetically susceptible (Rsrs). The female is genetically resistant to virus infection and so does not acquire antibody[03]. The "double unprotected" progeny have neither maternal antibody nor genetic resistance to protect them from early infection and subsequent development of tumours. These progeny then die of LL and the parents are rejected on the basis of the performance of their progeny. The picture is actually much more complex; however, considering all possible combinations of the two alleles, the selection pressure brought about by maternal antibody will decrease the incidence of the recessive allele within the flock. Since susceptibility is a prerequisite for infection and development of antibody, breeding on the basis of progeny performance may actually increase the genetic susceptibility of the flock to virus infection.

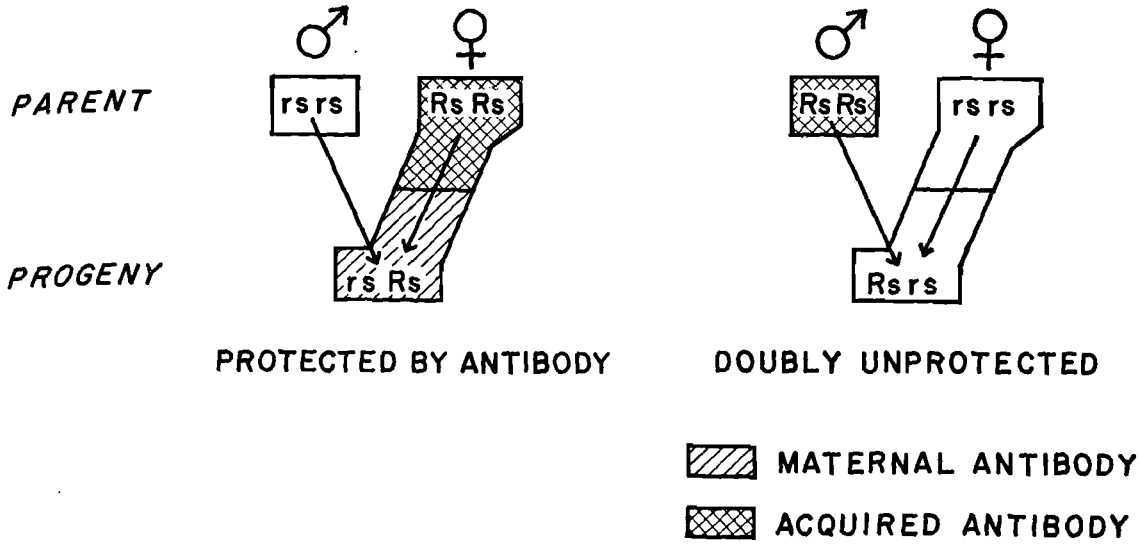

FIGURE 2—A model illustrating the relationship between the *selection* pressures exerted by maternal antibody and genetic resistance.

One other factor plays an important role and that is resistance to disease. Apparently there are many genetic factors involved. Inbred lines have been developed in which all the birds are highly susceptible to virus infection; however, none of them develop disease[29]. There are very few data on the nature and *modus operandi* of these factors. Many commercial flocks have a very low incidence of resistance to development of tumours. However, it is quite understandable that the cellular resistance will have an overriding effect since birds that do not initially become infected will not subsequently develop tumours.

CONCLUSION

Susceptibility to virus infection and to development of disease, early peripheralization of the bursa-depedent immune system and early virus infection predispose an individual bird to develop LL. Numerous small changes in the environment may sway the balance one way or another and upset the cycle of infection with LL viruses. The best advice currently available on controlling the disease makes use of present day knowledge to sway the balance in favour of a reduction in LL losses. This involves good sanitation and strict isolation during hatching and until the chicks are no longer susceptible to tumour development. This includes prevention of contact between chicks of different source flocks and of different age groups.

Eradication of the LL viruses has been highly effective in experimental flocks. When techniques become simpler and less expensive this will probably be the method of choice for the control of LL in the future.

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SUSPECTED HEREDITARY SPINAL ATAXIA IN CATTLE

L. VON MALTITZ*, P. A. BASSON** AND J. L. DE B. VAN DER MERWE**

SUMMARY

The occurrence of suspected hereditary spinal ataxia in cattle in the Gibeon district of South West Africa is described. Characteristic changes in the neurones of the spinal cord and some in the medulla oblongata were noticed in both cases in which specimens for histopathological examination were taken.

HISTORY

Thirteen years ago the owner of the affected cattle bought 20 cows and one bull, all Afrikaner, or Afrikaner crossbred types. The cattle received little attention, as they were acquired only as a sideline and the young bull calves were never castrated but marketed at the age of about 2½ years. Considerable inbreeding, therefore, resulted and under these circumstances it was impossible to identify the parents or grandparents of the offspring.

Nine years after the animals were purchased, a calf was born with signs of inco-ordination of the hind quarters. The owner noticed the abnormality when the calf was 6—8 months old and ascribed it to some injury to the back. No improvement was noticed during the following two months and the calf was eventually slaughtered.

The herd increased to a total of 80 animals and the owner became alarmed when he noticed a young bull, three heifers and a calf all of which suffered from inco-ordination of various degrees in the hind quarters. A veterinarian was consulted at this stage.

MATERIALS AND METHODS

The affected animals were examined clinically and compared with normal animals of the same age group. Two heifers, both 13 months of age, were slaughtered for examination. Entire brain, spinal cord and nerves and bloodvessels of the hind legs were collected in 10% formalin. The tissues were embedded in paraffin wax, cut with a sliding microtome and stained with haematoxylin and eosin (HE), haematoxylin-phloxin (HP)[1], acid-fushsin-methylene blue (AFMB)[2], Masson's trichrome stain[3], Hotchkiss periodic acid-Schiff (PAS)[3], Congo red[4], oil red O (ORO)[5], Berlin blue (BB)[6] and Schmorl's method for lipofuscin[5].

RESULTS

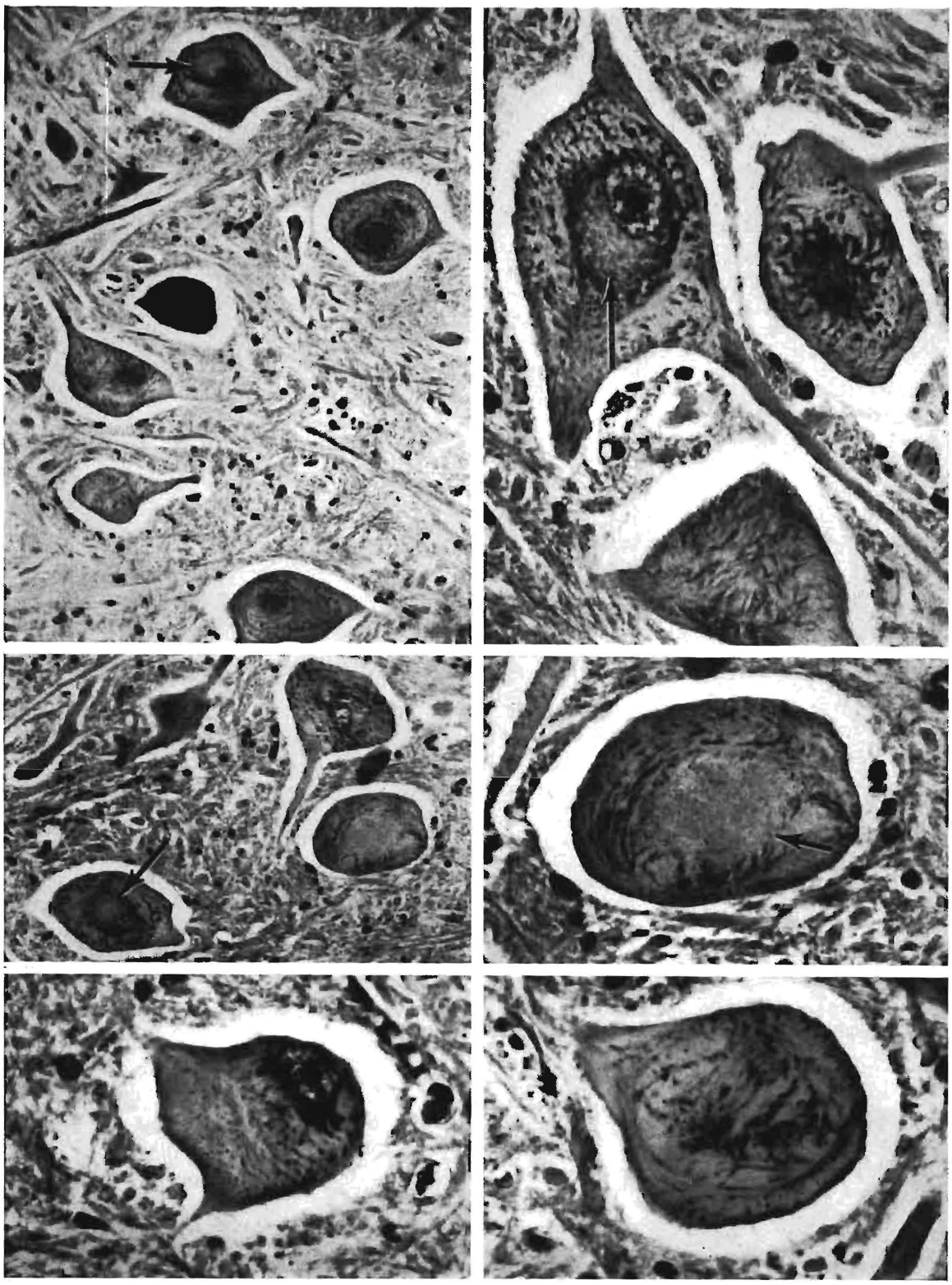
Symptomatology:

Out of a herd of 80, one bull and three heifers, all of which were approximately 13 months of age, and one three-week old calf were affected. The following were noticed on inspection and examination.

At rest no abnormality could be detected. During slow walking an occasional weakness or lameness was seen in either of the hind legs. This was not sustained, but usually repeated after 5—20 paces. The ataxia was aggravated with increased movement. During fast running the inco-ordination became marked with one or both hind legs frequently partially collapsing under the weight of the body. Balance was disturbed and the rump swayed from side to side. Occasionally the animals stumbled and dragged one or both hind legs. This was followed by alternating periods of recovery and incoordination. There was no improvement after a period of rest and prolonged running did not further aggravate the condition.

In spite of having had these symptoms for seven months, the four older animals never suffered from atrophy of any of the muscles of the hind legs or rump, nor was there any loss of sensitivity of the skin or

*State Veterinarian, Mariental, S.W.A.
**Veterinary Research Institute, Onderstepoort.



any part of the rump or hind legs. On palpation of the joints of all the affected animals, no signs of swelling, heat or pain could be detected.

Regarding the four older animals, there was no abnormality of body temperature, respiration, condition or general appearance in comparison to normal animals of the same age group exercised in an enclosure together with the affected animals. No sign of retention of urine or faeces, nor of any abnormality in the movement of the tails, could be seen.

The calf, in contrast to the four older affected animals, appeared slightly listless and was in worse condition than other calves of the same age. Movement appeared to produce pain in the hind legs, but body temperature and rate of respiration remained the same as in the other five healthy calves of the same age, both before and after they had all been chased around in an enclosure.

Macroscopic examination:

No changes were found in any of the two slaughtered animals.

Microscopic examination:

Most of the multipolar neurones in the ventral horn and the large neurones in the dorsal and lateral horns of the gray matter throughout the entire length of the spinal cord were enlarged and abnormal. The nucleus was frequently eccentric and surrounded by more or less concentric bands of Nissl substance and alternating lighter areas. A mildly eosinophilic and finely granular structure was frequently found immediately adjacent to the nucleus (arrows in plate). It was approximately circular, but not well demarcated and either encircled by dense Nissl bodies or a pale pinkish substance. This paranuclear structure usually exceeded the nucleus in size and its colour was more deeply eosinophilic than that of the surrounding cytoplasm. It stained reddish with HP, AFMB and Masson stains, failed to show positive reactions with PAS and Congo red and was negative for lipids and iron as indicated by the negative reactions with ORO, Schmorl's method and BB. The Nissl substance in the affected neurones was condensed in various patterns which were either circular, semi-circular, streaky or in curves. The impression was gained that this was caused by the increase of another substance, neurofibrils or organelles in the cytoplasm compressing the Nissl substance and possibly even replacing it in other areas. The ectoplasm in some of the cells was prominently enlarged. The eccentric nuclei were frequently found to be faded and probably degenerated.

Some of the neurones in the medulla oblongata were also affected, but their precise location was not determined. The rest of the brain and nerves appeared normal. No glial changes were noticed in either spinal cord or brain.

DISCUSSION

Various congenital and hereditary conditions of cattle, such as cerebellar hypoplasia, congenital ataxia due to a leucodysplasia, congenital posterior paralysis as a result of pallidal and reticular degeneration and cerebral pseudolipidosis have been described [7]. Other conditions like congenital posterior paralysis of Norwegian Red Poll calves, ataxia of cattle of the Meuse-Rhine-Yssel breed, epilepsy and spastic paresis were also reported without demonstration of lesions [7]. In none of these nervous disorders were lesions described which correspond to those found in the cases here presented such as the paranuclear structure and peculiar distribution of Nissl substance in an enlarged neurone. Various histochemical techniques proved the paranuclear structure to be negative for lipids, mucopolysaccharides and iron, but failed to clarify the nature of the changes observed. Changes in certain organelles such as the Golgi apparatus and an increase in neurofibrils would seem to be the most probable explanation for the peculiar appearance of the neurones.

The evidence of inbreeding suggests a strong possibility of the condition being hereditary.

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Veterinarians around the World 2

Die Veearts: Uêreldbeeld 2

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THE ROUTE OF MIGRATION OF *SCHISTOSOMA MATTHEEI* FROM THE LUNGS TO THE LIVER IN SHEEP

S. P. KRUGER*, L. P. HEITMAN**, J. A. VAN WYK** AND R. M. MCCULLY***

SUMMARY

Experiments were carried out on the migratory route of schistosomula of *S. mattheei* from the lung to the liver in sheep. It was shown that they migrated against the blood stream via the pulmonary artery, right ventricle and atrium, posterior vena cava and hepatic vein into the liver. The lung forms, which were also found on their migratory route to the liver, were more elongated than those described for *S. japonicum* or *S. mansoni*.

INTRODUCTION

Numerous studies in small laboratory animals on the life-cycle of *Schistosoma japonicum* Katsurada, 1904 and *Schistosoma mansoni* Sambon, 1907 have been conducted [1, 2, 5, 6, 7]. The present paper is the first study to be reported on experimental infestations of a normal definitive host, the sheep, with *Schistosoma mattheei* Veglia and Le Roux, 1929. The true migratory route of this parasite in one of its natural definitive hosts can therefore be determined.

The cercariae penetrate the skin and migrate to the lungs via the lymph or blood vascular system [1, 2, 3]. It appears that *S. mattheei* migrate to the lungs by the blood vessels, but experiments have not been extensive enough to exclude the possibility of migration via the lymphatics.

Many authorities believe that schistosomula pass via the capillary bed of the lung and the left heart into the general circulation to reach the portal system [1, 2, 4]. Others [3, 5] affirm that schistosomula rupture the lung capillaries, migrating through the lung parenchyma and visceral pleura into the thoracic cavity, through the parietal pleura and diaphragm to enter the diaphragmatic surface of the liver. It has also been suggested that schistosomula migrate via both routes [6, 7].

MATERIALS AND METHODS

1. The sheep used, were infested as follows:

   (a) Cercariae of *S. mattheei* were harvested and a known number applied either to the back or fore limbs of the sheep [3, 8]. Single or multiple infestations at intervals varying from one to eight days were used.

2. With the exception of Exp. 1 and 2, the following methods were employed:

   (a) In Exp. 3 and 4, and sheep 1 of Exp. 5, 10,000 I.U. heparin was injected intravenously a few minutes before slaughter by exsanguination from the large blood vessels of the neck.

   (b) The abdominal and thoracic cavities were opened. The blood vessels which are specified in the various experiments were clamped with haemostats. The surfaces of the lungs, livers, diaphragms and other organs were meticulously examined macroscopically for haemorrhages and other lesions. Then the blood vessels were either cannulated for perfusion, or opened for washing and aspiration (see below).

   (c) Various methods of flusing the worms from the blood vessels were used:

      (i) Perfusion with physiological or Earle's saline at 38°C. In the case of the Earle's saline, 10,000 I.U. heparin/L were added.

*Dept. of Zoology, Rand Afrikaans University, Johannesburg.
**Section of Helminthology, Onderstepoort.
***Lt. Colonel USAF, V.C., Staff Member of Geographic Pathology Division, Armed Forces Institute of Pathology (AFIP), Washington D.C. Temporary Assignment, Dept. of Pathology, Onderstepoort.

(ii) Wash - plus - aspiration technique. Earle's saline was applied to or injected into the relevant areas from a wash bottle. The washings were aspirated by a rotary suction pump and stub-nosed glass nozzle into an Erlenmeyer flask, and kept at 38°C and examined as soon as possible.

(d) The examination for schistosomula was carried out as follows:—

(i) Perfusion: The perfusate was filtered through a 400 mesh to the linear inch sieve, all the material on the sieve's surface collected and examined with the aid of a stereo-microscope [9].

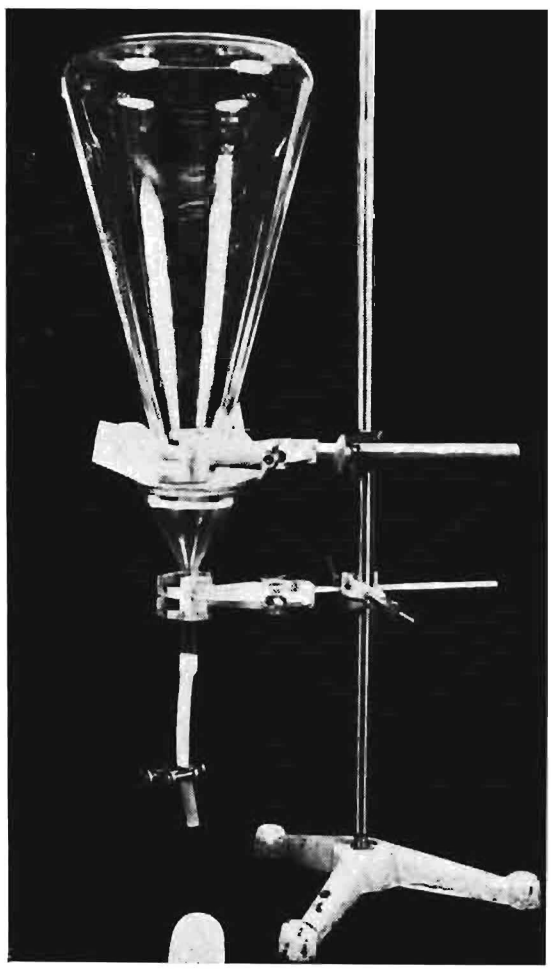

PLATE 1—Modified Baermann apparatus

(ii) Wash-plus-aspiration: Either the total volume of fluid was examined under the stereo-microscope or the schistosomula were concentrated by means of a modified Baermann apparatus [10] (Plate 1), before being counted. Schistosomula were kept alive for as long as 11 hours in Earle's saline at 38° in an incubator.

EXPERIMENTAL OBSERVATIONS

A. Two preliminary experiments were performed to determine the distribution of schistosomula in the host, and whether any haemorrhages occurred on the surfaces of the lungs, liver and diaphragm.

Exp. 1: One sheep was infested with 10,000 cercariae and killed on Day 6. Evans blue and heparin were injected intravenously [11] half an hour before slaughter. The surfaces of the lung and the diaphragm were carefully examined macroscopically for haemorrhages but with negative results.

Exp. 2: Two sheep were infested daily for 3 and 4 days respectively and slaughtered a day after the last infestation. Schistosomula were found only in the lungs.

