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Luis Patiño Camargo
(1891-1978)

Clásicos del INS

Con motivo de la celebración de los 25 años de *Biomédica*, el Comité Editorial desea rendir durante este año un homenaje a los investigadores colombianos que trabajaron en el Instituto Nacional de Salud e hicieron aportes destacados al conocimiento de las enfermedades infecciosas en Colombia.

En esta ocasión queremos recordar el trabajo clásico del doctor Luis Patiño Camargo y colaboradores sobre la fiebre manchada de Tobia, publicado originalmente en 1937 en el *American Journal of Tropical Medicine and Hygiene* y reproducido en este número con la debida autorización.

En palabras de sus autores: "El artículo tiene especial interés para los trabajadores de la salud pública en Colombia debido a que esta grave enfermedad ha pasado desapercibida hasta la fecha a pesar de su ocurrencia en lugares muy cercanos a Bogotá, a su carácter transmisible, a que presenta una tasa de letalidad mayor del 90% y tiene un carácter epidémico".

Casi 70 años después, vemos que su afirmación original continúa siendo vigente.

CLÁSICOS DEL INS

A SPOTTED FEVER IN TOBIA, COLOMBIA

PRELIMINARY REPORT¹

L. PATINO, A. AFANADOR, AND J. H. PAUL

Bogotá, Colombia

The spotted fever of Tobia, concerning which we wish to present a preliminary report for the benefit of other workers, was discovered by the Colombian Yellow Fever Service, a cooperative organization of the National Department of Health of Colombia and The Rockefeller Foundation, in the course of its epidemiological field studies.

The present paper is considered of special interest to public health workers in Colombia because this serious disease has hitherto passed unnoticed in spite of its occurrence so close to Bogotá and because it is transmissible, has a case fatality greater than 90 per cent, and has assumed an epidemic character. The paper is also of general interest to scientific workers, because laboratory findings demonstrate that the disease is one of the typhus group of infections not previously reported for this part of South America.

BRIEF HISTORY OF THE FIELD INVESTIGATIONS

In May 1935, the Yellow Fever Service received the first reports of an epidemic in Tobia, through the Director of Hygiene for Cundinamarca. This official supplied the Service with a memorandum prepared by the district school physician, who had observed various cases in the region and who stated that the

¹ The studies on which this report is based were made under the auspices of the National Institute of Hygiene, Colombia (Samper y Martínez), and the International Health Division of The Rockefeller Foundation, in cooperation with the National Health Department of Colombia.

disease could well be considered as yellow fever because of its epidemic character and clinical signs.

A few days after receiving this report one of the members of the Yellow Fever Service was sent to Tobia, but he found no active cases. He limited his activities to collecting epidemiological information and to the securing of samples of blood from contacts. All 29 of the specimens which he collected showed absence of protective antibodies for yellow fever when tested in mice.

In June of the same year, the Service studied several other cases, among which was that of Desgracias Martínez from whom a liver specimen was obtained. The liver showed no lesions characteristic of yellow fever.

In November another epidemic broke out, but again the yellow fever staff were unable to find active cases when they arrived in the district. From observations already made and from the reports of the inhabitants, it was at this time suspected that the disease was of the nature of typhus fever.

Finally, on December 3, the physician of the hospital in Villeta telegraphed that three patients suffering from the disease had just been brought to his institution from Tobia. On this occasion it was possible to carry out more complete clinical studies of various stages of the disease, and a special attempt was made to establish the virus in laboratory animals. Blood and urine cultures on these patients were negative, as were agglutinations with the typhoid-paratyphoid organisms. The examination of blood-smears for parasites and protection tests for yellow fever antibodies gave negative results. From the clinical signs and symptoms and from the behavior of the virus in laboratory animals, a provisional diagnosis was made of a spotted fever of the type of Rickettsioses (1-2). An analysis of these laboratory investigations forms the subject of the present report.

A DESCRIPTION OF TOBIA

The community of Tobia is part of the "township" of Juntas de Tobia in the "county" of Nimaima in the "state" of Cundinamarca. It is situated in latitude 5° 4' North, 0° 24' West of the

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meridian of Bogotá, and lies on the right bank of the Tobia River, at its confluence with the Villeta River. It is 118 kilometers from Bogotá, 78 kilometers from Puerto Liévano, and 8 kilometers from Villeta. The area involved is approximately 10 square kilometers, being 4 kilometers long and approximately $2\frac{1}{2}$ wide. It is limited on the north by the stream known as the Comba; on the east by the ridge called Cocunche; on the west by the Villeta River; and on the south by the Tobia River. The country is rough, with steep slopes rising from the river to the ridges behind. Sharp spurs branch in various directions to form narrow valleys. The altitude of the river bank at this point is approximately 700 meters, while the ridge behind is approximately 1200 meters high. The climate is temperate, and there is a pronounced dry season from July to August and from December to March. The hottest season is in August. Rainfall is variable, with an increasing tendency toward droughts because of the progressive denuding of the land.

