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No. 569
1909
Oct
Previous Paper
8015

NAIROBI LABORATORY REPORT
FOR HALF YEAR ENDING JUNE 30TH 1909

Transmits. Calls attention to valuable experiments
carried out especially as regards East Coast Fever.

P.D.

Print for the Tropical Diseases
Research Fund Paper as soon as
possible + return to me
at once.
H.J.R.

McLeod Print attached
Everyday
13/11

The Report is being included in the
Annual Report of the T.D.R.F. etc.
Send 3 copies of the print to
S. Bagsham. D.F.

at once
H.J.R.

SA

31/11

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[Ed. 4999] Feb. 1910

G. O.
36530
Recd
REGD 6 NOV 09

My Lord,

I have the honour to transmit herewith the report of the work done in the Nairobi Laboratory for the year ending June 30th, together with a covering letter from the Principal Medical Officer.

2. Your Lordship will observe that many interesting and valuable experiments have been carried out, more especially with regard to the causes of East Coast Fever.

I have the honour to be,

Your Lordship's humble
obedient servant,

[Signature]
Governor.

[Signature]

J. A. Principal Secretary of State
for the Colonies,
Downing Street,
London, W.

2611-15

Laboratory Report
Please read up

NAIROBI LABORATORY REPORT JANUARY - JUNE 30, 1909

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For the first four months of the year the work of the laboratory was carried on by Dr. Small during my absence on leave. This period is dealt with by Dr. Small in the annexed report. Owing to the outbreak of small pox and the consequent demand for vaccine a great deal of time during the months of May and June was taken up with vaccine preparation.

Vaccines.

The preparation of vaccine was continued with the strains described by Dr. Small. The prolongation of the incubation period has not been done away with, but, judging by the results of vaccination during the recent outbreak of small pox in Nairobi, the protective value of the lymph is not diminished, though delay in "taking" must always be a disadvantage in dealing with contacts. The strain derived from small pox patients has been carried to the sixth calf and it remains to be seen if there will be the same delay with this strain. During the months of May and June 8064 tubes of vaccine equivalent to at least 32256 vaccinations were issued from the Laboratory.

Congo Floor Maggot.

Kisumu	-	Mabi
Kisumu	-	Kiragu.
Kikuyu	-	Miaya
Kilumu	-	Nyandinik.

The Congo floor maggot was found by Dr. Wiggins in Nairobi gaol. I was able to procure specimens and find that it is well known to the natives apparently all the way from the coast to Uganda.

Smithsonian.

Ornithodoros.

Kisuhili	-	Kipe Papan
Kikumba	-	Katanga
Kikuyu	-	Katanga, Ngumba

A large number of *Ornithodoros* ~~sp.~~ *neubata* were obtained from near Nyeri. The Kishili, Kikumba and Kikuyu recognise this tick, but though some of them refer to immediate effects of the bite - local pain swelling and itching - they do not attribute to the bite any more remote ill effects. The Kikumba say that its usual habitat is beneath stones, especially near rivers and that it is not usually found in native huts except in old deserted ones. The first specimens seen by me in East Africa were caught in the open veldt in the Kidang valley during a mid-day halt.

Of the ticks brought from Nyeri, eight or ten were incubated all night and next morning fed on a monkey, this being repeated with fresh batches every day until forty five in all had fed. As the monkey's temperature remained normal the ticks were apparently not infected. All these ticks were finally sent to Professor Nuttall for use in his experiments.

Haemaphysalis leishmanii.

On June 1st smears from a swelling on a male were sent to the laboratory for examination. Stained by the method of Giardine these showed typical crystals of Rivolta. Other smears were stained by Leishman's method and it then appeared that one was dealing not with a yeast but with a protozoal parasite. Some of the discharge from the swelling was collected in 1% Nitrate

for further study which was continued while the supply lasted, photographs and drawings being made. I have since received the *Annales de l'Institut Pasteur* for May and find that the view that the parasite is a protozoon has already been put forward by Du Loux Thiroux and L'eppez. It will be seen that my observations agree closely with those of the latter authors.