B. Thereafter detailed migration studies were performed:—

Exp. 3: A sheep was infested with 71,200 cercariae and examined on Day 11 by perfusing the organs mentioned below with physiological saline. The schistosomula were collected on a sieve. The yields of schistosomula from the different locations were as follows:—

a. Posterior vena cava to intrahepatic vein	17,940
*b. Portal vein to posterior vena cava	42
c. Pulmonary vein to pulmonary artery	3
d. Pulmonary artery to pulmonary vein	0
e. Posterior aorta to extrahepatic portal vein	5

*After "a" had been accomplished.

Exp. 4: Two sheep received 13,500 cercariae and were examined on Day 4 and 5 respectively by means of perfusion with physiological saline.

Sheep 1 (4 days)

a. Pulmonary vein to pulmonary artery	157
b. Pulmonary artery to pulmonary vein	0
c. Posterior vena cava to intrahepatic portal vein	0
d. Aorta to posterior vena cava	0
e. The brachiocephalic trunk to jugular vein	0

Sheep 2 (5 days)

a. Pulmonary vein to pulmonary artery	113
b. Pulmonary artery to pulnary vein	0
c. Portal vein to posterior vena cava	0

Exp. 5: On five consecutive days 2 sheep each received a total of 54,000 cercariae.

Sheep 1

This sheep was bled to death when the schistosomula varied from 4—8 days of age. Perfusion and washing was performed with Earle's saline and heparin. The schistosomula were concentrated by means of the modified Baermann apparatus [10]. Yields of schistosomula were as follows:

A. Perfusion

a. Posterior vena cava to intrahepatic portal vein	29
b. Pulmonary vein to pulmonary artery	339

B. Washing

a. Posterior vena cava from diaphragm to heart	0
b. Right atrium and ventricle	18
c. Left atrium and ventricle	0
d. Thoracic cavity	0
e. Pericardial cavity	0

Sheep 2

The animal was killed, heparin was administered intravenously immediately before the animal was killed. The various organs were perfused and washed as initially explained. The total amount of fluid collected was examined for schistosomula and the yields were as follows:

A. Perfusion

a. Intrahepatic portal vein to posterior vena cava	7

B. Washings

a. Posterior vena cava from liver to heart	9
b. Right atrium and ventricle	3
c. Left atrium and ventricle	0
d. Pulmonary artery	52
e. Pulmonary vein	0
f. Thoracic cavity	0
g. Pericardial cavity	0
h. Anterior vena cava	0
i. Posterior vena cava posterior to the liver	0
j. The entire aorta	0

C. Microscopic observations on the morphology and activity of schistosomula.

Specimens freshly collected from the lungs and placed in Earle's saline were very active. They moved by a combination of rapid wriggling, contraction and expansion of the body. The same movements were observed when adult specimens of *Schistosoma hippopotami* Thurston, 1963 collected from

the posterior vena cava, the right heart and pulmonary vein of hippopotami [12] were placed in saline. By contrast the activity of the schistosomula differs markedly from the adult *S. mattheei* which moves comparatively sluggishly. It was, however, observed during perfusions that adult parasites could very rapidly attach themselves to the interior of a glass canula because of rapid neck and sucker action. The morphology of the schistosomula collected from the lungs (Plate 2) differs from that of those from the liver (Plate 3). The lung forms are elongated, thread-like organisms whereas the liver forms are more flattened in appearance [4]. The specimens collected from the right heart, the pumonary arteries and posterior vena cava were all similar in morphology to the lung forms and all of them had hematin pigment in the gut.

DISCUSSION

Experiments were carried out on the migratory route of schistosomula of *S. mattheei* from the lung to the liver in sheep. It was shown that they were present in the blood stream on the walls of the pulmonary artery, right ventricle and atrium, posterior vena cava and hepatic veins. It seems doubtful whether *S. mattheei* schistosomula migrate via the pleural cavities and diaphragm to the liver in sheep.

The absence of schistosomula in the left heart, pulmonary vein and aorta, while relatively large numbers were consistently encountered in the pulmonary arteries, right heart and posterior vena cava (from heart to liver), indicates that the schistosomula migrate against the blood stream. This is in contradiction to observations with other species, experimenting in laboratory animals [1, 2, 5, 6, 7].

When compared with the description for other species [3, 5, 7] the lung form of schistosomula of *S. mattheei* are more elongated. This observation is in agreement with the results obtained by Lengy [4] for *S. bovis* (Sonsino, 1876).

ACKNOWLEDGEMENTS

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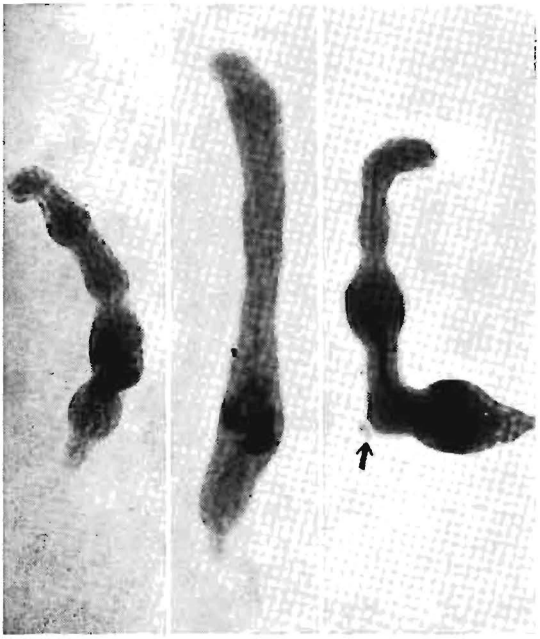

PLATE 2—Lung form of schistosomula showing hematin in gut. Arrow indicates lateral view of evaginated ventral sucker.

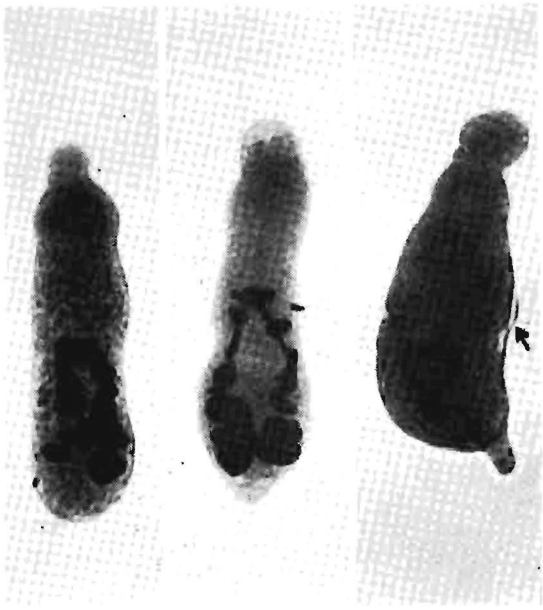

PLATE 3—Liver form of schistosomula showing greater concentration of hematin in gut. Arrow indicates lateral view of invaginated ventral sucker.

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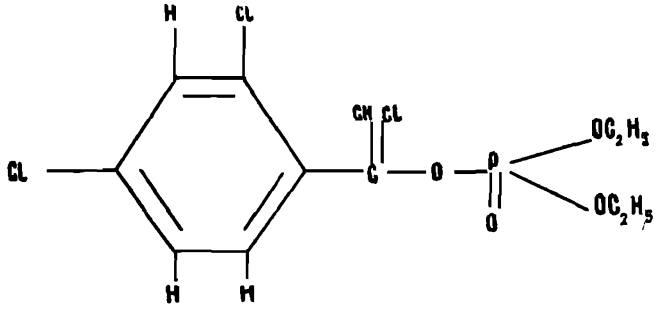

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Jl S. Afr. vet. med. Ass. 40 (1): 47-49 1969

THE DEGREE OF CYSTICERCOSIS INFESTATION OF CATTLE IN TERMS OF STANDARD MEAT INSPECTION PROCEDURES

L. W. VAN DEN HEEVER*

SUMMARY

In a survey at five of the largest abattoirs slaughtering 45% of the total South African cattle kill, 3.6% were found to be infested with *Cysticercus bovis*. On standard secondary examination 87% of these could be detained for freezing by virtue of 10 or fewer cysts having been revealed, 6.5% had 11—20 cysts and 6.3% had >20 cysts. By amending present legislation to allow of cattle with up to 20 cysts being detained, 94% of all infested cattle could be salvaged for food—representing an additional 3400 carcases p.a. at existing national cattle slaughter rates. The advantages and disadvantages of such an amendment are discussed.

INTRODUCTION

Since 1924, South African meat inspection regulations [1] have laid down standard procedures for the examination and evaluation of carcases with cysticercosis. Primary inspection requires two incisions into each masseter and one into each medial masticatory muscle, as well as an incision into each *M. triceps brachii*. The latter is of undoubted significance in detecting cysticerci [2]. Secondary inspection of "measly" carcases involves seven further incisions into the *M. triceps brachii*, one in the heart, one in the tongue, two in the muscular diaphragm, three in the pillars of the diaphragm, three in the fillet muscles and one in the brisket and chuck respectively [1]. Where primary and secondary examinations jointly reveal fewer than 10 cysts in carcase and offal, the carcase may be detained and subjected to freezing at −10°C for 14 days, under supervision of the authority but at the cost of the owner. The carcase as such may show no more than 6 cysts [1].

Why has 10 cysts been accepted as the maximum for light infestations permitting of treatment? Who not 20 or even more? These questions are periodically raised by cattle owners and their agents. Aesthetic considerations are of course important, as freezing renders the cysts non-viable without significantly altering their appearance.

Reference to meat inspection legislation of other countries reveals considerable variation in standards. New Zealand [3] permits no treatment of "measly" carcases. Denmark [4] requires heart, tongue, mastication muscles and diaphragm to be thinly sliced and where less than 10 cysts are thus found, treatment may be carried out, and this seems to be the basis of our South African regulations. Other countries are much less specific: France refers to "discretely" infested carcases which may be frozen; Netherlands [5] permits freezing where "one or only a few" cysts are encountered; Germany permits treatment only of carcases showing up to 1 cyst in areas of exposed muscle the size of the palm of the hand, whereas the U.S.A. [6] and Canada [7] will permit treatment of carcases showing up to 2 cysts on any palm sized area. Memo 3—*Meat* [8], of the United Kingdom, refers to "generalised" and "localised" cystocercosis without further specification, and permits treatment of carcases showing the latter. In Kenya, carcases showing more than 20 cysts are condemned, those with 1-6 cysts being frozen or cooked and those showing 7—20 cysts being passed to approved contractors after freezing or cooking. Rhodesian and Botswanaian regulations are virtually identical with those of South Africa. Only in Botswana, Kenya, Rhodesia, Denmark, the U.S.A. and South Africa are secondary incisions detailed so as to expose a specific muscle area for determining the degree of infestation.

To ascertain whether any practical benefit could be derived by increasing the maximum permissible number of cysticerci from 10 to 20 in carcases detained for treatment,

*Fac. Veterinary Science, Univ. Pretoria, P.O. Onderstepoort.

the Directors of Abattoirs in the five largest centres of the Republic were asked to collaborate and record the incidence of cysticerci in the course of routine and subsequent secondary inspection over a 12-month period. Their findings are recorded.

Table 1: THE INCIDENCE OF C. BOVIS IN CATTLE SLAUGHTERED AT FIVE LARGE ABATTOIRS DURING 12 MONTHS

Abattoir	Number slaughtered	Number infested (%)	No. (and %) of infested carcases showing: 1–10 cysts	11–20 cysts	>20 cysts
Cape Town	146667	1491 (1.02)	1381 (92.62)	21 (1.41)	89 (5.97)
Johannesburg	361671	12784 (3.53)	11468 (89.71)	614 (4.80)	702 (5.49)
Pretoria	100975	9597 (9.5)	7831 (81.60)	962 (10.02)	804 (8.38)
Durban	130703	3383 (2.59)	3049 (90.13)	212 (6.27)	122 (3.61)
Germiston	44044	867 (1.97)	793 (91.46)	24 (2.77)	50 (5.77)
Total + (Mean%)	784060	28122 (3.59)	24522 (87.199)	1833 (6.518)	1767 (6.283)

CONCLUSIONS AND DISCUSSION

1. Standard routine inspection on 784060 cattle slaughtered over a period of 12 months at five of the Republic's largest abattoirs revealed 3.6% with cysticercosis; 87.2% of affected cattle were lightly infested and therefore detained in accordance with existing regulations, the balance of 12.8% being condemned.

2. The incidence of cysticerosis in cattle slaughtered at the various centres ranged from 1.02% in Cape Town to 9.5% in Pretoria. At the largest abattoir (Johannesburg), the incidence was 3.53%; this figure is slightly higher than the national incidence revealed by statistics from 121 abattoirs [9].

2. The incidence of detained carcases appears to be inversely proportional to the incidence of cysticercosis at each centre: Pretoria, with the highest incidence of cysticercosis, detained 81.6%, whilst Cape Town with the lowest incidence detained 92.62% of infected carcases. Over the five centres 87.2% of infested carcases showed fewer than 10 cysts and were detained accordingly.

4. By raising the upper limit for detained carcases from 10 to 20 cysts, a mean of 94.15% of cysticercotic carcases could be made available for use by freezing.

5. These results are based on 44.9% of the 1.75 million cattle slaughtered in South Africa annually [10]. The collaborating abattoirs derive their slaughter stock from most of the Republic and from South West Africa. These results are therefore fairly representative of the whole country.

6. By increasing the upper limit for detained "lightly" infested carcases to 20 cysts, it is estimated that of the 52,500 carcases which are likely to be found infested with *Cysticercus bovis* in South Africa annually, 94% could be released as food after treatment to render the cysticerci non-viable. Such a step would make an estimated additional 3,400 carcases available.

7. At an average cost of 0.65c/lb. for freezing at −10°C for 14 days[11], 3400 carcases of an average carcase weight of 425 lb for all grades [11], could be rendered fit for use as food at the cost of some R9500. At R20.00/100 lb (controlled area auctions) the value of the carcases would, however, amount to some R289000.00.

8. The conversion of condemned carcases to carcase meal and tallow is undertaken at all large abattoirs, the yield being about 25% and 10% respectively. The current market price for carcase meal is R60.00 per ton and 6.25c/lb for tallow [12]. The yield of carcase meal and tallow from 3400 carcases would be R10837.00 and R9031.00 respectively, a total of only R19868.00.

9. This conclusion is not necessarily a recommendation for amendment of the regulations. The aesthetic aspects of an increase in upper limit of the number of cysts in carcases detained for treatment have not been investigated, nor have the possible effects on the supply of carcase material for conversion to protein animal feed meals, such as carcase meal, in abattoir rendering plants, been considered. An increase in carcase

freezing facilities at abattoirs would also be necessary, with a concomitant reduction in the need for by-products plant.

10. There exists a general feeling that a decrease in standards of inspection and evaluation would offend the public and that it would also be a deterrent to any efforts being made to effect at least effective control, if not total eradication of cysticercosis. The apparent increase in the incidence of cysticercosis in South Africa would support this contention inasmuch as even the present financial penalties imposed on the owners of "measly" livestock are not adequate to encourage countrywide eradication programmes.

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THE INFLUENCE OF VARIOUS TRANQUILLIZING AGENTS ON THE BODY TEMPERATURE OF SHEEP AT HIGH AND LOW AMBIENT TEMPERATURES

J. F. W. GROSSKOPF*, N. FAIRALL** AND D. VISSER**

SUMMARY

Various tranquillizers are being used in combination with neuroleptic and narcotic drugs for the immobilization of wild animals. Under tropical conditions a number of the so incapacitated animals often die showing severe hyperthermia before death. A number of these tranquillizing drugs viz. Chlorpromazine, Acetylpromazine, Triflupromazine, N-(3'-dimethylaminopropyl)-3-propionylphenothiazine, Haloperidol, Fluoperidol and Fluanizone was therefore tested for their effect on the body temperature of Merino sheep kept under different ambient temperatures.

It was found that administration of all these drugs caused hyperthermia in sheep at high ambient temperatures (over 95°F) and hypothermia at low ambient temperatures (less than 50°F). The greatest increase in temperature found in an individual sheep was 4°F above that of the untreated sheep (ambient temperature 100°F). Graphs are presented to illustrate the results.

Although some drugs gave a greater response than others at the high ambient temperatures it was not possible to draw any comparisons between them on these few results alone. No attempt was made to find an explanation for the thermic effects of tranquillizers.

INTRODUCTION

Among the wide variety of applications of tranquillizing agents in human and veterinary medicine is their use in the immobilization and capture of wild animals in combination with neuroleptic drugs such as oripavine hydrochloride (M-99) [1-4].

In hot climates, such as that prevailing in the Kruger National Park during the summer months, it has been experienced that captured animals under influence of these drugs, often die after showing hyperthermia [5-7]. Hanks described similar experiences in capturing waterbuck in East Africa and ascribed the heat stroke to the action of acetylpromazine [8]. In this country too the tranquillizers were suspected of causing this phenomenon.

It has been known that some substances such as 2, 4-dinitrophenol [9], ergotoxine [10] and naphazoline [11] produce hypothermia at low environmental temperatures and hyperthermia at higher temperatures. One of the tranquillizers, chlorpromazine, is known for its effect of lowering body temperature [9] and it has also been reported to inhibit defence reactions to heat [12]. Marsboom and Mortelmans [13] considered the phenothiazine derivatives to have a more powerful hypothermic action than the butyrophenone drugs. An example of the hypothermic action of chlorpromazine is reported by Bligh and Harthoorn [14]. A young captured giraffe treated with 1g of chlorpromazine intramuscularly before the implantation of thermistor probes for continuous radiotelemetric thermometry, showed a marked drop in deep body temperature during the first night. The lowest body temperature was reached approximately 12 hours after administration of the drug and it took another 12 hours to return to normal.

In the accompanying leaflet of only one of these drugs was mention made of possible hyperthermia after treatment while the majority of indications referred to hypothermia as one of the possible actions of the particular drugs.

It was therefore decided to investigate the problem of hyperthermia presumably caused by the administration of tranquillizers

*Dept. of Animal Science and Physiology, University of Pretoria.
**Post-graduate students, Dept. of Animal Science and Physiology, University of Pretoria.

and simultaneously to compare some of the tranquillizing agents used in the Kruger National Park, for their thermic reactions.

MATERIALS AND METHODS

Because antelopes are normally too excitable for temperature recordings and are not easily obtained, merino sheep were used as experimental animals and ten wethers and ten ewes of about the same weight and all carrying about 40—50 mm of wool were selected. As long woolled Merino sheep are rather resistant to changes in environmental temperature, it was thought that the use of such animals would provide a good test of the effects of the drugs.

The sheep were numbered and divided into two similar groups of ten each, hereafter called groups I and II.

For the treatments at high environmental temperatures all the animals were kept together in a ventilated thermostatically controlled heated room. A mixture of teff and lucerne hay was provided and water at the same temperature as that of the room was always available. The room temperature and relative humidity were continuously recorded by two thermographs placed 18 inches above the floor. Unfortunately the temperature of the room could not be maintained at the same level for all the trials due to low atmospheric temperatures on some days. Nevertheless all trials were conducted at high environmental temperatures varying from 95°F to 104°F. Relative humidity normally fluctuated between 50 and 65 per cent.

The body temperatures of the sheep were taken rectally approximately every 30 minutes over the first two or three hours and thereafter at longer intervals. Ordinary rectal clinical thermometers were used and held in position for exactly one minute. Care was taken to disturb the sheep as little as possible.

The normal routine was to put the sheep into the heated room the morning before the experiment. The next morning their temperatures were recorded in rotation and the experimental group injected with the drug. Groups I and II were alternatively used as experimental group and control group. Apart from the administration of the drug, both groups were treated identically.

As no suitable cold room was available, the treatments under low ambient temperatures had to be done at night in open pens. Unfortunately the coldest nights were not always selected and inconsistent results were therefore obtained. Otherwise the proceedings were similar to those in the warm room.