The whole area is under cultivation, the crops being chiefly sugar cane, corn, yuca, and bananas. A small part of the soil is left for pasturage. The first crop of corn is sown in March and harvested in June; and the second is planted in August for the November harvest. All of the farmers have dogs, pigs, and chickens, while a few have cats. Only a small number keep horses, mules, donkeys, or cattle.

A census made in the month of August 1936, showed that the area as delimited above supported a total population of 267. There were 149 persons who could be considered as natives to the region, while 118 had settled there from other places. The 136 males and 131 females reside in 43 houses. The age distribution is as follows:

0 to 9 years.....	75
10 to 19 years.....	60
20 to 29 years.....	41
30 to 39 years.....	33
40 to 49 years.....	33
50 to 59 years.....	14
60 to 69 years	7
Over 70 years.....	4

The people are of mixed racial origins, with a predominance of those whose white blood is mixed with Indian. There are no purely negro types, and the amount of negro blood in those of mixed origin is insignificant. The whole population is engaged in agriculture, principally the growing of sugar cane and the production of sugar products.

The community is surrounded by numerous areas of dense population and is in communication with important river ports and cities by railways and roads.

EPIDEMIOLOGICAL OBSERVATIONS

The records of the Yellow Fever Service show that between July 1934 and August 1936, 65 persons were victims of the disease under consideration; of these only 3 recovered. This gives a case-fatality of 95 per cent. Based on the present population of 267 persons, the disease has attacked about 20 per cent of the residents of the area.

The fever shows no characteristic relation to sex, age, or occupation. There were 33 fatal cases among males and 29 among females. The youngest patient who died was 2 years of age and the oldest 70. There was, however, a slight predominance of deaths in those in early youth, as is shown by the following table of fatalities by age groups:

0 to 9 years.....	12
10 to 19 years.....	5
20 to 29 years.....	19
30 to 39 years.....	9
40 to 49 years.....	7
50 to 59 years.....	4
60 to 69 years.....	5
70 to 79 years.....	1

Thirty-four deaths occurred among those who were native to the region and 28 among those who had come from other parts. Cases occurred indiscriminately among the workers in the fields and those employed in or about the houses.

The disease appears and disappears in the community with great irregularity, there being various months in which no cases have been observed. From the records it is possible to say that

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the larger number of infections occurred in the month of July, but considerable numbers are also reported for February, March, April, and August. It is most striking that the disease is very circumscribed in its geographical extension and that it has never passed beyond the strict limits as given above for the community of Tobia. No similar cases were discovered in neighboring rural areas or in the towns nearby, in spite of the fact that many of the victims had been removed to hospitals in Villeta, Bogotá, and Utica.

Another very characteristic finding is that the disease is restricted to certain individual houses. In one large family the disease appeared in September 1934 with 1 death; it reappeared in April 1935 with 5 deaths; in May there was another fatality; in October another. In December the 3 remaining members of the family were attacked, and only 2 of them survived. These 2 women, along with a youth of another house, were the only ones infected who are known to have survived the disease. The Triana family composed of 6 persons was completely wiped out in the month of July. The residents of the region having noticed that the contagion was so definitely localized to houses made a habit of burning the building with all of its contents whenever a death occurred. In this way 19 houses were burnt by the inhabitants.

Our observations show that all the residents and their domestic animals were heavily infested with various ectoparasites. The beds were usually made of rushes or split bamboo and harbored large numbers of *Ornithodoros venezuelensis* Brumpt,² *Cimex hemipterus*, and *Cimex lectularius*. *Pediculus capitis* was found on several persons; and the body-louse, *Pediculus corporis*, and larvae of the family Trombidiidae (harvest mites) were also collected. Ticks of the species *Amblyomma cajennense* F. were recovered from the clothing. Horses and mules carried both *Dermacentor nitens* Newman and *Amblyomma cajennense* F. Dogs were infested with ticks and fleas of the species *Rhypicephalus sanguineus* and *Ctenocephalides canis*. Ectoparasites were not found on the few cattle kept in the area. Blood-sucking mos-

² These classifications were made by Dr. J. C. Bequaert.

