

## Studies of the Rickettsias of the Typhus- Rocky-Mountain-Spotted-Fever Group in South Africa. I.—Isolation of Strains.

By R. A. ALEXANDER, J. H. MASON and W. O. NEITZ,  
Section of Protozoology and Virus Diseases, Onderstepoort.

RESEARCH work into heartwater, a disease of sheep, goats, and cattle caused by *Rickettsia ruminantium* (Cowdry 1926) has been hampered to a very great extent by the lack of a susceptible small laboratory animal. Up to the present all efforts to infect the guinea-pig, rabbit, rat, and mouse have been quite unsuccessful. It was, therefore, decided to procure one or more strains of the typhus group of rickettsias, which are pathogenic for guinea-pigs, to serve as a positive control for certain technique and experimental methods. During the course of this work other rickettsias were encountered and ultimately were compared with a strain of fièvre boutonneuse kindly supplied by Dr. Balozet of Tunis. The present series of papers is presented to record the observations that have been made, in the hope that the data may be of value in helping to clarify the somewhat confused position of the South African rickettsias in the current literature.

### A. STRAIN "APPLETON".

On May 5th, 1936, an assistant reported that a dog, belonging to him, had developed biliary fever and made a request for the animal to be treated. The history supplied was that 9 days previously the dog, a smooth-haired Fox Terrier puppy about 8 months old, had accompanied the owners to a picnic held on the banks of a river some sixteen miles north of Pretoria. During the following week many partially engorged ticks were noticed on the animal; these were removed from time to time by hand and were destroyed without identification. The dog then became ill; the symptoms described were listlessness, anorexia, anaemia, and fever. In spite of the history, which strongly supported a diagnosis of infection with *Piroplasma canis*, treatment was refused pending an accurate diagnosis and the owner was instructed to submit bloodsmears on the following day. A thorough examination of these preparations, stained by Giemsa, failed to reveal the presence of any piroplasms, nor was any sign of anaemia apparent. The owner was then instructed to present the dog for clinical examination. The puppy,

when seen the following morning, was in good condition but dull and disinclined to run about; temperature  $103.5^{\circ}$  F.; respiration slightly accelerated; pulse rapid, full, and not bounding; conjunctiva pink; urine normal; slight watery discharge from the eyes and nostrils; in addition it was reported that the patient had vomited several times during the night and had developed diarrhoea. Blood-smears were taken and revealed the presence of an occasional basophilic and polychromatic erythrocyte but no piroplasms. In the afternoon the temperature had risen to  $105^{\circ}$  F. so blood was taken from the jugular vein and injected, in 3 c.c. amounts, intraperitoneally into each of 3 guinea-pigs. The dog was returned with a tentative diagnosis of distemper and symptomatic treatment prescribed. Two days later it was reported that the dog had made an uneventful recovery.

The following is the history of the inoculated guinea-pigs:—

1. *Guinea-pig 1* showed a sharp rise in temperature to  $105^{\circ}$  F. on the afternoon of the 4th day; on the following morning the temperature had returned to  $\pm 103^{\circ}$  about which level it fluctuated for 7 days when the animal was killed by ether anaesthesia and a saline emulsion of the brain divided equally between two guinea-pigs by intraperitoneal injection.
2. *Guinea-pig 2*.—The temperature fluctuated slightly below  $103^{\circ}$  F. for 10 days and then rose to  $104^{\circ}$  F. As the guinea-pig appeared to be somewhat dull and flaccid it was killed and the brain injected intraperitoneally into two more guinea-pigs.
3. *Guinea-pig 3*.—During a period of 4 weeks, showed no rise in temperature nor apparent departure from normal health and was discharged.

Post-mortem examination of the two sacrificed guinea-pigs showed no abnormality other than a slight but definite enlargement of the spleen.