The tranquillizers and their dosages used, were as follows:—

1. *Chlorpromazine* (Largactil, Maybaker): 2.2 mg/Kg bodyweight intravenously.
2. *Triflupromazine hydrochloride* (Siquil, Squibb): 0.12 mg/Kg bodyweight intravenously.
3. *N-(3'-dimethylaminopropyl)-3-propionyl-phenothiazine* (hereafter called D-PP Phenothiazine) (Combelen, Bayer): 0.25 mg/Kg bodyweight intravenously.
4. *Haloperidol* (Serenace, Searle): 0.5 mg/Kg bodyweight intravenously.
5. *Acetylpromazine (Boots):* 0.12 mg, 0.25 mg and 0.45 mg/Kg bodyweight intramuscularly.
6. *Fluoperidol* (Azeperone or R1929, Janssen): 0.8 mg to 1.0 mg/Kg bodyweight intravenously.
7. *Fluanizone* (R2028, Janssen): 0.12 mg/Kg bodyweight intramuscularly.

Dosages were based on the instructions of the manufacturers. In the case of Acetylpromazine these were also exceeded.

RESULTS

Rectal temperatures were recorded in degrees F. The temperature of each sheep just prior to administration of the drug was regarded as the normal under the particular conditions. The mean deviations from this normal after administration of the drug were plotted graphically and can be seen as Figures 1 to 15. The ranges of temperature deviations of the individual sheep in the group are also indicated on these graphs.

Figs. 1 to 9: Changes in the rectal temperature of sheep injected with different tranquillizing agents and kept in a hot environment, as compared to untreated sheep in the same environment.

FIG. 1 ACETYLPROMAZINE (0·12 mg/Kilo)

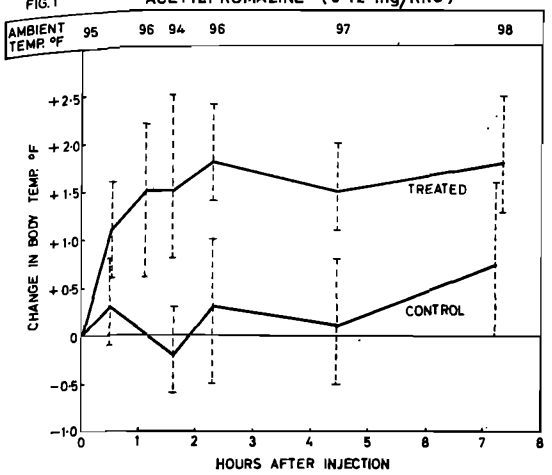


FIG. 2 CHLORPROMAZINE (2·2mg/Kilo)

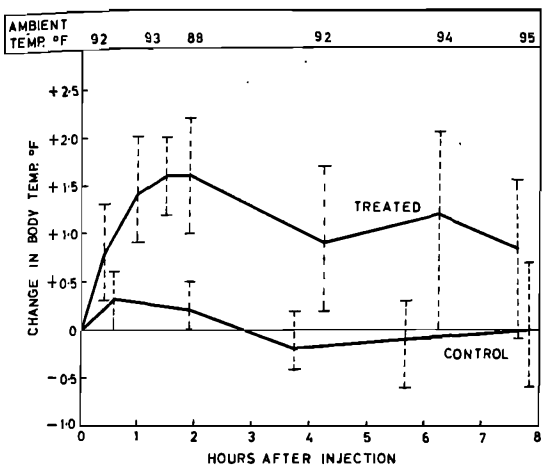


FIG. 3 D-P-P-PHENOTHIAZINE (0·25mg/Kilo)

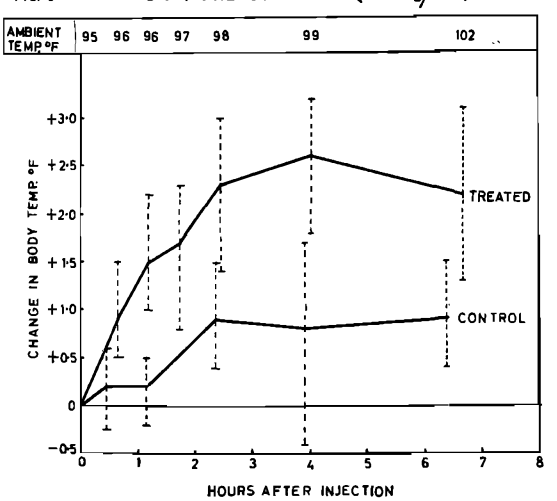


FIG. 4 FLUANIZONE (0·12mg/Kilo)

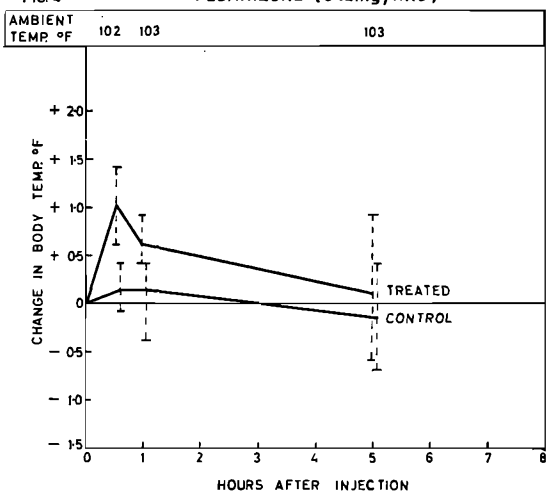


FIG. 5 HALOPERIDOL (0·5 mg/Kilo)

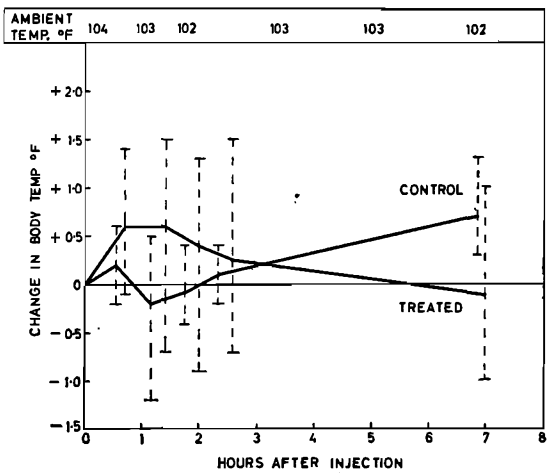
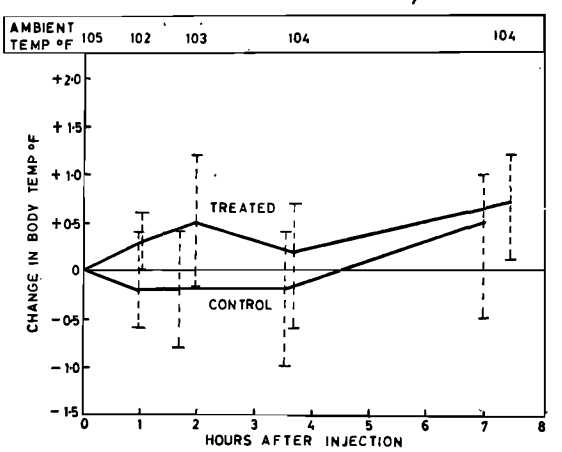


FIG. 6 TRIFLUPROMAZINE (1·2 mg/Kilo)



Figs. 10 to 13: Changes in the rectal temperature of sheep injected with different tranquillizing agents and kept in a cold environment, as compared to untreated sheep in the same environment.

FIG. 7 FLUANIZONE (0·11mg/Kilo)

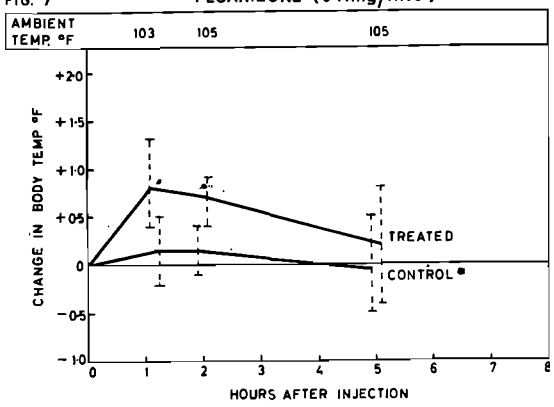


FIG. 8 ACETYLPROMAZINE (0·5mg/Kilo)

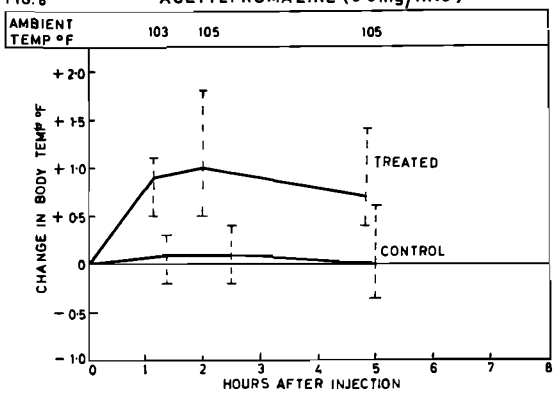


FIG. 9 FLUOPERIDOL (0·8mg/Kilo)

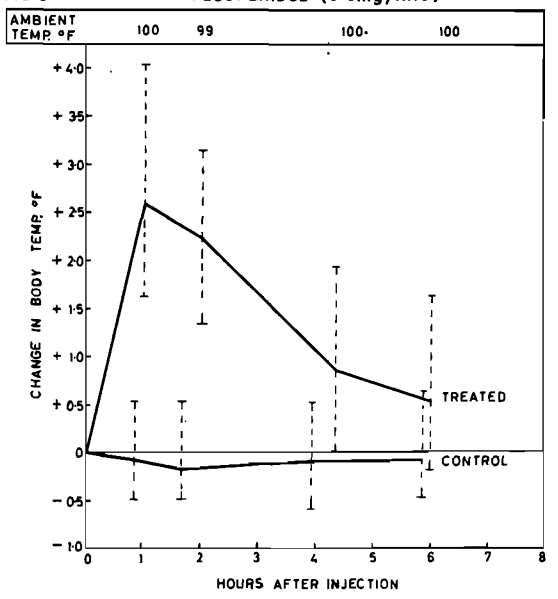


FIG. 10 ACETYLPROMAZINE (0·22mg/Kilo)

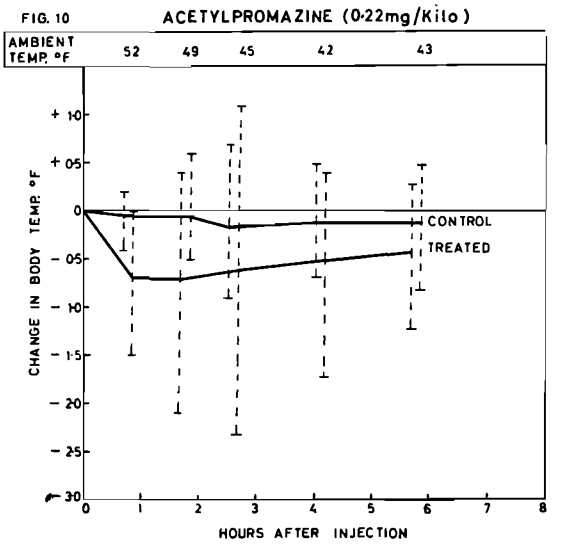


FIG. 11 CHLORPROMAZINE (2·2mg/Kilo)

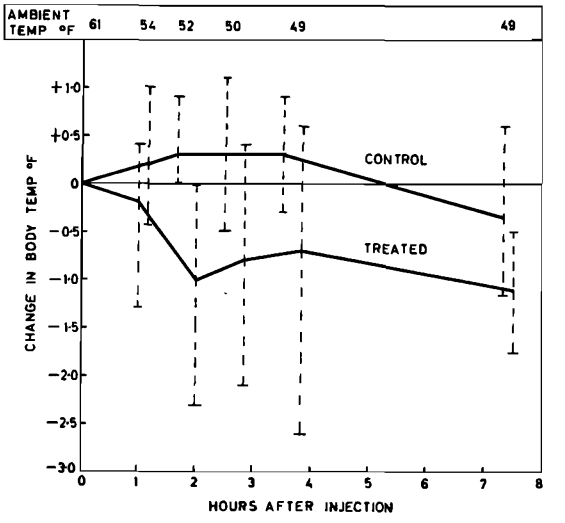


FIG. 12 D-P-P-PHENOTHIAZINE (0·25 mg/Kilo)

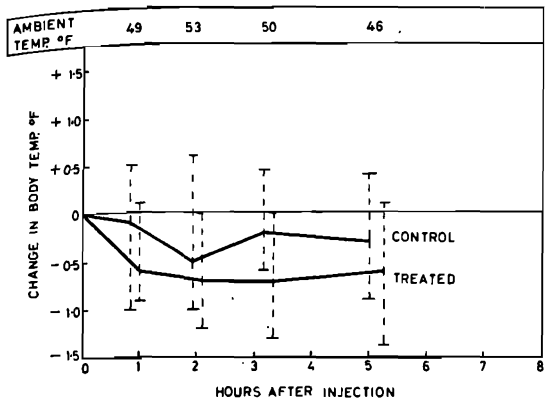


FIG. 13 HALOPERIDOL (0·5mg/Kilo)

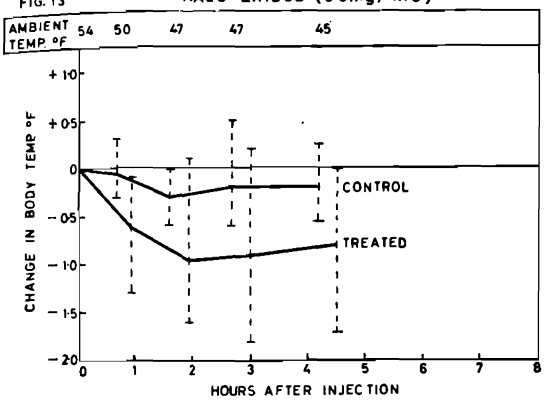
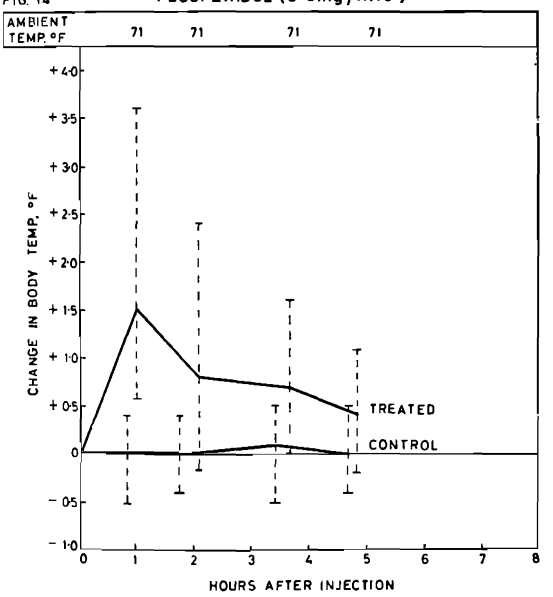
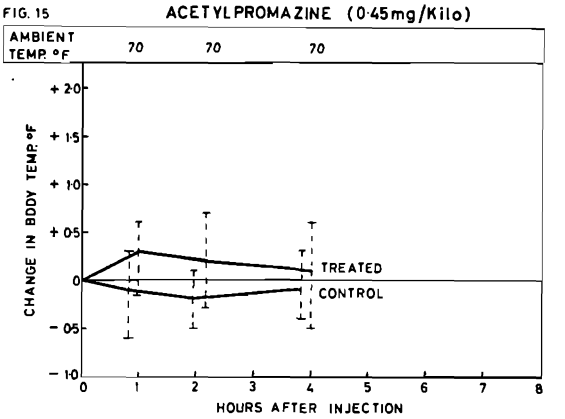


FIG. 14 FLUOPERIDOL (0·8mg/Kilo)



Figs. 14 and 15: Changes in the rectal temperature of sheep injected with Fluoperidol or Acetylpromazine and kept in a moderate environment, as compared to untreated sheep in the same environment.

FIG. 15 ACETYLPROMAZINE (0·45mg/Kilo)



As can be seen from the graphs the temperatures of the sheep treated with all the tranquillizing agents rose above those of the control sheep, when they were maintained at high ambient temperatures (above 90°F.). When kept in a colder climate (less than 50°F) the rectal temperatures of the treated sheep dropped lower than those of the untreated animals. At moderate temperatures (approximately 70°F) the rise in temperature caused by Acetylpromazine was less dramatic than at the higher temperatures but the Fluoperidol was still responsible for a marked increase.

The temperature reactions obtained were not consistent for any particular drug. A higher dosage level of acetylpromazine for instance, resulted in a lower temperature reaction than a smaller dose. The temperature reaction also bore no relation to the degree of tranquillization obtained.

The administration of Fluoperidol at a level of 1 mg per kilogram bodyweight caused a reaction in sheep which lasted a few minutes after injection. They showed muscular tremors, shaking of the head, chewing movements, myopia, incoordination of the limbs and retarded respiration. Approximately thirty minutes later they seemed to have an exceptional appetite, consuming anything in the line of straw or hay eagerly. The only available literature on Fluoperidol recommended 4 to 5 mg of the drug per kilo-

gram bodyweight for pigs. Sheep may be more susceptible to its action.

The treated sheep in which the rectal temperatures rose the highest showed signs of distress at times, trying to find a cooler spot and panting with open mouths. None of the control sheep ever showed any signs of discomfort in the warm room but they did pant rapidly.

CONCLUSIONS

All the tranquillizng agents tried in these experiments had an influence on the control of body temperature of sheep. At high ambient temperatures the body temperatures of the treated animals rose significantly higher than those of the untreated sheep while at low ambient temperatures the body temperatures of the treated animals dropped to a lower level than those of the control group. At a moderate ambient temperature (70°F) the effect of the drugs seemed to be less pronounced.

Because ambient temperatures could not be standardized and the dosage levels of the drugs were varied, no comparisons could be drawn between the actions of the different tranquillizers.

As a result of these findings the common assumption that a lower metabolism during tranquillisation could be responsible for the hypothermic state cannot be accepted. It is suggested that the disrupted temperature control is either caused by the anti-adrenaline action of the phenothiazine derivatives [15] or that the temperature control centre is, as are other hypothalamic nuclei, influenced by the drug.

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THE CONTROL OF SIMULIIDAE IN THE VAALHARTS IRRIGATION COMPLEX

C. J. HOWELL* AND G. W. HOLMES**

SUMMARY

During the past 15 years the population of cattlebiting midges (Simuliidae: Diptera) in the Vaalharts area has grown to pest proportions. Grazing has become progressively worse owing to droughts between 1961 and 1966 which has led to large numbers of stock being kept on riparian farms. Two different control methods based on the introduction of insecticides into the rivers and canal system are described. The results have proved that control of *Simuliidae* by these methods can be achieved easily but that rapid reinfestation occurs with increased flow rate of the water after heavy rains. Diapause eggs will have to be considered in future control measures envisaged.

INTRODUCTION

The family Simuliidae (Diptera: Nematocera) contains numerous species including some important pests of man and beasts. These biting midges with their characteristic well developed thorax are commonly known as "blackflies," "buffalo gnats" or "riviermuggies" in South Africa. *Simulium* midges occur in most parts of the world where suitable conditions exist for their aquatic immature stages to inhabit streams and rivers. They are present in many South African streams but only in certain areas, where the flow has been stabilised by water conservation schemes, have their numbers built up gradually over a period of years to proportions where control has to be considered.

BIOLOGY

The disperion of *Simulium* flies from their breeding habitat is affected chiefly by the surrounding vegetation, the availability of hosts and prevailing climatic conditions. The ideal breeding habitat of most species of economic importance is generally swift turbulent water well laden with small organic particles and rushing over a permanent rocky bottom. The males subsist on plant sap while the females of most species also require the blood of vertebrates which is essential for maturation of the ovaries.