In the smears from the swelling numerous bodies 1.5 μ to 3.0 μ in greatest width, ovoid, oval or circular in shape were seen. In each was a large mass of chromatin laid eccentrically and the rest of the body stained blue, an area near the chromatin being less deeply stained than the rest. The chromatin mass appeared to be situated to one side of an area slightly lighter in colour than the rest of the protoplasm. A few of these bodies were free between the cells but the great majority were contained within the large mononuclear and polymorphonuclear leucocytes. Many were in groups closely formed by the breaking down of a leucocyte, the group consisting of parasites and nuclear material from the leucocyte. Some of the leucocytes contained only one or two parasites but many were crowded with them containing as many as 10 or 12, one broken down group (v. fig.) showing 24 parasites. The free form showed a well marked capsule but this was rarely seen in parasites still within the leucocytes, the most as a rule to be seen in the case of these latter being a clear area between the parasite and the surrounding protoplasm.

Examination of wet specimens of the cited material

material showed the parasites as large numbers of roundish bodies chiefly within the leucocytes. Within these bodies to one side or end was an area clearer than the rest of the body and within this clear area a dark granule could be seen. The periphery of the parasite did not show any definite capsule. The citrated material was examined daily. For the first three days there seemed to be an increase in the number of parasites, judging however only from the appearance of stained smears made from the material; for no development was ever observed although fields showing many parasites with very active granules were kept under observation for several hours. No sign of budding was ever found either in fresh or stained specimens nor was there ever any hint of a flagellated form. After the third day there came a gradual diminution in the number of the parasites and disappearance of the leucocytes so that latterly only free parasites could be seen. After the ninth day the citrated material became covered with moulds and further observation was impossible, but up to and including the ninth day there could still be seen live parasites - that is parasites in which the granule could be seen to be active. The individual parasites whether seen in stained or in wet specimens had exactly the same appearance on the ninth day as had those seen on the first day.

The appearance of the parasite when stained with Leishman was so clearly that of a protozoan that the question at once suggested itself "Is the parasite" present in the leucocytes due to phagocytosis or

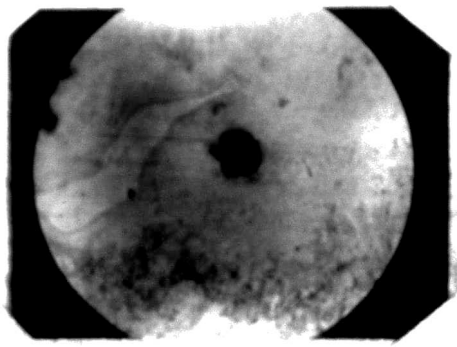
4. *Parasites in polyoma-like bodies*



c.

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5. *Parasites in polyoma-like bodies*



1954

the leucocytes so frequently become so crowded with parasites as to be destroyed made it seem more probable that the parasite developed within them. Had the presence of these bodies within the leucocytes been due to phagocytosis one would be inclined to expect that since such a very marked phagocytosis was set up by the presence of the parasites, removal of all viable disease by scraping cavity or excision would result in cure of the disease, the phagocytes being able to deal with whatever parasites might be left behind. But this is of course but seldom the case, the disease frequently recurring further up the lymphatic. These observations agree with those of Thiroux and I have seen that they tried to grow the parasite on various media and I tried to get Bordet's in a sterile solution. In their attempts as in mine there was an apparent increase at first, perhaps only due to escape of the parasites from the leucocytes and not a real increase. In my case observation of individual parasites prolonged over several hours failed to detect any sign of development.

The French Writers further note a similarity between this parasite and *Helicocoma tropicum*, they offer an explanation of the formation of the capsule seen in frozen specimens - that it is due to coagulation of the albuminous material in which the parasites are bathed and retraction of the parasite from this coagulated wall - and finally they name the new "Leucocytocoon piroplasmoides".

Trypanosoma transmission experiments.

The three experiments on transmitting *T. Gambiense* have been ended with a view to carrying out experiments on the lines of Kleins's work. Of these experiments one - interrupted feeding with *G. longipennis* - was ended by the death of the monkey - not of trypanosomiasis - after 297 flies had been fed during a period of 252 days.

In the second experiment (half hour interval) 232 *G. longipennis* were fed during a period of 184 days. This monkey is alive and uninfected.

In the third experiment (half hour interval) 237 *G. Fum.* were fed during a period of 195 days. This monkey is also alive and uninfected.

East Coast Fever.