All four guinea-pigs of the second generation showed a marked febrile reaction reaching a maximum of  $105.2^{\circ}$  F. after an incubation period of four days. They were killed on the 10th day after injection when the temperature showed a tendency to drop and the condition, subsequently shown to be due to a rickettsia (see Part 2), was maintained by brain-peritoneum sub-inoculation.

#### B. STRAIN "HARE".

On August 1st, 1936, a grey hare (*Lepus saxatilis*) was shot on a farm in the neighbourhood of Premier Mine in the Pretoria District. The hare was placed in a large china basin overnight and on the following morning the ticks which had detached were collected. A total of 12 partially engorged nymphae, identified as *Hyalomma aegyptium* var. *impressum*, were placed on the back of a guinea-pig being confined by a wire gauze cage held in position by

adhesive calico. Only 5 ticks attached and engorged to repletion, the remaining 7 being found dead in the gauze cage; engorgement was completed in 8 days (5.8.36 to 13.8.36). The guinea-pig showed no febrile reaction but from the 7th to 9th day after the attachment of the ticks it was noticed to be dull. On the 9th day it was killed by ether anaesthesia and a saline emulsion of the entire brain was divided between two guinea-pigs, the inoculum being given intraperitoneally. Both guinea-pigs developed a febrile reaction after an incubation period of 4 days and the disease, subsequently shown to be due to a rickettsia (see Part 2), has been maintained by brain-peritoneum inoculation from that time.

#### C. STRAIN "ROBERTSON". (TICK BITE FEVER.)

This strain was obtained from Dr. J. H. S. Gear of the South African Institute for Medical Research, Johannesburg, who in collaboration with Dr. Bevan (1936) has published a preliminary report on the disease in man. It was isolated from the blood of a human patient who became ill after being associated with the deticking of dogs. According to Gear (personal communication) confirmed by a limited number of cross-immunity tests by us, the strain is immunologically identical with several other strains he isolated from human cases on the Witwatersrand. Moreover, Gear and Douthwaite (1939) reported complete reciprocal cross-immunity with an additional strain isolated from a dog tick *Haemaphysalis leachi*.

#### D. STRAIN RAT TYPHUS.

This strain also was obtained from Dr. Gear. Originally it was isolated by Dr. M. F. Finlayson, Public Health Laboratory, Cape-town, who supplied the following particulars:—

"The strain of rat typhus which I gave to Dr. Gear was isolated from the brains of wild rats caught on a farm in the Klein Drakenstein area near Paarl in November, 1935. A case of typhus had occurred on this farm and I saw the case in convalescence but was unable to isolate virus from the blood. Apparently a focus of rat typhus infection occurs in this area as three years earlier a case occurred near Paarl and a strain of murine typhus was isolated from rats by Dr. Rhodes. My strain and that of Dr. Rhodes appear to be identical by cross-immunity tests and reactions. The strain has been passaged in guinea-pigs at ten-day intervals until recently when it was discontinued voluntarily. Takes have been most regular and I have no reason to believe that the strain has altered in virulence since its isolation."

#### E. FIÈVRE BOUTONNEUSE.

The strain of fièvre boutonneuse was isolated from ticks, *Rhipicephalus sanguineus*, kindly supplied by Dr. L. Balozet of the Pasteur Institute, Tunis. The ticks, which had been collected from dogs, were forwarded by ordinary mail in what appeared to be a section of bamboo or cane plugged with cork.

To establish infection in guinea-pigs two methods were adopted:

- (a) Intraperitoneal injection of saline emulsion of whole ticks.
- (b) Feeding ticks.

(a) *Injection of tick emulsion.*—This was the method adopted with success by Pijper and Dau (1936). Before emulsification the ticks were washed in ether for several minutes (ether changed twice) then in sterile saline (changed 4 times); they were then ground up with sterile precautions in a mortar, the requisite amount of saline being added in small quantities from time to time. To eliminate coarse particles the emulsion was spun lightly at 1,500 revs. per minute for 1 minute and the supernatant fluid was immediately injected intraperitoneally into guinea-pigs. The guinea-pigs were temperatured twice daily and subinoculations were made at any time between the 7th and 18th day when a febrile reaction appeared to warrant it. For subinoculation an emulsion of brain only was used, the dose for each guinea-pig being not less than one-quarter of a brain.