Male midges seldom venture from the breeding area where they are often seen hovering in swarms, but the females are known to fly as far as ten miles from the river in search of a blood meal before returning to deposit their eggs.

The elongate ellipsoidal eggs are laid on stones or foliage in at or just below the surface of the water. Myriads of females deposit in the same place forming conspicuous egg patches. When first laid the eggs are enveloped in a yellowish white slime which gradually becomes darker and harder. Normally only a certain percentage of eggs laid by one female hatch within a week, the remainder emerging in batches over a period of several weeks.

The small larva (5—11 mm long in the final sixth moult) has a characteristic club-like shape with two large cephalic fans on the head and a disc-like sucker provided with small hooklets on the much stouter posterior end of the body. A median proleg acts as an anterior sucker so that the larva is capable of considerable movement by alternate attachment of these two organs reminiscent of the motion of "measuring worms."

The larvae attach themselves by imbedding the hooklets of the posterior sucker into a silken pad deposited on the substrate, allowing them to hang passively in swift flowing water. Organic material is filtered from the oustretched cephalic fans, which are cleaned by the mouth parts from time to time.

*Department of Entomology, Veterinary Research Institute, Onderstepoort.
**Entomologist, Klipfontein Organic Products.

The larvae congregrate on the downstream surface of the substrate where the current is a its strongest, but considerable migration takes place under certain conditions.

If the larvae are disturbed, they will deposit a drop of gumlike saliva on the substrate and allow themselves to be carried downstream by the current, the saliva forming a silken thread which serves as an anchoring line. Larvae can find their way back to their original position by moving up the thread. Pupation takes place in the larval habitat, preference being shown for more sheltered areas.

The final sixth stage larvae spin a silken pocket-like cocoon through the opening of which a pair of branched respiratory filaments project and wave about freely in the water. During pupal life expired air from the spiracles of the developing adult is trapped withinn the pupal skin as a bubble in which the adult fly rises to the surface from the pupal case.

The duration of the aquatic life cycle varies under different climatic conditions and from species to species. Below the Vaalharts diversion weir it appears that *Simulium chutteri* Lewis, 1965 requires approximately 14 days in summer to develop from egg to sixth stage larva. In the Vaal river near Parys *Simulium damnosum* Theobald, 1903 shows a peak in the number of larvae every 14 days, suggesting that this is the time required for a complete life cycle[1]. Both species continue to breed through the winter in the Vaalharts area but it is not know to what extent diapause eggs play a role during the cold months.

DESCRIPTION OF THE VAALHARTS AREA

The water stored by the Vaalharts diversion weir, six miles above Warrenton, stretches for a distance of 25 miles to a point half a mile above the Christiana road bridge. Below the weir the water flows for a hundred miles over an irregular rock bottom dropping 556 feet in elevation in two major steps before being joined by the Harts river a few miles below Delportshoop. The first rapid fall of 243 feet in elevation occurs in the 28 miles between the weir and Windsorton (Fig. 1 A to E) and the second of 244 feet in the 24 miles between Barkly West and Delportshoop (Fig. 1 H to J). In both areas numerous rapids are formed which may be permanent or semi-permanent, many being caused by the coffer dams built by alluvial diamond prospectors. These all provide ideal stony runs for the filter-feeding larvae of *Simulium* and assist in keeping the fine organic food particles in suspension.

The Harts is a small river which runs almost parallel to the Vaal. Below the level of the Vaalharts weir the distance between the two rivers varies from 16 to 24 miles. The drop in elevation is less, and hence the rate of flow much slower, than that of the Vaal, and along the length of the river, which collects all the seepage water of the irrigation farms in the Jan Kempdorp and Hartswater areas, the substrate varies from stone to hard clay.

Between the Vaal and Harts rivers there are hundreds of miles of concrete lined canals. The main canal below the Vaalharts diversion weir has a maximum capacity of 900 cusecs of which 65 cusecs flow down the Klipdam canal supplying the Barkly West/Delportshoop areas, 45 cusecs down the West canal and the remainder to the irrigation holdings in the Jan Kempdorp and Hartswater areas.

Along the banks of both the Vaal and Harts rivers an area of dense bush exists which thins out to fairly open thorn veld tending to be very rocky in parts, but which has a moderate grass coverage along the Vaal above Windsorton. Below Barkly West and along the Harts river the veld is sparse and rocky. The limited livestock supported by this sparse veld reduces the *Simulium* population by limiting the available food supply. This is most probably the reason why *Simulium* has not reached pest level in this area as it has below the weir. South of the Vaal river the thorn veld soon gives way to open grass veld, limiting the spread of flies due to the lack of shade and vegetation.

THE VAALHARTS PROBLEM 1963

Periodic outbreaks of *Simulium* along the Vaal river, affecting in particular horses, are remembered by the older members of the farming community. Since the competition of the Vaalharts diversion weir approximately 35 years ago, a constant flow of not less than 200 cusecs, being the requirements of Kimberley, Barkly West, etc., has been assured. This stabilisation of flow along the 40 mile

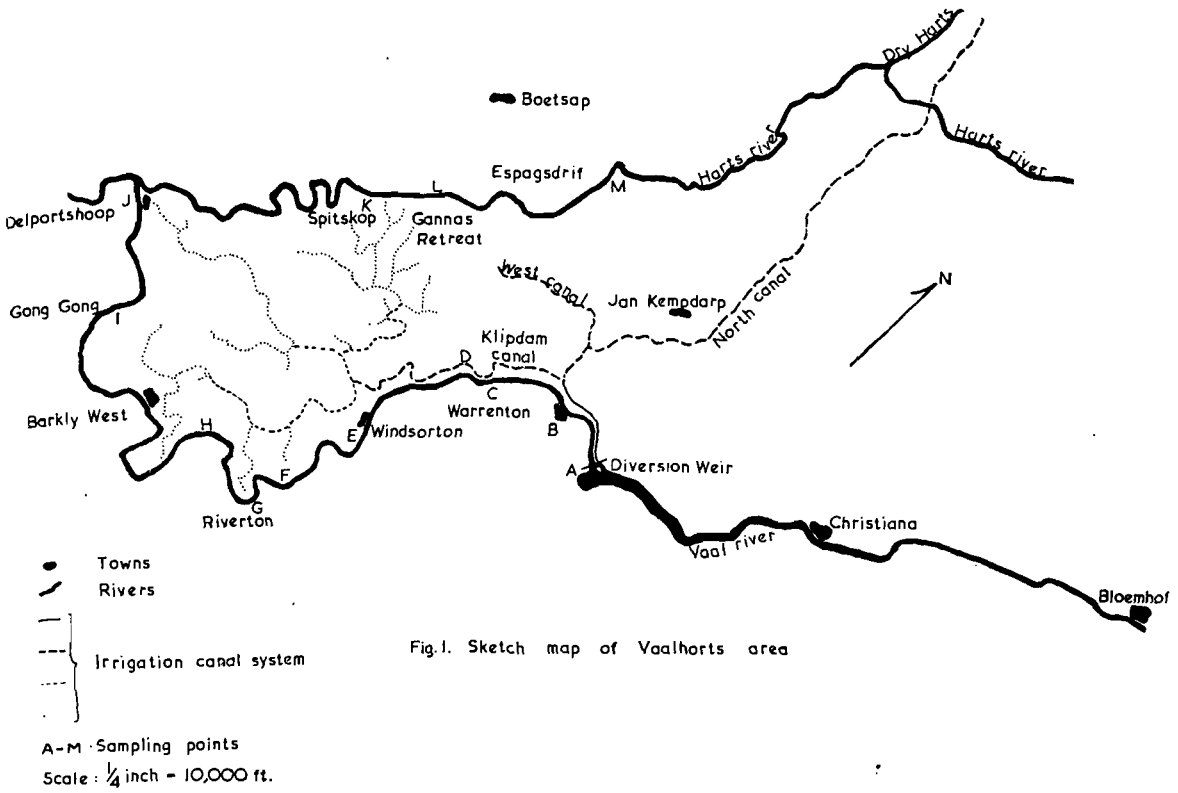

Fig. 1. Sketch map of Vaalharts area

stretch of swift flowing turbulent water has allowed *Simulium* populations to build up unhampered by periodic droughts for at least the past decade.

A survey of the area between the diversion weir and Windsorton, conducted by this Institute, indicated the necessity for some measure of control in the near future. A hydrobiological evaluation of the area by the National Institute for Water Research (N.I.R.W.) confirmed the fact that biological control was of a very low order [2]. The physical condition of the river bottom was not suitable for predacious hydropsychid Trichoptera to establish a population which would have a controlling effect on the larvae of *Simulium*. These conditions were again mainly due to the alluvial diamond digging operations in existence since the turn of the century, which have left the river bottom devoid of sand and small stones required by the *Trichoptera* in the construction of their cases and mats.

Chemical analysis of the water by the National Institute for Water Research showed a high nitrate value at all the sampling points below the diversion weir, suggesting a plentiful supply of suspended organic material [2]. As no significant source of enrichment could be found below the weir, and the absence of high populations of *Simulium* in the river above Christiana in spite of an ecologically suitable environment it seems possible that the 25 mile stretch of water trapped above the weir provides the necessary enrichment.

This abundance of suspended organic material, together with the suitable stony runs, the high current speed and constancy of flow, the availability of suitable host animals along the river banks, and the suitable vegetative covering extending from the northern and western banks, have resulted in the build up of large populations of *Simulium*.

During 1963, livestock on the riparian farms below the weir was concentrated on the grazing along the river banks due to the prevailing drought, which by then had been in existence for about two years. Due to

mass attacks by *Simulium*, cattle and sheep sought protection by lying down in the thickest vegetation during the day instead of grazing, thereby losing condition on what was considered to be reasonable grazing.

The Simuliidae attack cattle and sheep and show a particular preference for horses, but no vertebrate is free from their attentions. Certain *Simulium* spp. attack man. Several are known to transmit human and animal onchocerciasis and although the disease does not occur in the area in question, the possibility of its introduction cannot be ignored.

In the Warrenton area the midges are a pest mainly because of the irritation they cause. They attack stock where the skin is thinnest or fairly hairless, and their bites do a lot of damage to ears, udders, genitalia, etc. The bite is painful and causes a pustular swelling which may persist for week. Ciurea and Dinulescu [3] record that in 1923 in Roumenia 16,747 domestic animals died as the result of the attack of *Simulium reptans columbaczense* Schiner. These deaths could not be attributed to any pathogenic organism, but the rapidity of the afebrile reaction and the fact that the flies were present in hordes seem to suggest some form of toxicosis.

In April 1963 the Warrenton Agricultural Society approached this Institute for assistance. A visit to the area by officials of the Department of Entomology, Onderstepoort revealed the abundance of these tiny biting midges, which appeared to be confined chiefly to the west bank of the Vaal river between Warrenton and Windsorton. The midges occurred in countless thousands and were observed in clouds over and around herds of cattle. In Warrenton itself human beings were frequently driven indoors in the late afternoon due to hordes of midges which descended upon them and caused extremely painful bites [4].

Control of the problem by the application of an insecticide into the water every 14 days for five successive treatments to eradicate the larval stages was suggested by the Department of Entomology following up a discussion of the problem with officials of the Natoional Institute for Water Research. Control measures would devolve upon the Veterinary Division, while the National Institute for Water Research would undertake the investigation of the water biology so that accurate assessment of the control programme could be obtained [5]. Arrangements were made for a team of research officers from the National Institute for Water Research to survey the problem area before and after the 18th October, 1963 on which day application of DDT at a concentration of 0.14 parts per million was to be undertaken. The introduction was to be made above the sluice at the diversion weir, through an apparatus which would provide a constant flow of insecticide for a 30 minute period. However, permission to continue with the programme could not be obtained and the project was suspended.

THE CONTROL PROGRAMME 1965—1966

The control programme was initiated in October 1965 in answer to persistent agitation by the Warrenton farming community whose position had been aggravated by the prevail ing drought which forced a concentration of cattle near the river.

The control of Simuliidae by the use of insecticides is not new, and has been found to be effective in many parts of the world. In South Africa control of the midges has only recently become necessary and a thorough scrutiny of literature on the subject indicated that insecticides could be used with safety provided certain concentrations were not exceeded.

The chlorinated hydrocarbon DDT is the insecticide most frequently used and was described by Brown [6], for the control of *Simulium* larvae, as "the perfect weapon for the perfect target." The insecticide is adsorbed onto clay particles in the water and the *Simulium* larvae being filter-feeders, ingest these particles with the adherent insecticide. This allows a very slow dosage rate to be used, as the result is not dependent on external contact with the insecticide, but on the fact that it is ingesed cumulatively.

It is evident that no insecticide available is specific for *Simulium* only, and that aquatic invertebrates other than Simulium are affected by the treatment to a greater or lesser degree. Because of its stability, the use of DDT suggests the possibility that persistent treatment of streams over a period of years may result in a cumulative effect. This could adversely affect stream arthropod populations, especially those forms playing a part

in biological control of the midge larvae. Jamnback and Eabry[7] found little overall change in the arthropod population after nine or more years of treatment of eleven streams involving two or more treatments annually provided the concentration used remained within prescribed levels.

The control programme in November 1965 was planned to comprise five fortnightly applications of DDT at 0.15 parts per million for a period of 30 minutes each, through an apparatus suspended above the sluice gate at the diversion weir. The apparatus consisted of mixing drums connected to a centrifugal pump feeding into a header tank to obtain a constant flow during the 30 minutes of introduction. From the header tank, a pipe fed into a perforated pipe system spanning the width of the sluice which discharged the insecticide into the water rushing through the sluice.

Before introduction of the insecticide on 18th November, 1965, sampling points were selected in the area between the diversion weir and Windsorton (fig. 1, A, B, C, D. and E) and the relative population density estimated to observe the effect of the insecticide. For the purpose of estimating larval population numbers, a crude but effective method was employed at the different sampling points. Ten submerged stones were removed at each sampling point and on each of these an area five centimeters in diameter showing the greatest density was selected and all larvae counted. The average larval count of ten samples was taken as the larval population density at the particular sampling point. The presence of all six larval stages as well as pupae was a constant finding before control measures were employed. Other invertebrate fauna were present, but not in very great numbers.

On the 18th November, 1965 the first introduction of DDT into the river was completed and biological and chemical samples taken to determine the result. The larval population density counts taken up to 36 and 48 hours after exposure were very significant and clearly indicated the effect of the insecticide. (Chemical samples taken at various points were discarded in subsequent operations as the quantity of DDT in the water was too low to be determined with reasonable accuracy).

The result of this first introduction was very promising, consisting of a total reduction of the larval population of 97.7% after 24 hours. The most distant sampling point (18 miles—fig. 1 D) gave a mortality figure of only 79.4% after 32 hours and it was concluded that the flow rate of 1300 cusecs was barely sufficient to transport the insecticide over that distance.

The second application on 30th November, 1965 was also carried out at a flow rate of 1300 cusecs and although the pre-treatment figure of the larval population again showed an increase, overall post-treatment mortality was 99.5%. The most distant sampling point gave a figure of 98.5% after 48 hours. In an attempt to improve its effectivity, a similar formulation of DDT with the specific gravity adjusted from 1.004 to 0.9996 was prepared for the third application, as the small focus left at the most distant sampling point after each treatment could upset the whole programme. Unfortunately the prevailing drought had by now become very serious, and by the 14th of December, 1965, the flow rate had dropped to 180 cusecs. As this was much too low for our purpose, it was arranged to allow a flow of 1300 cusecs for our experiment for the 30 minutes period of introduction only.

The result was extremely disappointing, as the insecticide gave only 68.5% reduction of larvae at the second sampling point (5.5 miles—fig. 1 B) and no reduction at any of to E). The biological findings indicated that the more distant sampling points (fig. 1 C almost one third of the DDT had already disappeared after 5.5 miles, whilst at the most distant point, 18 miles away, larval counts actually increased during the 48 hours following introduction. Continuation of the programme at this rate could, therefore, only result in the major portion of the DDT settling out on the bottom of the river over the first ten miles where it could have no effect on the Simuliidae, and might only upset the entire arthropod population. It became clear that as long as the flow rate was the factor on which the success or failure of the campaign depended, no useful purpose could be served by continuing the introduction of insecticide into the water. The operation was postponed at this stage pending more favourable conditions as it was obvious that the effect of the lower specific gravity formulation employed could not be evaluated on this application as conditions were totally different.

Good rains in January, 1966 relieved the drought in many parts of the country and caused a sudden increase in the river flow. Immediately the *Simulium* population started to increase, and the two species, *S. damnosum* and *S. chutteri,* initially appeared to be present in more or less equal numbers. By the end of February the simuliid population had risen to the previous high levels, but it was seen that *S. chutteri* dominated the breeding sites, whereas *S. damnosum* represented only about five to ten per cent of the larval population.

The flow rate of the river soon subsided to between 200 and 300 cusecs, a volume totally inadequate for transportation of the insecticide. Introduction at various points along the river was considered, but the topography of the area is of such a nature that this would be physically impossible.

Aaerial application then seemed to be the only practical method of introducing the insecticide into the infested water as this would ensure equal distribution of the material over the area. An additional advantage to be gained would be the treatment of the marginal vegetation which would result in a marked reduction in numbers of male and female simuliids sheltering in the plant growth at the river's edge. By March the *Simulium* population had built up to their previous level and the first aerial application of the new series was made on the 31st of March at the rate of 0.28 lb DDT per acre (0.1 parts per million) over the 28 mile stretch of river below the weir.

This resulted in a 100% mortality over the whole treated area after 8½ hours, while a general reduction in adult population along the river was noted. Numbers of small bottom feeding fish, *Labeo capensis* (A. Smith) Boulenger (Cyprinidae), were seen dead or moribund after this first aerial application, their mortality probably being due ot inges tion of large numbers of dead larvae settling on the bottom. To reduce the risk of further fish mortality, the following four applications were made at 0.23 lb DDT per acre (0.075 parts per million), with perfectly satisfactory results. After the third application no larvae could be found anywhere in the treated area. The Harts river, the main canal and the Klipdam canal were included in the fourth and final application after *Simulium* were found breeding in this river and on stones in the canals. It appeared as if almost complete control had been achieved after the final application in May, and a survey of the whole area in conjunction with the National Institute for Water Research was planned for the following year.

SURVEYOF THE VAALHARTS COMPLEX 1967

Between January and June, 1967 the area was examined extensively by a team from the Institute, as well as by officials of the National Institute for Water Research, and a draft programme of research in which both Institutes would take part was drawn up. The field work unfortunately was frequently interrupted and had to be postponed several times due to flood conditions prevailing after the heavy rains which started in January.

Apart from the two species, *S. damnosum* and *S. chutteri,* which were always found in large numbers, *Simulium adersi* Pomeroy, 1922 and *Simulium mcmahoni* de Meillon, 1940 were also recorded in the Vaal river. The presence of immature *S. chutteri* and other species on the floor of the canals reiceiving permanent water, as well as their presence on the carapaces of crabs in the canal, was recorded for the first time, while the two species *Simulium griseicolle* Becker, 1903 and *Simulium ruficorne* Macquart, 1838 were found infesting the Harts river and a small flowing stream on the farm "Stonehill" adjacent to the Vaal river. This stream had its origin from a leak in the canal above the farm.

The distribution of the adults of the important species appears to be dependent on the shelter provided by the shrubs and trees. Appreciable numbers of flies could be seen up to 10 to 15 miles from their breeding sites on the north bank, where the fairly dense bushveld coverage was extensive, while the area south of the Vaal river, being devoid of trees and shrubs, was completely free of the midges, and farmers in the grassveld area had never seen them.

Examination of the Vaal river was extended as far as Delportshoop. In spite of restricted sampling due to the flood conditions, well established breeding was present from the diversion weir to Delportshoop. Furthermore, the canal system was also found to be heavily infested. Infestation of the Harts river was less severe because the stream is slower and the flow less permanent.