I first saw Koch's granules or as we call them locally " blue bodies " in 1904. As a result of many examinations I came to the conclusion that the presence of these bodies was pathognomonic of the disease and for some years a positive diagnosis of East Coast Fever has been given only when these bodies were found. As Koch stated that these bodies are present in spleen and glands before the intracranial parasites appear in the peripheral blood, Dr. Small devised a method of spleen puncture which has been made considerable use of locally. This procedure has been of the greatest use to determine

a diagnosis in doubtful cases where only a few ring and red parasites could be found in blood smears. Before this method was introduced such cases were regarded as suspicious until they either died or recovered. If they recovered the presumption was that they had been suffering from *P. mutans*; if they died blue bodies would almost invariably be found in the spleen. In only two cases in my experience were these bodies absent from the spleen after death of animals whose blood had showed a high infection with ring and red parasites. A further use might be made of Dr. Small's method in determining what percentage of animals attacked by East Coast Fever in this country recover. Dr. Small also arranged for smears from spleens of cattle slaughtered at the local abattoir. In one of his series of 168 smears and in two of my series of 120 smears blue bodies were found; in the latter cases the temperatures unfortunately were not sent.

Shelby H. Ross

Bacteriologist

August 14 1909

HUMAN EXAMINATIONS.

January 1st to April 30th. May 1st to June 30th.

Negative	42.		19.
Malaria.			
Sub-tertian	4.		6.
Benign tertian	2.		2.
Quartan	2.		2.
Differential leucocyte counts			
(a) Large mononuclear increase & pigment 4.			2.
(b) No L.M.N increase non pigment..... 3.			19.
Sputa for tubercle	1		1.
- 7			4.
Proctum exam	- 1.		0.
Fecal tests	4.		4.
- 4.			1
Plaques	1.		
- 2.			
Gonorrhoea	- 4.		
Urethral exam	2.		5.
- 1			
Stools	1		
Urine	2.		
		Gonorrhoea	1
		Chemical & microscopic	11.
		Bilharzia	1.
Total C.O.	<u>87</u>		<u>78</u>

Human Examinations. Contd.

Total B.F.	87.	78.
Feces	1.	1.
Urine	1.	-
Water Analysis	6.	4.
Aspirates	-	1.
Sections of tissues	1.	8.
Various	-	2.
<hr/> Total.	<hr/> 96	<hr/> 94.

VETERINARY EXAMINATIONS.

January 1st to April 30th.

May 1st to June 30th.

Negatives	647	318.
Simulium.		
1 cattle. Rings & rods.	58.	24. (of which 1 showed P. bigeminum & 1 Spirochaetes in addition)
Hyalobodies	56.	In smears P.M. 23. (2 P. bigeminum)
		By Spleen pure ture 23 (2 P. bigeminum)
P. Bigeminum	4.	In blood. 3.
"	0	P.M. 2
"	0.	Spleen pure tures 2.
Marginal points	1	
<hr/> Total	<hr/> 796.	<hr/> 395. 0.0.

	B.P. Total	796.		396.
<i>P. equi</i> (donkey)	2		(mule)	1
<i>P. ovis</i>	1			
<i>P. canis</i>	1			2
Trypanosomes Cattle	1			6
Horse	16		Donkeys	2
Mule	1			-
Anaemia	14			13
Ulcerative lymphangitis	23			4
Tubercle (fowl)	2			1
Spirchaete mule	1			
Ostrich (nil)	1			
Anthrax (ox)	1			
Spleen smears from Slaughter house.	-			120.
Epi oeth lymphangitis	-			1.
		<hr/>		<hr/>
		860		545
Sentient Texas Fever Spleen	1		Ulcerative Lymphangitis	1
Tubercle Guinea pig	1			
Fowl Liver	1			
Anthrax Spleen & Kidney	2			
Calcified glands of ox	1			
Pharyngeal	1			
Thickened large intestine	1			
Heated lung sheep	1			
" " calf	1			
Liver of fowl (T.B.)	1			
		<hr/>		<hr/>
Total		871		546

GAME.