Altogether 3 attempts were made to establish a strain in this way without success, as follows:—

1. On 11.10.37 200 completely or partially engorged nymphae, immediately on arrival from Tunis, were emulsified and divided among 6 guinea-pigs. Serial passage through guinea-pigs was carried out for 11 generations without establishing infection, and the attempt was abandoned.
2. On 13.11.37 it was found that 50 nymphae, which had been maintained in a constant humidity (80 per cent.) and temperature (80° F.) room, had recently moulted to adults. These ticks were fed on 4 guinea-pigs; after being attached for 5 days (i.e. partially engorged) they were removed, emulsified and the emulsion divided intraperitoneally among 4 guinea-pigs. Subinoculations were carried out in the usual manner for 4 generations but infection was not established.
3. On 2.12.38 an emulsion of approximately 50 adults and nymphae of a second batch of ticks received from Tunis was divided intraperitoneally among 3 guinea-pigs. On the 5th day after injection one of these guinea-pigs commenced a febrile reaction, which attained a maximum of 105.2° F. on the 8th day when the brain was subinoculated into 4 guinea-pigs. All the guinea-pigs of the second generation succumbed to peritonitis as a result of bacterial infection.

(b) *Feeding Ticks.*—On 11.11.37 about 12 adults were placed on the backs of each of 5 guinea-pigs. The ticks attached readily. All the guinea-pigs showed a slight febrile reaction between the 6th and 10th day after tick infestation though only in one case did the temperature rise above 105° F. Two, which showed the most severe reaction, were subinoculated on the 10th and 11th day respectively, with the following results:—

1. All three guinea-pigs succumbed to peritonitis of bacterial origin.

2. Both guinea-pigs commenced a febrile reaction on the 6th day after injection, the temperature rising to a point above 105° F. on the 9th and 10th day respectively. Both guinea-pigs were destroyed for serial passage on the 10th day and the disease has been maintained by serial brain-peritoneum inoculation.

*Comment.*—The demonstration of rickettsia in smears prepared from the tunica vaginalis of the testes has indicated that a rickettsial infection is being maintained. Morphologically these rickettsias closely resemble those described by Hass and Pinkerton (1936). In spite of this it is admitted readily that there is no direct experimental evidence to prove that the rickettsia infection isolated is that to which the name *fièvre boutonneuse* has been applied in the literature. However, there is little reason to doubt the contention for the following reasons:—

1. Balozet informed us that no strain of *fièvre boutonneuse* is maintained in guinea-pigs in the laboratory at Tunis. Whenever a strain is required for experimental purposes it is isolated from ticks (*R. sanguineus*) collected from dogs at the Institute. It is from these ticks that our strain was established.
2. We have carried out sufficient work at Onderstepoort, involving the serial passage of guinea-pig brain material in guinea-pigs to be reasonably certain that the available stock of guinea-pigs do not harbour a transmissible rickettsial infection.

For these reasons we have not hesitated to label the strain “*fièvre boutonneuse*”. It is quite possible that minor differences in respect of some properties may be established when compared with other strains described in detail in the literature but for the purpose of these studies the North African strain was the one which could be most easily obtained.

#### SUMMARY.

Details are given of the origin and methods of isolation of five strains of rickettsia.

1. Strain “*Appleton*” from a dog.
2. Strain “*Hare*” from ticks *Hyalomma aegyptium* collected from a hare (*Lepus saxatilis*).
3. Strain “*Robertson*” (Tick-bite fever) from a human patient.
4. Rat Typhus from a rat.
5. *Fièvre boutonneuse* from ticks (*Rhipicephalus sanguineus*) collected from dogs in Tunis.

#### BIBLIOGRAPHY.

See conclusion of Part IV.