Botha, Hoffman and Eksteen [8], in their interim report, concluded that re-establish-

ment of breeding in the Vaal river in the area under consideration could have been due to entry from outside the DDT treated area by flying adults, or due to eggs and larvae which survived the treatment. They also found that physical conditions did not appear to favour the development of a predacious fauna that would itself control the *Simulium* population and suggested that flow manipulation as a means of control should be investigated.

During the course of examination of the canal floors, during a two-week maintenance period when the canals were closed, it was found that *Simulium* larvae could survive in shallow standing water for 70 hours or more and recover quickly on being placed in flowing water. This, together with the fact that *Simulium* larvae can survive for a couple of days in the moist mud under stones, appears to exclude such treatment as a control method..

The fairly high *Simulium* population found in the winter of 1966 suggested that an outbreak of some magnitude was imminent in the spring, and as no alternative to applying insecticide to the river had been discovered, the screening of 19 different insecticides to find a more specific, less stable larvicide was undertaken at Warenton. In these tests, the different insecticides were introduced into plastic-lined troughs in which the larvae were placed. River water was pumped through the apparatus and the larvae readily attached to small stones put into the troughs prior to being exposed to he drug. The plasic material lining the troughs was discarded after each test to avoid contamination by a previously used insecticide.

During these tests it was observed that control larvae left overnight on the dry plastic liners and exposed to the cold, were easily revived by being transferred to running water the following day. It is not yet known for how long a period these larvae can survive this type of treatment but it affords a very definite indication that they have some form of protective mechanism which enables them to bridge adverse conditions.

THE CONTROL PROGRAMME 1967—1968

Experience gained during the first control programme clearly indicated that a flow rate of less than 1500 cusecs was not sufficient to convey the insecticide applied at a single point for the required distance for treatment of the Vaal river between the diversion weir and Windsorton.

Aerial application of the material was commenced on 19th September, 1967 at a calculated rate of 0.075 parts DDT per million based on a flow rate of 300 cusecs, but this proved to be only 0.05 parts per million as the flow was found to be 500 cusecs instead of the 300 cusecs as stated. The *Simulium* fly population was so heavy that the leading edges of the aircraft wings and the windscreen were coated with a grey layer of dead flies, the red gut contents of engorged females being very evident on the white wings of the plane after each run.

The treatment of the Vaal river with insecticide extended from the weir to Windsorton (fig. 1 A to E) at the first application. The Harts river was sprayed for a distance of about 60 miles from a point north of Espagsdrif to beyond Spitskop (fig. 1 M to K) and the canal system was treated simultaneously by the introduction of 0.1 parts per million DDT into the main canal at the weir for a two-hour period.

A survey of the sprayed area 14 days later disclosed the rather unexpected result that not a single larva could be found anywhere at any sampling point along the 26 mile section of the Vaal river. This sudden apparent cessation of breeding following upon high breeding activity all winter and which occurred after only one application of insecticides was remarkable. To ensure that complete cessation of breeding had in fact taken place, a second application was performed in the Vaal river including the following 12 miles below Windsorton as far as the farm "Morrisdraai" (fig. 1 A to F3). A survey of the treated area the following day revealed a total absence of *Simulium* larvae, and the number of flies seen was extremely low.

The third treatment on 17th October, 1967 was applied to the 42 miles between the De Hoop weir and Delportshoop (fig. 1 H to J) as the portion of the Vaal river sprayed on the two previous occasions was still free of any breeding. Treatment of the canal system and the Harts river was included throughout the programme to ensure that no flies could possibly reinfest the problem area from these sites.

Once again heavy rains in the Vaal catchment area affected the flow volume which had increased from 300 to over 2,000 cusecs by 31st October when the fourth aerial application was to have been conducted. As the whole area between the diversion weir and Delportshoop (fig. 1 A to J3 had now been treated, including the Harts river and canal system, further treatment was supended until such time that the flow rate had subsided. The treated areas remained free of *Simulium* larvae and it appeared justified, therefore, to suspend treatment further but to observe the area fortnightly as the whole programme had not been completed. Reinfestation would thus be observed at an early stage and the way in which it occurred could possibly be discovered.

Within 18 days of the increase in flow, a high population of *Simulium*, with a dominance of first to third instar larvae, appeared on all stones examined which had been covered by this increased flow. This heavy infestation of *Simulium* larvae could not be attributed to eggs laid by a possible few adults still flying about, and strengthened the suspicion that diapause eggs, which are capable of withstanding dehydration and remaining viable for a considerable period [9], were responsible for the rapid reinfestation. This interesting observation arose from the fact that reinfestation invariably took place only after inundation of stones which had been out of the water at low flow levels. The time lapse between high flow levels during the first control programme in 1966 was approximately five months, yet reinfestation took place immediately after a high volume of water had caused the submersion of previously exposed stones. During the second programme in 1967 reinfestation took place 18 days after the increased flow in spite of complete cessation of breeding due to the treatments. To determine the validity of this tentative conclusion several stones above the water level which bore seemingly desiccated egg patches were exposed to flowing water in the laboratory at the Institute. *Simulium* larvae were observed hatching from these dry stones within a few days.

CONCLUSIONS

The control programmes described have shown that outbreaks of cattle biting midges (Simuliidae: Diptera) in the Vaalharts area can be controlled temporarily. Each of the control programmes consisted basically of five fortnightly applications of DDT (sluice or aerial applications) based on the time required by a *Simulium* larva to develop from egg to final (sixth) larval instar in the summer. The lifespan of the adult flies is assumed to be approximately six weeks, hence the five fortnightly applications. The particulate-feeding *Simulium* larvae are fortunately very sensitive to low dosage of insecticides capable of adhering to these particles. The following facts have emerged from the investigation of Simuliidae in the Vaalharts complex.

1. Adequate vegetation on the north-west bank of the Vaal river and the presence of host animals confine the severe outbreaks to the first 40 miles between the diversion weir and the farm "Morrisdraai."

2. The distribution of *Simulium* flies, which is dependent on the availability of suitable shelter (trees and shrubs), appears to vary from 10 to 15 miles from breeding sites.

3. The floor of the canal complex is capable of supporting large populations of *Simulium* larvae and pupae.

4. The Harts river is capable of supporting a low population, but its flow is erratic, and the numbers will probably not increase.

5. In the Vaal river below the diversion weir and in the canal complex *S. chutteri* is the dominant species. Six *Simulium* spp. occur in the area.

6. Eggs, which are capable of surviving long periods of desiccation on the lower surface of stones, are responsible for the large outbreaks of *Simulium* after periods of increased flow. The factors responsible for diapause occurring in eggs are not known but it would appear that water temperature plays a role in the breaking of this condition i.e. rising water temperature in spring. *Simulium* continue breeding at a slower rate throughout the winter in this area of the Vaal river.

7. *Simulium* larvae are capable of surviving for over 70 hours in shallow standing water or mud and are capable of complete recovery when subsequently brought into contact with flowing water.

8. Biological control in the area is of a very low order due to the physical condition of the river bed. This form of control cannot contribute substantially towards decreasing the periodic outbreaks.

9. Manipulation of the flow will not control the problem due to the resistance to desiccation shown by both the larvae and the eggs.
10. Insecticidal treatment of the Vaal river water over a period of weeks results in the development of slimy mats due to the elimination of invertebrates which keep the microflora of the stones in check and prevent their excessive development. This condition gradually disappears after the insecticidal treatment has been discontinued and generally is cleared instantly after sudden high flow levels.

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The authors wish to express their gratitude to the following persons who were involved in the control programme and without whose advice and assistance the project could not have been undertaken:—

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The staff, Department of Entomology, Onderstepoort.

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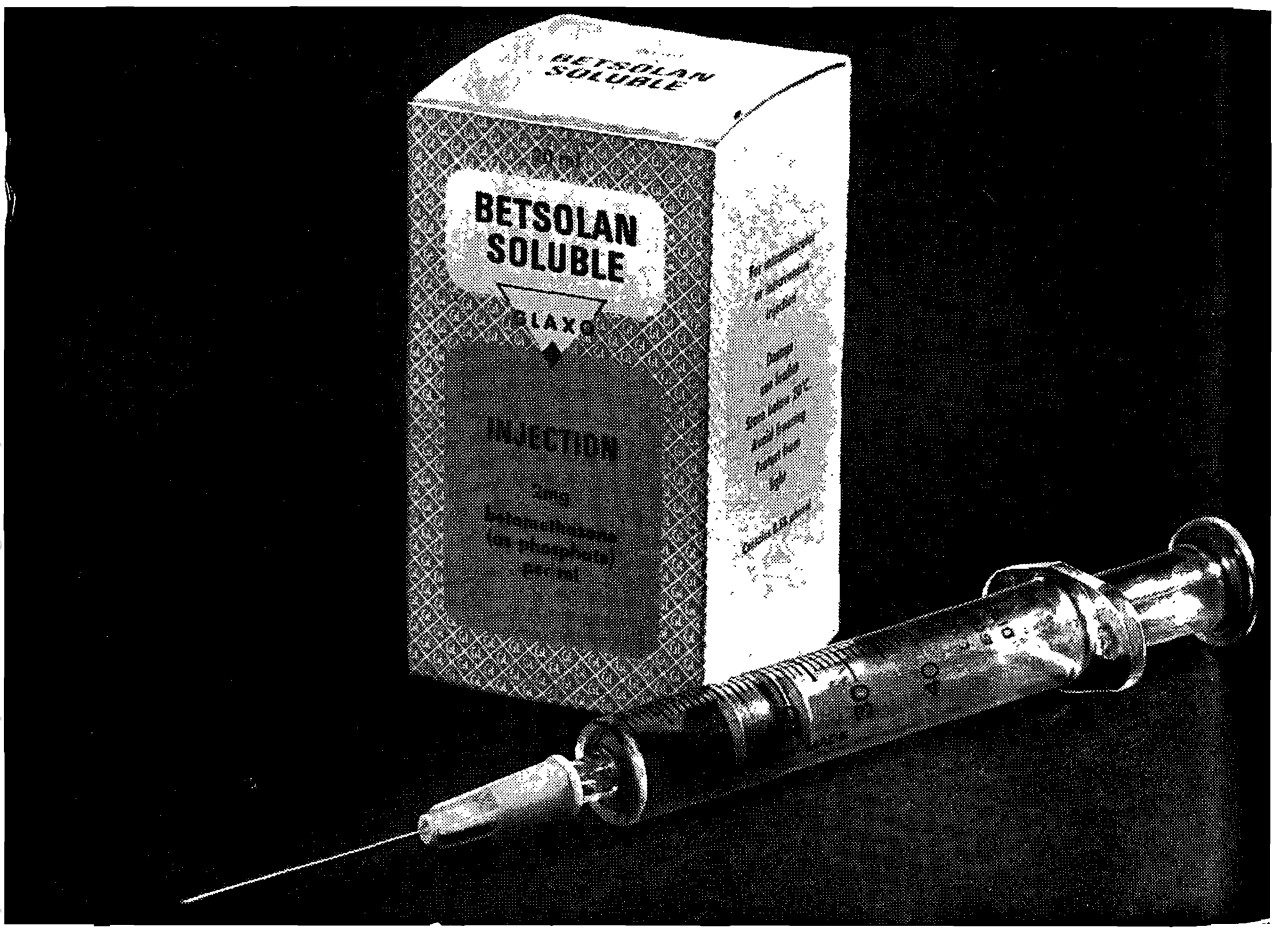

Jl S. Afr. vet. med. Ass. 40 (1): 71-72 1969

KARYOTYPE OF THE GEMSBOK AND SPRINGBOK

IRMGARD G. HEINICHEN*

A survey of diseases and parasitism of wild life in the Etosha Game Park in the northern part of South Africa was undertaken in July, 1967. During these investigations bone marrow biopsies of two Gemsbok, *Oryx gazella* (Linn., 1758), a male and a female, and a female Springbok, *Antidorcas marsupialis* (Zimmermann, 1780) were collected immediately after they had been shot. Spreads were made and treated according to the standard procedure in use here and chromosone counts done on these animals as shown in the tables below:—

The gemsbok and springbok were found to have morphological identical chromosones (fig. 1, 2 and 3). The diploid chromosone number being 56. In both species two pairs of submetacentric chromosones and 25 pairs of acrocentric chromosomes were found. The sex-chromosomes of the female in both spe cies consisted of a large acrocentric X-chromosome pair while in the Oryx male a large acrocentric X- and a small acrocentric Y-chromosome was found.

These findings support those of Wurster and Benirschke (cit. Hsu & Benirschke[1]) and Hsu and Benirschke[1].

ACKNOWLEDGMENTS

Greatful appreciation is expressed towards Prof. H. P. A. de Boom for arranging the excursion to South West Africa and towards Mr. de la Bat, Dr. H. Ebedes and Staff of the Etosha Game Park for their enthusiastic co-operation.

Table 1. CHROMOSOME COUNTS OF **ORYX GAZELLA.**

Animal	Sex	Time of collection	2n chromosome number 54	55	56	57	58	Total No. of spreads counted
Gemsbok	Male	8.00 a.m.	5	6	34	5	0	50
Gemsbok	Female	10.00 a.m.	5	6	41	1	0	53
Total			10	12	75	6	0	103

Table 2. CHROMOSOME COUNTS OF **ANTIDORCAS MARSUPIALIS.**

Animal	Sex	Time of collection	2n chromosome number 54	55	56	57	58	Total No. of spreads counted
Springbok	Female +	10.00 a.m.	54 3	55 6	56 37	57 4	58 0	50

* Veterinary Research Institute, Onderstepoort.

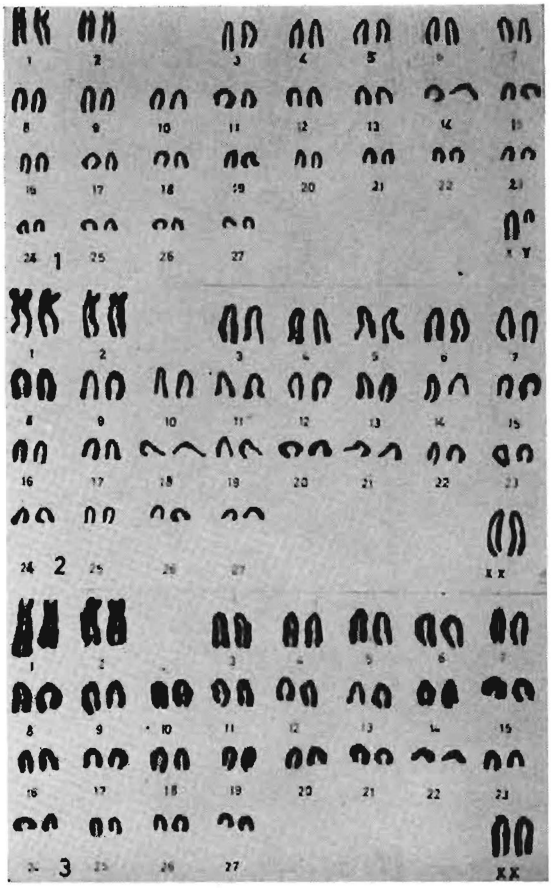

Fig. (1) A male and (2) a female karyogram of **Oryx gazella**. (3) A female karyogram of **Antidorcas marsupialis.**

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Jl S. Afr. vet. med. Ass. 40 (1): 75-80 1969

RECENT STUDIES ON CATTLE TRANSFERRINS

D. R. OSTERHOFF* AND L. P. NEETHLING**

SUMMARY

Quantitative studies were performed *in vitro* on the relative iron-binding ability of bovine serum. With the use of Isotope Fe^{59} it was shown that the iron-binding ability differs in the different transferrin genotypes and that the amount of iron exchanged in the different electrophoretic bands varies considerably.

In another experiment, the relationship between transferrin types and adaptability was investigated. Different haematological values gave good indications that animals possessing the Tf^E allele in homozygous form were better adapted because their blood values underwent the least changes during a stress period.

The apparent half-life time of autologous bovine erythrocytes was determined in 50 Afrikaner cows possessing different transferrin types. A relationship between the half-life of erythrocytes and the transferrin type of the animal could be established.

Preliminary investigations on a subdivision of the Tf^D allele proved that the further differentiation into Tf^D1 and Tf^D2 largely increases the practical application of the Tf locus.

INTRODUCTION

The differentiation of globulins according to their molecular size by electrophoresis with starch gel as supporting medium has been widely used for the fractionation of human and animal serum proteins, especially the beta-globulins. Giblett *et al*[1] were able to prove that these genetically variable beta-globulins are the specific iron-binding proteins, called transferrins.

Four different aspects of cattle transferrins have been studied by the authors, and will be discussed uder the following headings:

1. Radio-isotope studies on cattle transferrins
2. Transferrins and adaptability
3. Red cell survival in transferrin varients
4. Subdivision of the transferrin allele Tf^D.

Since details of materials and methods of most of the experiments have been described elsewhere[2, 3, 4, 5], only the most important results of these studies will be given.

RESULTS AND DISCUSION

1. *Radio-isotope studies on cattle transferrins*

The quantitative distribution of iron in the various electrophoretic bands of the transferrin types was studies. Using Isotope Fe^{59} it was proved that the ironbinding ability differs in the various transferrin genotypes and that the amount of iron exchanged in the different bands varies considerably. Table 1 gives the relative iron-binding ability (RIBA) of Afrikaner cattle serum of different transferrin types.

Table 1: THE RELATIVE IRON-BINDING ABILITY OF CATTLE SERUM OF DIFFERENT TRANSFERRIN TYPES

Transferrin Genotype	No. of animals*	No. of samples	Average count/ 4 min.	Relative iron-binding ability (RIBA)
Tf^A/Tf^A	20	33	12530	2.9
Tf^D/Tf^D	14	22	7332	1.7
Tf^A/Tf^E	9	12	6431	1.6
Tf^A/Tf^D	8	13	6053	1.4
Tf^D/Tf^E	11	20	4665	1.1
Tf^E/Tf^E	11	20	4273	1.0

*All experimental animals were Afrikaner cows of the same age and kept under veld conditions.

*Faculty of Vet. Science, Univ. of Pretoria, P.O. Onderstepoort.
**Section Radaition Biology, Veterenary Research Institute, P.O. Onderstepoort.

Although a certain degree of variation appeared within the different genotype groups the difference in the RIBA values of the various genotypes were remarkable. A statistical analysis of the variation within and between groups was rejected in view of the fact that individuals differ in the amount of iron already bound to transferrin at any given time. Nevertheless the six transferrin genotypes could be grouped into three subgroups, namely:—

GT 1 consisting of genotypes Tf^A/Tf^A,

GT 2 containing genotypes Tf^D/Tf^D, Tf^A/Tf^E and Tf^A/Tf^D, and

GT 3 having the remaining genotypes Tf^D/Tf^E and Tf^E/Tf^E.

This classification is based on the differences in RIBA values, since the ratios GT 1 : GT 2 : GT 3 approximately equals 3.0 : 1.5 : 1.0.

The ratio of the iron-biding ability of each electrophoretic band in the various types was calculated by referring to the C-band arbitrarily chosen as unity. These results are given in Table 2.

Table 2: RATIO OF IRON-BINDING ABILITY OF DIFFERENT GENOTYPES

Transferrin genotypes	β-globulin bands						Average counts of C-band
	A'	A	B	C	D	E	
Tf^A/Tf^A	0.16	0.76	1.40	1.00			3774
Tf^A/Tf^D	0.02	0.20	0.66	1.00	0.45		3598
Tf^D/Tf^D		0.19	0.58	1.00	0.67		3005
Tf^D/Tf^E		0.09	0.39	1.00	1.30	0.61	1376
Tf^A/Tf^E		0.15	0.63	1.00	1.00	0.92	1738
Tf^E/Tf^E			0.39	1.00	1.90	1.30	931

The interesting observation was made that the band carrying the greatest percentage of iron in the different genotypes varied between the groups but not within the groups. Thus in GT 1 the main band is B, in GT 2 it is C whereas in GT 3 band D carried most of the iron.