Jan 1 to April 30

GAME.	Result	Negative	Total
Thomson's Gazelle	-	4.	4
Vongoni	Anaemia 2	-	4
"	Cysticercus 1	1	
Roodbuck		3	3
Celebes Monkey	Spiræ hæmæ 1	-	1
Waterbuck		1	1
Jackson's Hartbeest		1	1
Elephant		1	1
ebra		1	1
D.iker		4	4
Small green monkey Haemaphysalis		2	2
Small green monkey Haemaphysalis	1	-	1
Sykes monkey	1	-	1
Bush pig		1	1
Gribi		1	1
Hare		1	1
Wild Pigeon Halteridium	1	-	1
Green "		2	2
Stork		1	1
Florian Proteosoma	1	-	1
G.Fowl		3	3
L.Bustard	Proteosoma 1	-	1
Spur Fowl		1	1
Red Winged Partridges	Leucocytozoon 1	-	1

May 1st to June 30th

Chinese fowl	Halteridium	5	1	6
Carbon			5	5

Total

48

17

NAIRNBI LABORATORY REPORT

January 1st to April 30th 1909.

The experiments in transmission of T. Gambiense by means of G. Fusa and Longipennis as described in former reports have been continued.

- | | |
|--|------------------|
| (1) G. Fusa with half an hour interval | (142 flies used |
| (2) G. Longipennis " " " | (125 " " |
| (3) " " interrupted | (141 " " |

These experiments have been up to the present time unsuccessful.

Some difficulty has been experienced in maintaining a sufficient supply of monkeys for experimental purposes owing to the high mortality during transit and shortly after arrival.

Suitable hutches have now been obtained and should help to obviate this difficulty.

Ymains. It was noticed that the incubation period of the strain of lymph in use here was unduly prolonged. Fresh seed lymph was immediately ordered for and was expected to arrive on April 22nd. A supply was also sent for from Dar-es-salaam and 100 tubes of glycerinated lymph prepared in Dresden obtained.

In the meanwhile an attempt was made to exalt the virulence of the old strain by direct inoculation through a series of calves, and afterwards two calves were inoculated from particularly good vesicles on healthy human subjects.

These methods were only partially successful in that although the vesiculation became more typical the incubation period still remained longer than normal. On the appearance of small pox in the country a calf was inoculated with lymph obtained from several bad confluent cases.

The fourth calf of this series has now been reached but it is too early to make any report about this strain.

Two small tubes of dried lymph from the "Institut Sere - therapeutique and vaccinal Suisse" at Bern were received and a series of three calves inoculated, the result being unsatisfactory this strain was discontinued. Some "Ivory Points" obtained locally were tested on the human subject and gave no reaction. The vaccine at present issued by this Laboratory is derived from the following sources:-

- (1) Lister Institute (the original strain).
- (2) Dresden lymph from Dar-es-salaam.

During the period covered by this report lymph equivalent to 71706 tubes has been issued of which 1,200 were supplied to Zanzibar.

EAST COAST FEVER.

Several small outbreaks of this disease have occurred with a view to showing that Koch's granules or blue bodies are not found in spleen smears from cattle not suffering from East Coast Fever all animals slaughtered in Nairobi since last December have been so examined. Blue bodies were found in several cases and as all those

animals were examined previous to being slaughtered by an experienced Inspector they may be presumed to have been apparently healthy. Arrangements were then made with the Chief Veterinary Officer to have the temperature of each such animal taken and recorded on its respective slides.

During the first four months of the year 168 such slides were examined, blue bodies being found in one only whose temperature was 107 F and whose red cells showed a large infection of rings and rods.

As the diagnosis of East Coast Fever has been for some time in this Protectorate based on the presence of blue bodies, particularly in the spleen and a means of diagnosis during life being desirable a method of spleen puncture has been elaborated. This operation is simple and bad results have not yet been reported. Up to the present 162 such examinations have been made by Veterinary Officers and after a little instructions by Stock Inspector also. In several cases repeated punctures have been made on the same animal apparently without resulting. The method is as follows:-

The hair being removed from the spot and the skin cleansed a small stab is made through the cuticle with a scalpel about an inch internal to the outer border of the longissimus muscle in the second intercostal space from the costal margin. A fairly large bore hypodermic needle is inserted into the cut and driven sharply inwards and forwards. The capsule of the spleen being somewhat tough it found convenient to drive the needle in with a sharp tap to avoid simply

the capsule and pushing the spleen aside. The syringe is then attached and a little fluid drawn into the barrel. The needle is then withdrawn and the fluid squirted into a sheet of white paper on which the spleen pulp is easily seen and a satisfactory smear of it can be made. The whole operation should of course be performed with due attention to cleanliness. Of these 162 puncture 62 were taken from the survivors of a herd of infected cattle some three weeks after the last death, no blue bodies were found and rings and rods in only two. In 47 taken on a farm from which several cases at long intervals had been reported, one showed bodies which although not typical were strongly suggestive of blue bodies; the animal was in very poor condition; four showed rings and rods. In an animal placed on infected ground blue bodies were found on the first day of the disease.