The actual physical meaning of RIBA is not quite clear, since only the iron-exchange ability of the various bands is measured and not the iron-binding capacity but the ability to exchange iron and the iron binding capacity must be related in some fashion. It is believed that the differences obtained in Table 2 might indicate physiological differences between the genotypes.

In Table 3 the daily gain of Afrikaner bulls over a 140-day test period and the corresponding feed conversion rate are related to the percentage active iron in the B-band and the RIBA values of the corresponding genotypes.

Table 3: POSSIBLE RELATIONSHIP BETWEEN TRANSFERRIN POLYMORPHISM AND ECONOMIC TRAITS IN AFRIKANER BULLS

Transferrin genotypes	Results of growth performance*			Results of iron-binding ability	
	No. of animals	Daily gain(lb)	Feed conv. rate (lb)	% active iron in B-band	RIBA
Tf^A/Tf^A	36	2.14	8.07	42.2	2.9
Tf^D/Tf^D	23	2.09	8.05	23.8	1.7
Tf^A/Tf^E	61	2.07	8.59	17.0	1.6
Tf^A/Tf^D	55	2.06	8.19	28.3	1.4
Tf^D/Tf^E	26	1.95	8.67	11.4	1.1
Tf^E/Tf^E	16	1.94	9.04	8.5	1.0

*In a test period of 140 days at the Irene Bull Testing Station.

The agreement is remarkable, taking into consideration that the results were obtained from two entirely independent groups of animals. The tentative subdivision of genotypes into three sub-groups also applies here. The group GT 1 (Tf^A/Tf^A) having the highest RIBA values showed the greatest daily gain and almost the lowest feed conversion rate, whereas the group GT 3 (Tf^D/Tf^E and Tf^E/Tf^E) had the lowest RIBA index, the lowest daily gain and the highest feed conversion rate. Group GT 2 was intermediate.

The existing relationship is manifested in the percentage active iron in the B-band since it is clear that the higher this value the greater is the daily gain and the lower the feed conversion rate. Whether the RIBA index or the iron present in the protein of the B-band is responsible for the trend obtained could not be established.

It appears from the work of Berg & Gall[6] and Makarechian & Howell[7] that no correlation exists between transferrin types and production traits in the case of mainly Hereford cattle. This points to the danger of evaluat-

ing transferrin genotypes alone and not also considering the individual contributions of physiological components of the genotype. The present study strongly indicates that the B-band and its ability to bind iron could be of great physiological importance with regard to growth.

2. *Transferrins and adaptability.*

While the allele Tf^A was apparently correlated to fast growth during the 140 days feeding period, the allele Tf^E could possibly be correlated to adpatability. Ashton [8] suggested the relatively high frequency of Tf^E in Zebu-type cattle as an indication of the well-known climatic and ecological tolerance of these cattle. The aim of the experiment to be described was to bring forward evidence for Ashton's theory.

Although it has been shown that direct climatic effects of temperature, humidity, radiation, altitude and air movement influence animal performance, it is certain that the indirect effects produced by disease, nutrition and traditional management practices are usually more important. Of all these factors nutrition is the one which can be controlled most easily.

Three groups of Afrikaner cows of different transferrin genotypes (15 Tf^E/Tf^E, 22 Tf^A/Tf^A, 18 Tf^D/Tf^D) were selected from the Onderstepoort experimental herd according to their transferrin types, weight and condition, age, number of calves produced and stage of pregnancy.

All animals were kept in special unfavourable nutritional conditions during wintertime and all possible records were collected at regular intervals. When the first animal, actually belonging to the DD group, died, the animals were transferred to better veld. Shortly after the change four further animals —all pregnant cows—three of them belonging to the DD- and one to the AA-group were lost.

Fig. 1 indicates the loss of weight of the three groups representing the different homozygous transferrin genotypes.

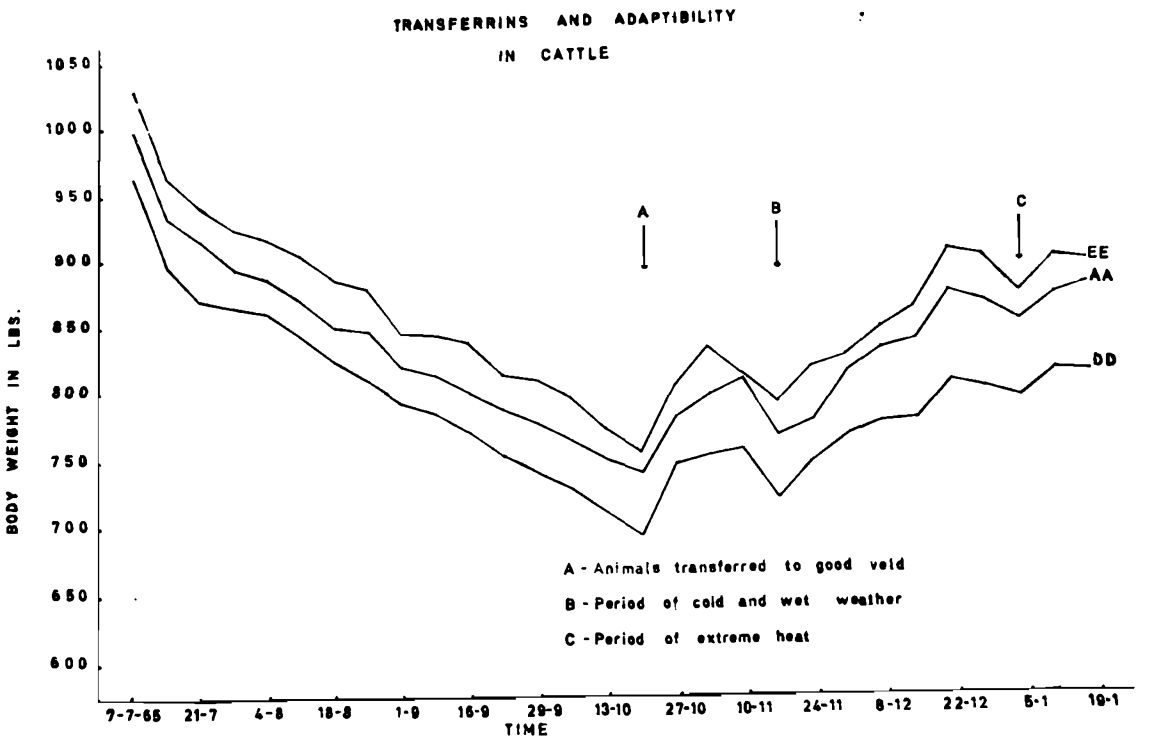

FIG. 1. Transferrins and adaptability in cattle.

There was no significant difference between the three groups in the weight losing or regaining phase. Animals were arranged in order of decreasing weight at different stages of the experiment. Here, a clear pattern was obtained: in all three groups the heaviest animals at the start of the trial remained the heaviest throughout the experimental period. In he case of the lighter animals this sequence also changed very little.

A significant increase in total plasma proteins was established over the six month period investigated. Simultaneously a definite drop in the albumin values and a marked increase in the globulin content was observed. The haemoglobin values dropped significantly together with the haematocrit readings—both values being closely correlated with each other. These values remained low even in December when the recovery of the animals had not yet had an influence on the haemoglobin figures.

In Table 4 the changes which actually took place are calculated as percentages and shown for the three groups.

Table 4: PERCENTAGE CHANGE OF SOME BLOOD VALUES IN THE STRESS PERIOD FROM JULY TO DECEMBER

	Group AA	Group DD	Group EE
Total Plasma Protein	+12.9	+13.3	+10.0
Albumin	−21.3	−22.0	−21.6
Globulin	+47.5	+48.2	+40.0
Haemoglobin	−22.1	−19.6	−12.5
Haematocrit	−14.4	−18.6	−11.3
Body weight	−16.5	−19.4	−16.5

From Table 4, which depicts the percentage deviation of the December from the July values, the group EE appears to be the most constant one, i.e. changes in blood values are the smallest of the three groups.

These results and the fact that no animal of the EE-group was lost, can be taken as an indication that animals possessing the Tf^E allele in homozygous form possibly could stand the stress to which they were subjected better than animals possessing either Tf^A or Tf^D allele in homozygous form. Further work on animals possessing the Tf^E allele in heterozygous form is necessary to confirm these results.

3. *Red cell survival studies in transferrin variants.*

In the search for physiological factors which could be related to inherited blood components it was decided to study the life-span of bovine erythrocytes.

From a large experimental herd of Afrikaner cattle 50 cows of various ages were selected according to their transferrin types.

In order to label the erythocytes *in vitro* the following procedure was adopted: 30 ml of blood was collected from each animal using heparin as anticoagulant. The blood samples were centrifuged and the cells washed once with 40 ml ACD solution. The excess ACD was removed and one ml of an isotonic solution of sodium chromate (Cr 51) was added (specific activity greater than 20 mC/mg Cr: Philips Duphar, Petten, Holland). After incubation for 30 minutes at 37°C, the samples were washed twice with saline to remove all excess activity and reconstituted with saline to the original haematocrit value. The autologous labelled blood was reinjected into the jugular vein of the animals. After 30 minutes a 5 ml sample of blood was collected in heparin from the opposite vein for counting purposes. Thereafter the animals were bled at regular intervals for a period of at least 45 days in order to establish the life-span of the erythrocytes.

The calculated halflife of the erythrocytes varied between 11.9 and 16.3 days. There seems to be a relationship between the half-life of erythrocytes and the transferrin type of the animal as depicted in Table 5.

Table 5: THE RELATIONSHIP BETWEEN TRANSFERRIN TYPES AND HALF-LIFE OF ERYTHROCYTES

Transferrin genotypes	No. of animals	Apparent half-life of erythrocytes (days)	Standard deviation (days)
Tf^D/Tf^D	8	15.5	0.9
Tf^A/Tf^A	7	14.0	1.1
Tf^A/Tf^E	9	13.8	1.1
Tf^A/Tf^D	9	13.5	1.2
Tf^D/Tf^E	9	13.4	1.1
Tf^E/Tf^E	8	12.7	1.2

The animals possessing the Tf^E allele have the shortest red blood cell halflife, which implies that they have a higher basal metabolic rate than those possessing the other transferrin alleles. This is in complete agreement with the findings given in Table 3 where animals with the Tf^E alleles showed by far the poorest feed conversion rate. A high metabolic rate implies less storage of reserve nutrients which is a possible explanation for a low RIBA value. The ideas in the literature as to the possible advantage of animals possessing the Tf^E allele in regard to climatic and ecological tolerance can now be viewed in a different light. Stresses of various kinds may be overcome more easily by animals possessing higher metabolic rates inherent in the hormonal regulation of metabolism.

Table 6: SUB-DIVISION OF THE ALLELE Tf^D FOR TRANSFERRINS IN SIX BREEDS

Breed	No. of animals tested	Transferrin alleles			
		Tf^A	Tf^D1	Tf^D2	Tf^E
Afrikaner	56	.38	.02	.16	.44
Ayrshire	205	.26	.36	.17	.21
Brahman	21	.22	.19	.28	.31
Brown Swiss	20	.35	.30	.28	.07
Charolais	34	.34	.35	.31	.00
Simmentaler	20	.15	.15	.70	.00

There appears to be no doubt that the better adaptability of the Tf^E animals is in fact a manifestation in their greater ability to resist stress.

4. *Subdivision of allele Tf^D.*

Further improvement in the technique of electrophoretic separation of iron-binding proteins made a subdivision of the allele Tf^D possible [9].

Fig. 2 shows the actual subdivision of the electrophoretic bands together with a diagrammatic explanation.

The implications of this subdivision may be far-reaching and the radio-isotope and red cell survival studies should be repeated with them in mind. Adaptability aspects must also be reconsidered, not only with regard to the comparison of heterozygote animals, but also to the different types of new transferrin alleles. Differences between the transferrin genotypes Tf^D1/Tf^D1, Tf^D1/Tf^D2 and Tf^D2/Tf^D2 should be investigated, particularly as a relationship between Tf^D and milk production has been indicated [10].

The value of transferrin determinations used in conjunction with blood typing in parentage tests and twin diagnosis has been discussed elsewhere [5]. The differentiation of Tf^D into Tf^D1 and Tf^D2 alleles increases the practical applicability of the Tf locus in parentage exclusion tests to a great extent.

TRANSFERRIN TYPES IN CATTLE

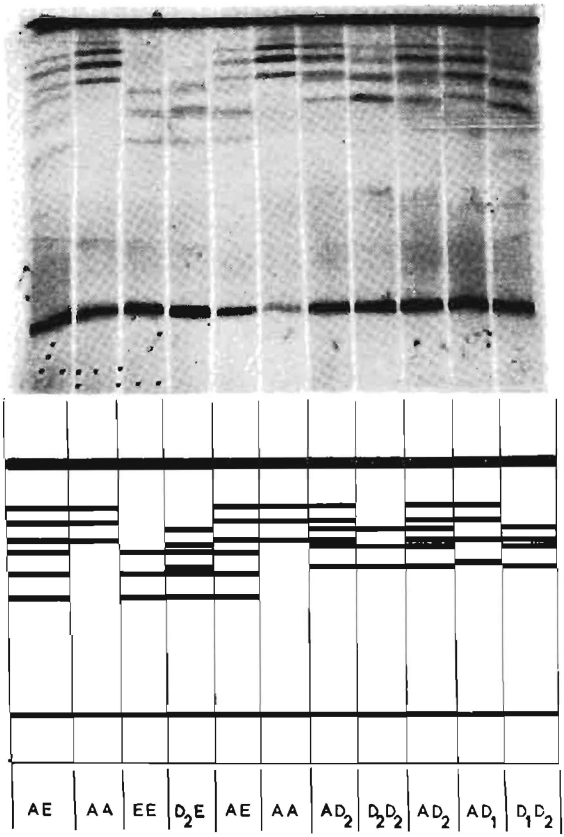

FIG. 2. Transferrin types in cattle (Subdivision of allele Tf^D)

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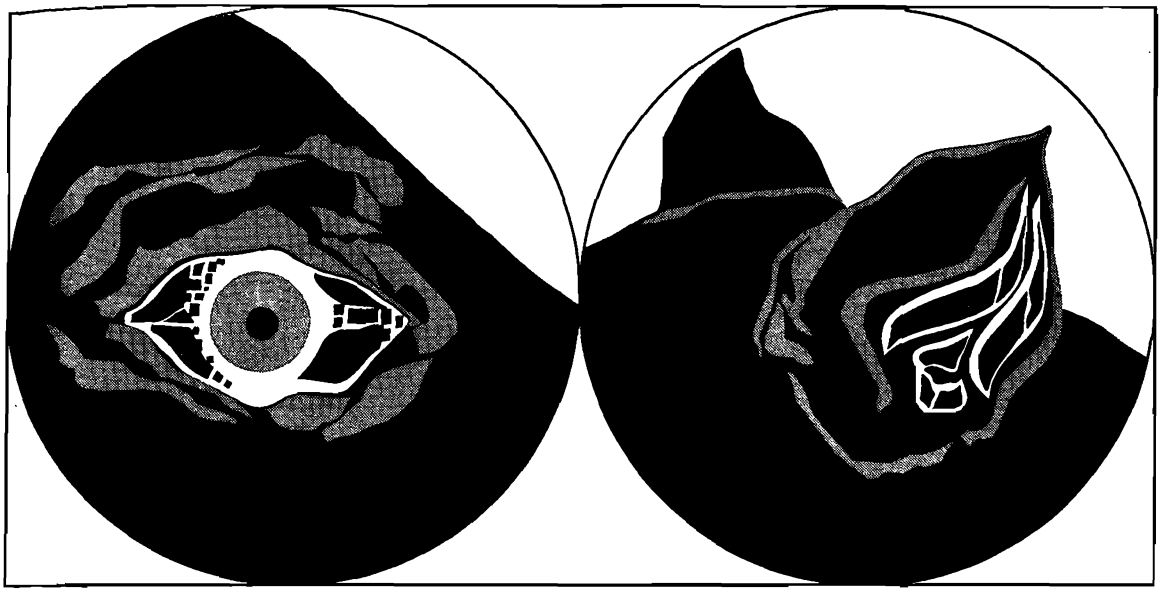
In superficial eye and ear conditions of domestic animals...



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THE AFRICAN BUFFALO AS A SOURCE OF FOOD AND BY-PRODUCTS

E. YOUNG* AND L. W. VAN DEN HEEVER**

SUMMARY

The mean weights of carcases and organs as well as the mean dressing out percentage and size of slaughtered adult buffaloes of the Kruger National Park (K.N.P.) are recorded as well as the approximate yield of deboned meat, biltong, bones, free fat and blood. More than 40 different diseases and parasites of buffaloes from the K.N.P. are listed and the incidence of five of the more significant of these conditions in carcases and organs is given. The frequency distribution of parasitic and non-specific lesions encountered in the different portions of 116 carcases are tabulated. Some of the more significant diseases are briefly discussed.

INTRODUCTION

Commercal exploitation of game is not the policy of The National Parks Board except when reduction in numbers becomes necessary in the course of nature conservation.

More than 40 different diseases and parasitic infestations have been recognised in African buffalo in the K.N.P. and the significance of these is still largely unknown. Some undoubtedly affect the suitability of meat and organs for use as food.

Buffaloes shot at random, mainly in the southern part of the K.N.P. were made available for detailed study, and data was collected concerning yield of food and by-products as well as the incidence and extent of diseases and infestations. This information should assist in caculating the potential nett production potential of *Syncerus caffer* (Sparrman, 1779) off the natural South African lowveld.

MATERIALS AND METHODS

The normal routine was to immobilize a randomly selected animal with succinylcholine chloride administered by means of a van Rooyen crossbow and dart-syringe. The animal was immediately bled to death by severing the jugular veins and carotoid arteries. The blood was collected and weighed.

The animal was hoisted by the hindlegs, the head amputated at the atlanto-occipital joint and the legs severed at the carpal and tarsal joints. The carcase was measured after it had been skinned and eviscerated. The length of the carcase was taken as the distance between the hock and the lowest point of the hanging carcase. The widest part of the carcase was measured over the thorax and the maximum depth was represented by a line drawn from the highest point of the shoulders (hump) to a point in line with the carpal joints.

Information on the live weights and dressing out percentages were collected in co-operation with Mr. N. Fairall who will publish this and other information on carcase analyses of this species in another paper.

The organs and all the fat which could be separated were weighed and the mean weight of the different edible components calculated.

Two dressed carcases of adult bulls, weighing 678 and 436 lb respectively, and one carcase of an adult cow with a weight of 537 lb, were deboned. The meat and the skeletons were weighed separately for calculation of the meat to bone ratio. Nine pieces of meat, each weighing 200 g, were obtained from different parts of the carcase of one bull, cut into thin strips, salted and hung in a cool shady place for two weeks. The resultant dry biltong was weighed and

*Division of Veterinary Services, Kruger National Park, P.O. Skukuza.
**Fac. Vet. Sci., Univ. Pretoria, P.O. Onderstepoort.

the yield of dry biltong from fresh meat and from an average adult buffalo carcase was calculated. Meat of the same buffalo was also dried at 100°C for 40 hours for determination of the moisture content.

At post mortem examination, the usual incisions employed in routine inspection of domestic cattle for cysticercosis were made.

Apart from diagnoses which were made at autopsy on 117 animals, some of the conditions were diagnosed by means of serological and other laboratory examinations and field observations.

A detailed description of the pathology of diseases and parasitic conditions encountered in the 100 randomly selected buffaloes will appear elsewhere.

RESULTS

The data on body component yields are summarized in Tables 1 and 2.

Infectious diseases and parasites which have been encountered in buffalo of the Kruger National Park are listed in Table 3, the incidence of the most significant parasites in buffalo carcases and organs are indicated in Table 4 and the frequency distribution of parasites and non-specific lesions in usable parts of buffalo carcases is summarized in the Table 5.

DISCUSSION

The size of hanging carcases is of importance in the planning of transport and storage facilities and was therefore recorded.

As shown in Table 1 the mean dressing out percentage of adult buffalo (40.44%) is lower than the corresponding figures of 57.71% for adult wildebeest [12] and 57.40% for adult impala [13] of the Kruger National Park. The results of the carcase analyses will be discussed elsewhere in greater detail by Fairall. Taking into account the data on yield, the reproductive potential of the randomly selected group of animals [14] and the information regarding edible products (Tables 4 and 5) one can arrive at more accurate figures for the meat yield of free living buffaloes. It should however be realized that breeding and mortality rates, and carcase weights of buffaloes, as in all other wild ungulates, may fluctuate tremendously from one year to another. A reliable range of the nett production by this species can therefore

Table 1: MEAN WEIGHTS (lb) AND MEASUREMENTS (inches) OF CARCASES AND ORGANS OF ADULT BUFFALOES (OVER 3 YEARS)

Weights / Measurements	Males		Females		Total	
	Mean	No.	Mean	No.	Mean	No.
Length of hanging carcase	116.06	15	112.15	13	114.25	28
Depth of hanging carcase	42.13	15	39.07	13	40.71	28
Width of hanging carcase	30.43	15	30.92	13	30.64	28
Live weight	1298	22	1121	22	1209	44
Weight of dressed carcase	644	22	552	22	598	44
Dressing out percentage	49.61	22	49.26	22	49.44	44
Weight of tongue	4.45	11	3.42	7	4.05	18
„ „ tail	3.89	19	3.89	19	3.89	38
„ „ heart	6.84	19	5.73	19	6.28	38
„ „ both lungs (without trachea)	12.78	19	11.36	19	12.07	38
„ „ liver	12.84	19	12.00	19	12.42	38
„ „ spleen	3.63	19	3.27	18	3.45	37
„ „ both kidneys	2.63	19	2.26	19	2.44	38
„ „ blood	27.46	15	18.62	16	22.90	31
„ „ free fat	5.94	15	6.68	16	6.32	31
„ „ wet skin	115.21	19	88.94	19	102.07	38
„ „ head and lower limbs	108.63	19	76.05	19	92.65	38

Table 2: MEAN YIELD OF MEAT, BILTONG AND GREEN BONE FROM CARCASES OF THREE ADULT BUFFALOES

(TWO ♂, ONE ♀)

Dressed carcase weight	550 lb
Total yield of deboned meat	369 lb
Meat suitable for conversion to biltong	±300 lb
Moisture content of fresh meat	70.39%
Weight of biltong (2 weeks old) as percentage of fresh meat	33.72%
Green bone and tendons	181 lb

Table 3: LIST OF INFECTIOUS DISEASES AND PARASITES DIAGNOSED IN BUFFALOES OF THE KRUGER NATIONAL PARK

Diseases/Parasites	Remarks	Reference	Diseases/Parasites	Remarks	Reference
Rinderpest	(a)	1			8
Bluetongue	(a)	2	**Haemonchus bedfordi**		8
Wesselsbron disease	(b)	3	**Haemonchus** sp.		8
Ephemeral fever(?)	(b)	3	**Thelazia rhodesii**		8
Lumpy skin disease(?)	(b)	4	**Thelazia** sp.		8
P.L.G.V. infection(?)		—	**Onchocerca synceri**		—
Anthrax	(a)	5	Filariasis	(c)	—
Brucellosis		6	**Stomoxys** sp.		—
Theileria lawrenci	(c)	7	**Lyperosia** sp.		—
Coccidiosis		—	Hippoboscid	(c)	—
Sarcocystis sp.		—	Keds	(c)	10
Paramphistomum microbothrium		8	**Haematopinus bufali**		10
Cotylophoron cotyphoron		8	**Linognathus** sp.		11
Schistosoma mattheei		8	**Boophilus decoloratus**		11
Parabronema skrijabini		8	**Hyaloma transiens**		11
Avittelina centripunctata		8	**Rhipicephalus evertsi**		11
Cysticercus sp.		—	**Rhipicephalus appendiculatus**		11
Echinococcus sp.		—	**Amblyomma hebraeum**		—
Trichuris globulosa		8	**Demodex** sp.		7
Oesophagostomum radiatum		8	**Sarcoptes** sp.		7
Agriostomum gorgonis		8	**Psoroptes** sp.		8
Trichostrongylus sp.		8	**Linguatula serrata**		—
Cooperia fuelleborni		8	Mites around horns	(c)	

(a) These diseases were diagnosed serologically. Bluetongue was however, also successfully induced in experimentally infected buffalo calves.

(b) Typical symptoms and/or post mortem lesions of these conditions have been observed in buffaloes but the diagnoses have not yet been confirmed.

(c) These parasites have not yet been identified.

Table 4: THE INCIDENCE OF FIVE OF THE MOST SIGNIFICANT PARASITIC INFESTATIONS IN CARCASES AND ORGANS OF 116 BUFFALOES

Parasite	No. examined			No. positive			% frequency		
	♂	♀ +	Ca.	♂	♀ +	Ca.	♂	♀ +	Ca.
Onchocerca synceri	56	52	8	49	36	0	87.50	80.76	0
Sarcocystis	56	52	8	17	15	0	30.35	28.85	0
Cysticercus sp.	56	52	8	12	4	0	21.42	9.61	0
Linguatula serrata	56	52	8	33	30	0	64.28	61.53	0
Echinococcus cysts	56	52	8	3	2	0	5.36	3.85	0

♂ = Bulls older than 6 months.

♀ + = Cows and heifers older than 6 months.

Ca. = Calves of both sexes under 6 months.

TABLE 5: FREQUENCY DISTRIBUTION OF PARASITIC AND NON SPECIFIC LESIONS IN AFFECTED PARTS OF ONE HUNDRED AND SIXTEEN BUFFALO CARCASES

Aetiology	Parts of carcases affected								
	Skin	Tongue	S.M.	Ser.	Heart	Lungs	Liver	Kidneys	Tripe
Sarcocystis sp.	–	+ + +	+ + +	–	–	–	–	–	–
Schistosoma	–	–	–	–	–	–	+ +	–	+
Cysticercus	–	+	+	–	–	–	–	–	–
Echinococcus	–	–	–	–	–	+	+	–	–
Onchocerca	–	–	+	–	–	–	–	–	–
Demodex	+	–	–	–	–	–	–	–	–
Sarcoptes	+	–	–	–	–	–	–	–	–
Psoroptes	+	–	–	–	–	–	–	–	–
Linguatula	–	–	–	–	–	–	+ + +	–	–
Non specific	+	+	+ +	+ + + +	–	+	+	+	+

S.M. = skeletal musculature (excluding the musculature of the tongue)
Ser. = serous membranes.
Tripe = empty stomach, intestines and attached mesenterium.
\+ = 1—25% incidence of occurrence.
\+ + = 26—50% incidence of occurrence .
\+ + + = 51—75% incidence of occurrence.
\+ + + + = 76—100% incidence of occurrence.
– = not observed at meat inspection.

only be determined after information on the population dynamics and production capabilities of this species have been collected over longer periods and under a greater variety of environmetal conditions.

Our preliminary results indicate that about two thirds of the weight of fresh muscle is lost in the production of biltong and that about 300 lb of suitable meat may be obtained from an adult animal. The yield of biltong therefore amounts to approximately 100 lb per adult buffalo.

Organs, bones, blood, free fat and hides have commercial value as human food or as raw material for the production of byporducts such as liver meal, carcase meal, bone meal, meat meal, blood meal, oil and leather. Their weights are therefore important.

Diseases and parasites can affect the nett production of meat producing animals in various ways. Some may affect parts of carcases macroscopically and may render these repulsive. Others may influence meat production indirectly by virtue of their effect on the natality-, growth-, and mortality rates of the species and a third group of infections may present a disease hazard to human or animal consumers of infected parts of carcases.

Parasites which may cause lesions in the skins of buffaloes include filariae, *Lyperosia*, *Psoroptes*, *Sarcoptes* and *Demodex* infestations, tick infestations complicated with secondary infections and mites around the bases of the horns of some of the buffaloes. A variety of traumatic factors may also play a part. Lice infestations were never associated with macroscopic pathology. Local areas of chronic dermatitis on the sides of the abdomen and thorax, around the base of the tail and in the vicinity of the eyes and horns as well as cutaneous nodules due to demodeciosis and scars of traumatic origin (frequently caused by lions) were the most significant cutaneous lesions encountered. Sarcoptic and psoroptic mange were only encountered in very young calves.

Sarcosporidiosis, cysticercosis and onchocerciasis represent, in our opinion, of the most significant parasites of edible parts of buffalo carcases. These conditions were diagnosed in the northern, central and southern parts of the Kruger National Park.

Sarcosporidiosis was not diagnosed in young calves but at meat inspection 88% of 56 bulls and 81% of 52 cows and heifers older than 6 months were found to be infected. *Sarcocystis* cysts (Miescher's tubes) were glistening and varied from 2.5 cm. long and

0.5 cm. in transverse diameter to the smallest which could hardly be seen with the naked eye. In some animals the cysts were all very small and of similar size. The cysts were generally oval-shaped in the subcutis or in the loose connective tissue surrounding the larynx while larger and thinner, more typical Miescher's tubes were usually found in active msucles. Miescher's tubes were seen in the tongue, around the pharynx and larynx, in the oesophageal musculature and in the musculature of the neck, shoulders, diaphragm and thighs. The tongue, pharyngeal region and dorsal part of the cervical musculature were, however, most frequently affected.

Cysticercosis was diagnosed less frequently than sarcosporidiosis but the former may be of more significance, depending on its potential infectivity for man. The identity and host range of this parasite is yet to be determined. Cysticerci or "measles'" were found in the masticatory, cervical and subscapular musculature, in the muscles of the hump, shoulders, brisket, diaphragm, thighs, tongue and heart and also in the supramammary lymph node of one animal. As many as 10 cysts were found in a single heart and, in this organ the parasites occurred in the ventricular walls, septum and apex and could sometimes be seen through the epi- and endocardium.

Onchocerciasis in the buffalo is manifested by typical nodules particularly in the subcutis and superficial musculature of the thoracic and sternal regions. The parasites have been identified by Kruger [8] as *Onchocerca synceri*. Infested nodules also occurred in the masseter, sternocephalic, triceps, external abdominal and preputial muscles.

Non-specific lesions of the subcutis and musculature included areas of greenish discoloration over the neck, hump, thorax, shoulders, axilla, abdomen and thighs. The greenish discoloration of the subcutis and superficial musculature could have been due to an eosinophilic reaction towards parasitic migrations such as *Parafilaria bovicola* which have been described in domestic cattle from the Northern Transvaal [9]. Small greenish nodules were also observed in the musculature of some of the animals and this could have a similar aetiology. Bruising, poor bleeding with resultant filling of the blood vessels, and agonal blood "splashing" in the musculature, as well as hydraemia, associated with emaciation, were also encountered.

Lesions of the heart included subendocardial haemorrhages in the auricles and ventricles, whitish patches under the endo- and epicardium, endocarditis and focal dis seminated chronic active and non-active epicarditis over the ventricles and auricles, and inflammatory lesions over the bases of the larger blood vessels where they enter or leave the heart. In one case a fibrous adhesion resulted in attachment of the apex to the pericardium.

Pericarditis, pleuritis and peritonitis of a fibrous nature were rather frequently diagnosed in buffaloes of both sexes and all age classes from different parts of the Park. These lesions, often so extensive as to cause adhesions between organs, were sometimes accompanied by hydropericardium, hydrothorax and ascites. The lungs were for instance quite frequently attached to the costal pleura and in a few cases even to the diaphragm. These lesions, together with the frequent affection of lymph nodes, signs of nervous depression, pneumonia and pyrexia in young calves, very low survival rates of young animals in certain herds and the finding of unidentified inclusion bodies in smears of affected serous membranes and lymph nodes suggest the possibility of infection with a virus of the psittacosis-lymphogranuloma venereum group. Other possible aetiological factors have been suggested but this syndrome still requires elucidation.

Hydatid cysts were encountered in the lungs and livers of a few animals but in no case were the organs affected as severely as, for instance, those of zebras of the same Park.

Pentastomiasis was diagnosed in 64.28% of the 56 bulls and 61.53% of the 52 cows and heifers examined. Eight calves under six months of age were also examined but no parasites could be found. The tongue-shaped parasites, usually in the liver, the atria and ventriculi of the heart and in some of the larger bloodvessels, were identified as nymphal stages of *Linguatula serrata* [8]. The adult stages of this parasite have been found in the nostrils of lions of the Kruger Park. The immature stages also commonly infest blue wildebeest but have only seldomly been found in impala. In the liver of buffalo these parasites may be found in the absence of any macroscopic pathological lesions. In other cases, liver lesions associated with *Linguatula* infestations included focal necrosis of the liver parenchyme, rarely

encystation of the parasites, ulceration of the liver capsule and, in many cases, chronic lesions of peritonitis of the liver capsule resulted in villi-like projections. The parasites themselves were usually most conspicious in the hepatic veins but also occurred directly under the liver capsule or deeper in the substance of organ.

Lesions due to schistosomiasis were encountered in the livers and the splanchnic vessels of some of the animals. *Schistosoma mattheei* was found to be the responsible organism [8]. Some of the affected livers had a repulsive appearance and were, for aesthetic reasons, not fit for consumption.

Other macroscopically visible affections of edible organs include cloudy swelling, cirrhosis, focal disseminated necrosis and abscessation of the liver, cystic kidneys in three animals, and a few instances of nephrosis and nephritis.

Apart from direct losses as result of pathological conditions of edible parts of buffaloes, diseases such as rinderpest [1] and anthrax [5] may be responsible for high natural mortality whereby the nett production of free-living populations can be detrimentally reduced. Other diseases and parasites may, depending upon various factors, also contribute to descreased productivity of this species in its natural habitat. Our knowledge of the significance of the various pathogens mentioned as decimating factors of buffalo populations is unfortunately as yet incomplete at this stage, but preliminary observations have indicated that diseases and parasites may play an important role in the natural regulation of population numbers.

Infectious diseases, common to man and food animals are of known public health importance. Zoonoses diagnosed in buffalo of the Kruger Park and which may be transmissible via infected carcases include anthrax, brucellosis, Wesselsbron disease and probably taeniasis and sarcoptic mange.

Other organisms, which are pathogenic for the buffalo, like *Schistosoma mattheei* and probably the *Echinococcus* sp., may indirectly infest man via another host. Trichinosis, another zoonosis of significance, could not be diagnosed in specimens from buffalo carcases [15].

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RESEARCH NOTE

INCORPORATION OF A MACRO-SLIDE IN THE MICRO-TECHNIQUE OF IMMUNO-ELECTROPHORESIS

I. S. WARD-COX*

In the application of electro-phoretic analysis to the study of immuno-genetics, exceptional resolution in the alpha and beta zones is required for accurate precipitin arc investigation. In an attempt to improve this resolution, various buffers were tried. These included the conventional Michaelis type veronal buffer [1], the veronal buffer described by Hirschfeld [2], a lithium hydroxide buffer, the various Tris combinations [3] and many others. None of these gave the required result and the difficulty was ascribed to the limitations of the microscope slide, particularly to the short distance of migration of antigen particles. A larger container was therefore designed to fit the cassette of the LKB apparatus in use.

The container was made entirely of Perspex with the following outside dimensions: Length 27.2 cms., width 5.6 cms., height 0.8 cms. The inside depth was 2.0 cms. at the sides and 1 cm. at each end. The base was constructed of $\frac{1}{8}$" and the sides of $\frac{1}{16}$" thick material. These dimensions coincided with those of the conventional slide holders and could therefore fit side by side in the cassette.

The lateral dimensions of the trough and wells remained unaltered, i.e. the wells retained their diameters of 1.5 mm and the trough remained 2.0 mm wide, both wells being 4.0 mm from the trough. The trough, however, was made 22 cms long and was cut with a razor blade and straight edge. Upon widening the wells to 2 mm diameter however, better distribution of the antigen was accomplished. A conventional technique was used with the exception of an extension of the duration of the electrophoresis run from $2\frac{1}{2}$ to 3 hours.

Figure 1 shows the larger pattern alongside the conventional one while Figure 2 illustrates the structural details of the apparatus. It will be noticed ha considerable improvemen was achieved with the resolution of the alpha and beta zones, eliciting arcs that have heretofore been impossible to resolve otherwise.

It was found that drying the agar prior to staining was unpractical since the material warped and cracked considerably due to the extent of the agar area. Staining was therefore carried out on the undried agar and the plate photographed, after decolorizing for 36 hours. The limitations of the method are therefore (1) the impracticability of storing the slides, and (2) the exclusion of all water-soluble stains, especially those for lipoprotein fractions.

ACKNOWLEDGEMENTS

Prof. D. R. Osterhoff is thanked for his interest and helpful suggestions.

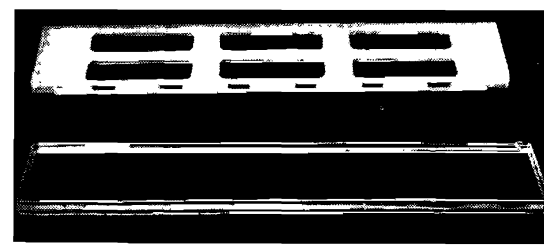

Fig. 2

(Fig. 1 on page 92)

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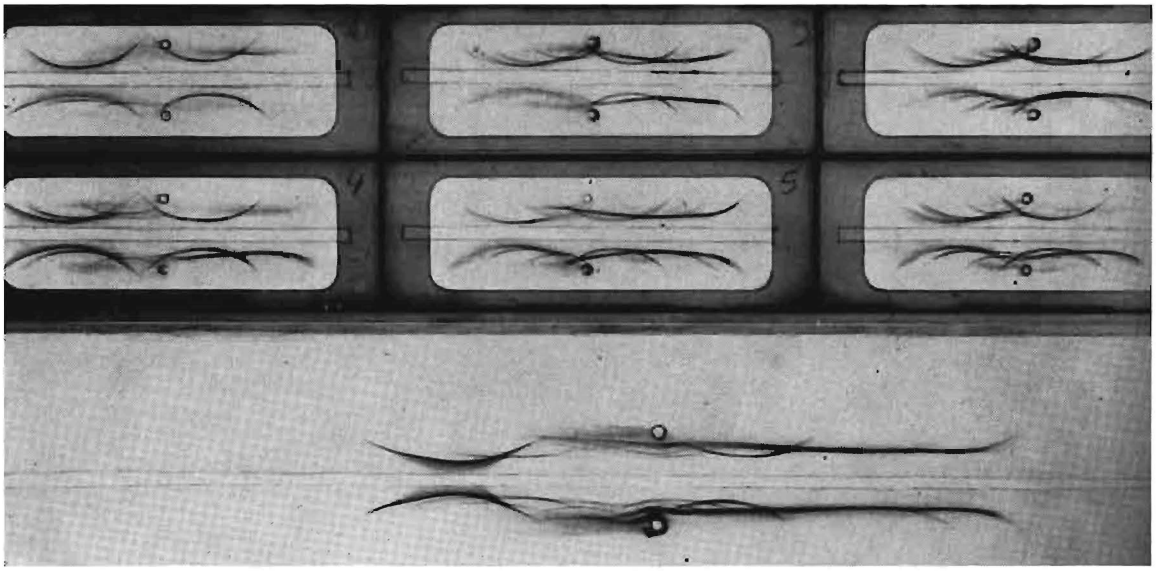
*Immuno-genetics unit, Veterinary Research Institute, Onderstepoort.