Ulcerative Lymphangitis. This disease is still prevalent, the bacillus having been found in 23 examinations. Acting on a case reported by Valtier in which improvement had followed the administration hypodermically of antidipteritic serum the Chief Veterinary Officer obtained a supply and the investigation of its value is now being carried out without however much success. An attempt was made to procure a high state of immunity to this organism in two donkeys. Unfortunately however before the conclusion of the experiment the animals died of an intercurrent disease. Two bullocks were also inoculated and a local lesion only produced in both cases from which the bacillus was recovered.

Experimental work in this and other directions has

been greatly impeded by the large amount of routine work and latterly by necessity of vaccine production on a large scale.

~~Sd/~~ H. Small
Acting Bacteriologist.

P.M.O's Office
Mairbh,
22nd September 1909.

36530
REC'D
REGD 6 NOV 09

Sir,

I have the honour to transmit the half yearly report on the work done in the Laboratory for the six months ending June 30th. The delay in forwarding the Report has been due to my absence on inspection duty.

The work of the laboratory was much interfered with owing to the outbreak of small pox, which practically confined for many weeks, the whole efforts of the staff to the preparation of vaccines.

The Trypanosom experiments mentioned in the last report - namely those conducted with a view to determine how long the Tsetse flies, *Glossina* *Fusca* and *Longipennis* could carry the infection of Trypanosom Gambiense - have been abandoned. The net result of the experiments is negative; that is to say, if there is any delay in transferring an infected *Fusca* or *Longipennis* to a clean animal, Trypanosom Gambiense does not assert itself in the blood of that animal.

The range of the *Gnathodorus Moubata* (the tick that carries spirillum fever) and the *Cheyletiella* *Wigglesworthi* has been further extended. It is more than probable that both these will be ultimately proved to inhabit all parts of the *Protonotaria*. The danger of this lies in the risk of the ticks becoming infected with the *Spirillum* *Duttoni*, and so spreading tick fever. In the last four years

Secretary,
Administration,
Mairbh.

years, only one sporadic case has been brought to notice.

The cause of the epizootic lymphangitis described by Dr. Ross, is, in his opinion, due to a definite protozoan which he describes at some length as inhabiting chiefly the leucocytes. Though efforts to cultivate the parasite in media failed, he does not consider that their presence in the leucocytes is merely accidental i. e., due to phagocytosis. An attempt to immunize donkeys so as to obtain a serum for injection proved abortive.

Investigations into the cause of East Coast Fever have been continued. Both Dr. Ross and Dr. Small, who devoted a good deal of time to the subject, agree that the presence of Kmh's granules or "blue bodies" in a spleen smear taken from a dead animal are pathognomonic of East Coast Fever. Following up this idea, Dr. Small instituted a method of spleen puncture in the living animal; an operation which, though looked upon with suspicion on the human subject, can be apparently performed with impunity on cattle. The value of this method of diagnosis can hardly be overestimated, as it enables an operator to at once decide whether a herd is infected or not, or what animals will succumb.

The usual statistical tables are appended detailing the ordinary routine examinations performed both medical and veterinary.

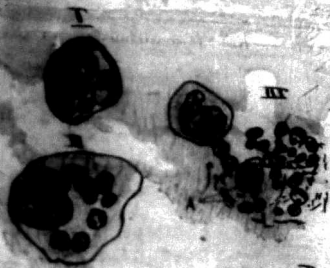
I have, the honour to be,

Sr.

Your obedient servant,

D. B. F. W. S.

Principal Medical Officer.

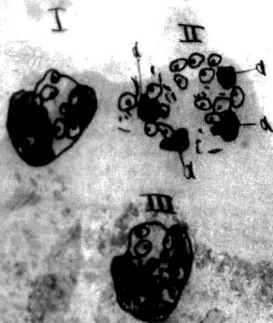


A Remains of nucleus

I and II Parasites in polymorphonuclear and large mononuclear leucocytes
Freshly taken smear

After five days in citrate solution

276
 cap 7
 Lacks of Deep 24
 206 Colonial Mice
 by N. M. ...



A Portions of nucleus of leucocyte

I and III Parasites in leucocyte
II Leucocyte broken down releasing parasites