(Fig. 1, Research Note, page 91)

BOOK REVIEW

SOME DISEASES OF ANIMALS COMMUNICABLE TO MAN IN BRITAIN

EDITOR: OLIVER GRAHAM-JONES

Pergamon Press, Oxford, 1968. Pp XV + 338, Tab. 70, Figs. 29, Price 126/-

This book constitutes the Procedings of a symposium organised by The British Veterinary Association and The British Small Animal Veterinary Association, held in London, June 1966. Some 37 eminent workers in the medical and veterinary fields contributed papers, which dealt with some of the more important zoonoses to which the human population in Britain is exposed. The authors dealt expertly with facts and figures emanating from recent research, investigation, surveys and experience, and the Proceedings also contain details of the discussion and the summaries which followed each session.

Papers on the following diseases are presented, usually by veterinary and medical contributors who each deal with certain facets: Salmonellosis, Shigellosis and Pasteurellosis; Toxacariasis, Cysticercosis, Trichinosis, Hydatidosis and Fascioliasis; Newcastle Disease, Louping Ill, Cat Scratch Fever, and Ornithosis; Rabies and B-virus infections; Leptospirosis and Listeriosis; Q-fever and other rickettsioses; and finally, Brucellosis.

The book presents readers interested in infectious and parasitic diseases in general, and in the zoonoses in particular, with a clear and concise exposition of the most recent developments concerning aetiology, diagnosis, control, evaluation and significance of the various diseases of animals to which man may be exposed. It is a most useful reference work to both veterinary and medical workers in public health, being clearly and well illustrated, clearly printed, and provided with details of references after each paper. The book is not indexed, and it is also rather a pity that it should appear a full two years after the Symposium was held.

L. W. v. d. H.

Jl S. Afr. vet. med. Ass. 40 (1): 95-96 1969

RESEARCH NOTE

A SAFETY DEVICE FOR LARGE SCALE ELECTROPHORESIS

I. S. WARD-COX*, A. M. G. PRETORIUS**, H. E. S. FOUCHE* AND T. G. POTGIETER*

The introduction of high voltage electrophoresis has made the danger of accidental electrocution a very real one. The open system has to be used where funds are limited.

A recent introduction, by a Swiss firm manufacturing an apparatus capable of 5000 V output, has been a highly efficient "safety cell" which makes provision for cooling as well as an automatic switch which cuts off the current whenever access to the medium is required. However this device has the disadvantages of only accommodating a single run at a time and the current across the medium cannot be monitored. When multiple runs are carried out concurrently the problem becomes more complicated and the danger more acute. The apparatus to be described has been evolved to solve these problems.

Such a device had to fulfill the following requirements:—

(a) accommodation for more than one electrophoretic unit,

(b) each unit had to function independently,

(c) no access to the medium whilst the current is on,

(d) sufficient cooling of the medium while in operation, and

(e) facilities to monitor the current whilst in operation.

The cabinet is made of plate glass in a wooden frame with a durable base. It is divided into six compartments separated from each other by stainless steel gauze and has a solid wooden rear panel. The length of the cabinet depends on the number of units to be accommodated, each unit compartment being 2 feet long. For less than six units only one fan is required whereas a second fan is necessary over greater distances in order to maintain even cooling of gels. Fans capable of variable and reversible air flow are installed at each end. The front glass panels slide over each other to provide access to each compartment and operate microswitches that cut off the current upon opening. Two terminals are installed in the frame at the bottom of each front panel and are connected to leads that can be inserted into holes drilled into the ends of each gel plate for monitoring the current while the panels are closed. The wiring in each compartment is connected in parallel in such a way that each can be switched off independently. Each compartment is pro-

*Veterinary Research Institute, Onderstepoort.
**Institute of Pathology, Department of Medicine, University of Pretoria.

vided with an outside plug for the power supply, connected through its respective microswitch, and a pilot lamp inside to denote that the current is on to the respective compartment. This pilot lamp will remain on as long as the outside switch is on whereas the pilot lamp on the power supply will denote whether the microswitch is operating. All compartments are interconnected leaving only one plug for current supply.

After placing the loaded media in the units and connecting the leads to the outside terminals and covering the gels with wetted gauze, the front panels are closed, the fans switched on and the current regulated from the power supplies which are placed on top of each compartment. Cooling can be further accomplished by placing cooling coils at the ends of the unit in front of each fan, the unit being switched on 30 minutes before operation.

From the photograph it is clear that the unit can be comfortbaly accommodated on a work bench, standing on short legs to facilitate cleaning.

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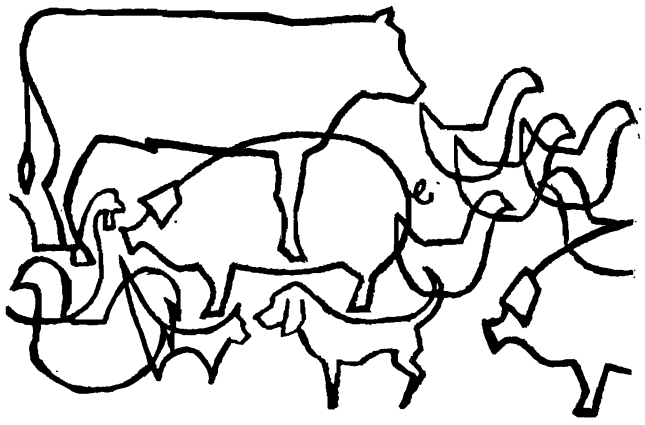

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Jl S. Afr. vet. med. Ass. 40 (1): 99-100 1969

KARYOLOGICAL STUDIES ON SOUTHERN AFRICAN PERISSODACTYLA*

IRMGARD G. HEINICHEN**

A cytogenetic survey was undertaken on the southern African species and subspecies of the order Perissodactyla, taking advantage of the relatively recent improvements whereby chromosome number and karyotypes could be established with greater accuracy.

Material was obtained from male and female animals either chemically immobilized, caught or shot in various game parks or game farms in South Africa, South West Africa, Rhodesia and Mozambique. The bone marrow biopsy technique based on that of Sandberg, Crosswhite & Gordy[1] with some adaptations[2] was employed. Several, up to about 50, good chromosome spreads were counted and karyograms were constructed. Simultaneously blood smears were made and the nuclear appendages on 500 neutrophil polymorphonuclear leukocytes counted to determine the feasibility of polymorphic sexing.

The following results were obtained:

Species	2n Chromosomes	Metacentric (=meta-submetacentric) chromosome pairs	Acrocentric (=acro-subtelocentric) chromosome pairs	Number of animals
Ceratotherium simum	82	0	40	5
Diceros bicornis	84	4	37	1
Equus burchelli	44	18	3	15
Equus zebra	32	13	2	8

The chromosomes as they appear in the four species of the Perissodactyla are shown in figures 1 (1, 2 and 3) and 2 (1, 2, 3 and 4).

The subspecies of *Equus burchelli*, namely *E. b. burchelli, E. b. antiquorum* and *E. b. crawshaii (=selousi)* and intermediate types between the latter two all have the same chromosome number, namely 2n=44, and morphologically apparently identical karyograms. The same applies to the subspecies of *Equus zebra, E. z. zebra* and *E. z. hartmannae*, with a diploid chromosome number of 32.

The karyotypes of the different species of the Perissodactyla were compared with each other. There is a great variation in number and morphology of the karyotypes, so that no morphological relationship between the autosomes was found, although a similarity was recognized in the sex chromosomes throughout the order. The sex chromosomes of the rhinoceroses resemble those of the horse.

Karyotype evolution among the Perissodactyla was difficult to explain. Robertsonian fusion, whereby a decrease of chromosome number is accompanied by a decrease in number of acro-subtelocentric chromosomes could not be the only reason for the existence of such a wide range from 32 to 84 chromosomes in this order. Robertsonian fusion must have been accompanied by other phenomena, such as tandem fusion, translocations with subsequent loss of heterochromatic centromeres, translocations reverting to acrosubtelocentric chromosomes as a result of pericentric inversion or possibly the loss of very small chromosomes. Polyploidy could not be offered as an explanation here.

These cytogenetic studies could not assist in clarifying the taxonomic problems among the zebras at subspecies level. Nevertheless, it has confirmed the existing classification at species level ,with every species of this order having its specific diploid chromosome count.

It was concluded that an identical chromosome number and an identical karyotype may not be advanced uncritically for the identity of species, neither may differences in chromosome number be accepted as proof of difference in species, unless one excludes

*A summary of a thesis submitted in partial fulfilment of the requirements for the degree of Master of Science (Agric.) in the Department of Genetics, Faculty of Agriculture, University of Pretoria, Pretoria. December, 1968. To be published in Kudu No. 13, 1970 (Journal for Scientific Research in the National Parks of the Republic of South Africa).

**Dept. of Anatomy, Faculty of Veterinary Science, Onderstepoort.

chromosome polymorphism.

Although no chromosomes of hybrids were studied, the findings of other authors on hybrids were discussed, showing that in nearly all known hybrids of the Equidae, the diploid number of the hybrid was equal to the sum of the haploid number of both parents and that all Equidae hybrids, excluding a few exceptional mules, were sterile.

Suggestive evidence was found for the existence of a mitotic cycle with peak activity during about 9 to 11 o'clock a.m. Although not specifically investigated, indications were found that activity of the animal (and thus external factors influencing that activity) may play a rôle, yet that physical stress, excitement, and delay in collection of bone marrow after immobilization may possibly depress the number of mitotic figures obtained, presumably due to circulatory changes in the bone marrow.

Clear-cut sex differences exist in all the species and subspecies examined; the female sex can be determined by counting typical "drumsticks" only.

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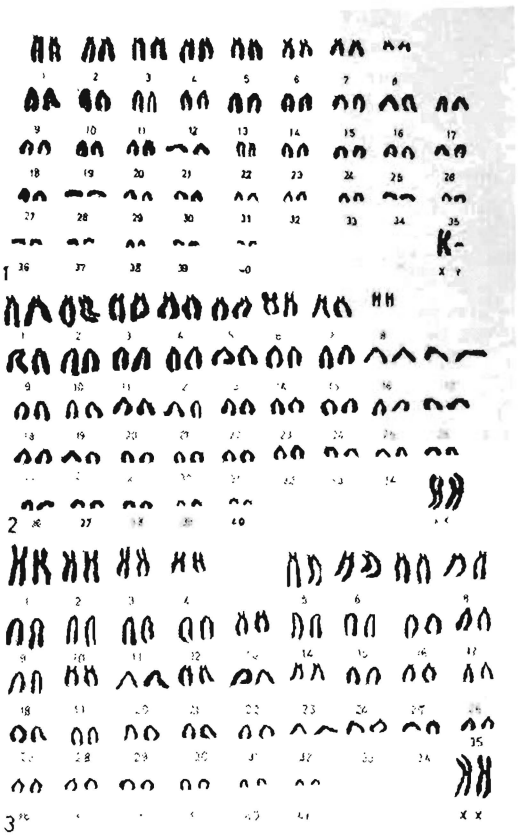

FIG. 1. (1) Male and (2) female karyogram from mitotic chromosomes of **Ceratotherium simum.** (3) Female karyogram from mitotic chromosomes of **Diceros bicornis.**

FIG. 2. (1) Male and (2) female karyogram from mitotic chromosomes of **Equus zebra.** (3) male and (4) female karyogram from mitotic chromosomes of **Equus burchelli.**

BOOK REVIEW

ADAPTATION OF DOMESTIC ANIMALS

EDITED BY E. S. E. HAFEZ

Bailliere, Tindall & Cassell, London 1968. pp. 415, 177 illus. 16 plates. Price 165s.

This very interesting book is the work of no less than 27 contributors. It covers a very wide field and its scope can best be indicated by outlining the contents which consists of five main parts.

Part I. "Ecological and Bioclimatological Aspects"—The six chapters of this section deal with the physical environment, the principles of adaptation—morphological physiological and behavioural, the effects of the environment on production and the world distribution of domestic animals.

Part II deals with basic physiological mechanisms of adaptation and with biological rhythms.

Part III is entitled "Adaptation to Specific Environments" which include tropics and deserts, cold, high altitude and stress and disease. (The last named chapter is contributed by K. van der Walt and B. C. Jansen).

Part IV deals with adaptation in cattle, sheep and goats, swine and poultry and has a chapter on comparative functions of ruminants in hot environments.

The fifth part describes techniques of measuring physical factors of the environment and physiological and behaviour responses thereto. This is a particularly useful section as it describes modern electronic, including telemetric, equipment.

The appendices include a list of supplementary references and one of sources of equipment.

As is to be expected in a book handling such a wide field, only the principles of each aspect have been dealt with but the references given after each chapter make further study easy.

Two minor points of criticism are:—

1. The table on page 9 indicates that sled dogs occur in the Kalahari (bracket misplaced) and on page 71 it is implied that the "water buffalo" of Asia and South Africa are the same species.

The main lesson of the book is that modern research into adaption in animals requires the collaboration of physiologists, physicists and animal husbandry experts as well as sophisticated equipment.

This book should be studied not only by those interested in research but also by all who are interested in the physiology, management and productivity of domestic animals. That all these aspects are intimately affected by the environment is made abundantly clear.

R. C.

B.V.Sc.V.-PRESTASIES - 1968

Ten tye van die gebruiklike geleentheid by die Fakulteit Veeartsenykunde van die Universiteit van Pretoria aan die einde van die akademiese jaar 1968, is die volgende toekennings aan die nuwe B.V.Sc.-graduandi bekend gemaak:

1. Die S.A. Biologiese Vereniging se THEILER-MEDALJE ("For diligence and merit") is aan P. Bland van den Berg toegeken.

2. Die Tak Witwatersrand van die S.A.V.M.V. se KLINIESE MEDALJE is deur R. E. Turner verower.

3. Imperial Chemical Industries (Pharm) S.A. Bpk., het hulle prys vir GENEESKUNDE EN INFEKSIESIEKTES aan P. Bland van den Berg toegeken en hulle prys vir CHIRURGIE EN GENESIOLOGIE is verower deur K. O. P. Shires.

4. Maybaker (S.A.) Bpk. se KLINIESE prys is toegeken aan G. E. Perchman.

5. Pfizer (S.A.) Bpk. se PFIZER-PRYS is deur S. S. van Berg verower.

6. Agricura Laboratoria Bpk. se AGRICURA-PRYS is toegeken aan P. Bland van den Berg.

7. Lilly Laboratories (S.A.) (Edms) Bpk. se prys vir PLUIMVEESIEKTES is deur P. Bland van den Berg verwerf.

Die nuwe graduandi en die prys- en medaljewenners word van harte gelukgewens.

B.V.Sc. V - 1968

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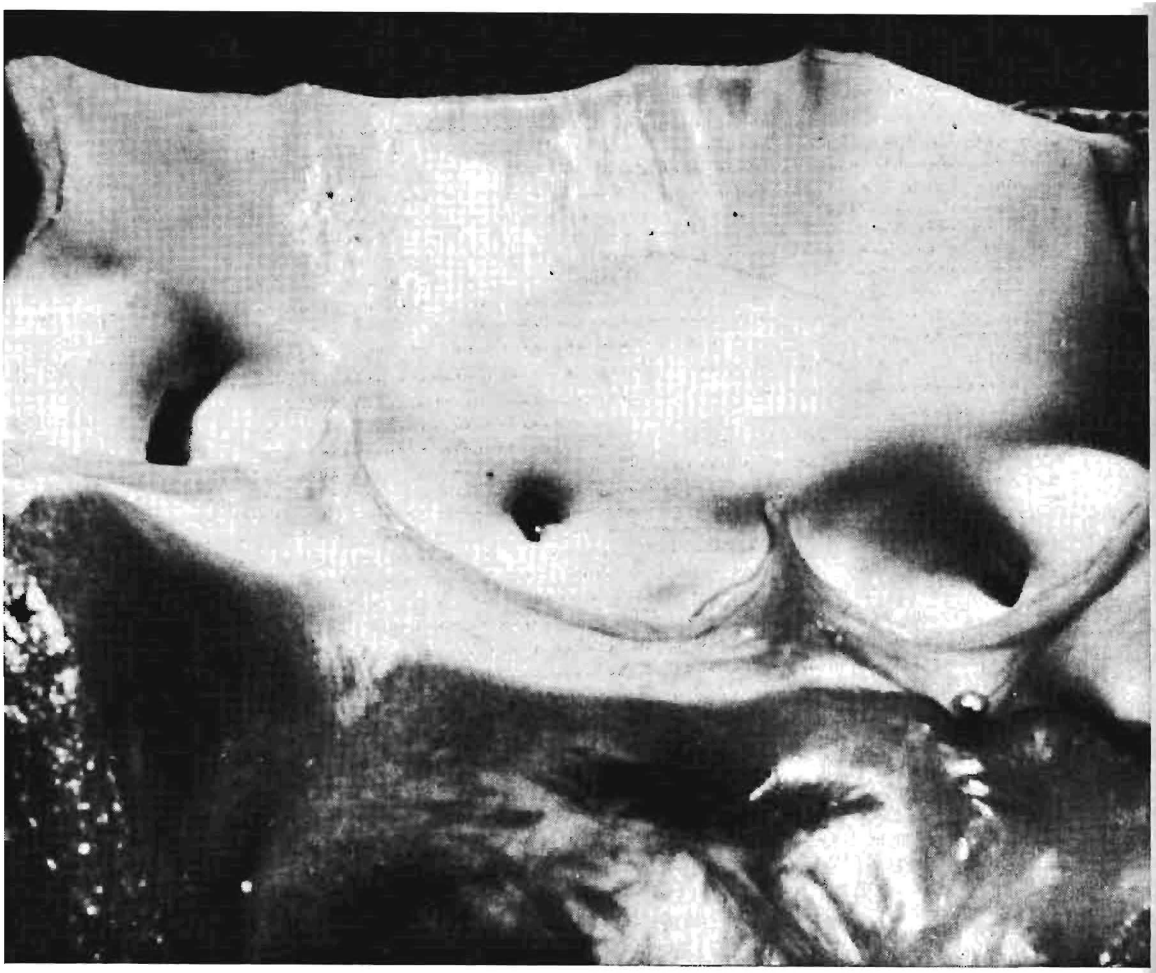

ELAND:

AORTA WITH THREE CORONARY OSTIA

P. A. Basson and R. M. McCully, Dept. Pathology
Veterinary Research Institute, Onderstepoort.

Contrary to the occurrence of two coronary ostia in our domestic animals and man, the eland (**Taurotragus oryx**) has three, one in each aortic sinus (see plate). Of seven eland of both sexes that have so far been examined specifically for this anatomical feature, this was a constant finding. Four kudu (**Tragelaphus strepsiceros**) and four blue wildebeest (**Connochaetus taurinus**) have subsequently been autopsied and one out of each group also had three ostia. In one kudu bull the cranial sinus contained six small ostia and the right caudal none. A number of gemsbuck, springbuck, buffalo, zebra, giraffe, impala, red hartebeest and elephant that have been examined to date all had only two coronary ostia.

ELAND:
AORTA MET DRIE KORONÊRE OSTIA

P. A. Basson en R. M. McCully, Dept. Patologie,
Veeartsenykundige Navorsingsinstituut, Onderstepoort.

In teenstelling met die voorkoms van twee koronêre ostai by ons huisdiere en die mens, is daar in die eland (**Taurotragus oryx**) drie, een in elke aortiese sinus (sien foto). Uit 'n totaal van sewe elande van beide geslagte wat tot dusver spesifiek vir hierdie anatomiese verskynsel ondersoek is, was dit 'n konstante bevinding. Vier koedoes (**Tragelaphus strepsiceros**) en vier blou wildebeeste (**Connochaetus taurinus**) is vervolgens ook nadoods ondersoek en een uit elke groep het drie koronêre ostia gehad. In een koedoe bul is ses klein ostia in die kraniale aortiese sinus gevind en in die regter kaudale sinus geen. 'n Aantal gembokke, springbokke, buffels, zebras, kameelperde, rooibokke, rooi hartebeeste en olifante wat dusver ondersoek is, het almal egter slegs twee koronêre ostia gehad.