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quitoes were relatively rare in the region; only a few samples of adult *Psorophora ferox* Humboldt and *Aedes angustivittatus* were captured.

The systematic burning over of the land during the dry season has practically destroyed all of the wild animal life of the area so that today there remain only a few specimens of the following species: *Dasyurus novemcinctus* (armadillos), *Agouti paca* ("lapas"), *Dasyprocta fuliginosa* ("carmos"), *Trinacodus apollinaris* (field rats), *Cerdocyon thous* ("zorros"), and *Didelphis paraguayensis* ("runchos"). The classification of these animals was made by comparing specimens with the labelled types in the Museum of Natural Science in Bogotá, which had been determined by the Reverend Brother Apolinar.

CLINICAL OBSERVATIONS

The disease is characterized by an exanthema, profound stupor, and general congestion with marked involvement of the central nervous system. There is high fever, rapid pulse, constipation, and albuminuria.

A few patients were seen who were able to describe the onset of the disease. They reported that for about 2 days preceding the high fever they suffered from pain in the joints, dizziness, and general malaise. It appears, however, from other records that the onset is usually more sudden. The duration of illness was from 4 to 10 days, with an average of about 7 days in the 62 fatal cases investigated. In 2 out of the 3 survivors, the disease lasted over 2 weeks, with a prolonged period of convalescence.

The onset of the attack was characterized by headache, muscle and joint pains, chills, and fever. A few persons reported nausea and vomiting. There was always congestion of the conjunctiva and skin, with photophobia. The tongue was coated, dry, trembling, and very red at the borders and the tip. A few patients showed a slight jaundice. Another constant sign was persistent constipation.

Beginning on the fourth day the clinical picture was chiefly interesting because of the central nervous manifestations: lassitude, stupor, and delirium in all its manifestations. In the

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majority of cases it was necessary to tie the patient to the bed. Two of the patients who had been removed to Villeta walked out of the hospital and into the streets, causing great alarm to the populace.

The fever is very high, with slight remissions during the morning; 41.5°C. might be considered the maximum and 39° the minimum in a typical case. The pulse is rapid, running around 140. In the case of the 2 women who recovered from the infection, it was observed that in spite of very high temperatures their pulse rate never reached more than 110 per minute.

The most characteristic sign of the infection seems to be the skin eruption which begins on the third day and rapidly spreads over the whole body. The spots are papular and of various colors ranging from pink through violet to frank hemorrhagic areas. They appear first on the wrist, neck, feet, and face, but soon cover the whole body, including the scalp. On the cheeks the eruption forms a continuous patch of a reddish color. After death the body is completely covered with violet spots.

Albumin in the urine is a constant sign and it usually appears in large amounts. In one case 2.5 grams were found per liter. In 5 cases studied at the hospital a slight increase in the number of white cells (11,000 to 14,200) was observed, with a relative increase in neutrophiles.

LABORATORY STUDIES

1. On December 5, 1935, two guinea pigs were inoculated intraperitoneally at the Hospital in Villeta, each one receiving 5 cc. of the blood of Julia Olaya, who was in the 7th day of her illness and was showing a temperature of 40° and a slight eruption. One of the animals died 3 days after the inoculation and the other after 8 days, both having exhibited a febrile reaction. The autopsies showed: hypertrophy of the inguinal and axillary glands, a blood-tinged serous fluid in the peritoneal cavity, and congestion of several organs. Smears made from the walls of the peritoneal cavity showed endothelial cells containing *Rickettsia* bodies (1, 2). Passages were continued in animals until February 28, by which time the virus had been through 17 ani-

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mals in 9 series. In the earlier passages there was a marked congestion of the testicles in the males and of the genital organs of the females, with *Rickettsiae* in peritoneal scrapings. All the animals inoculated died. Repeated blood cultures on the patient from whom the virus was isolated proved to be sterile. Agglutinations were performed with the serum from the patient in the acute period of the disease, at the beginning of convalescence, and after recovery. They were always negative for typhoid and paratyphoid bacilli and for *B. proteus*.

2. On December 5, 1935, two guinea pigs were inoculated intraperitoneally at the Hospital at Villeta, with 3 cc. of the blood of Benilda Olaya, who was in the fourth day of the disease and had a fever of 40°. Two other guinea pigs were inoculated in the same way, each receiving 2 cc. of urine collected by catheter. The two pigs inoculated with blood became sick on the 3rd and 5th day respectively, showed a fever of 40.5°, and died on the 7th and 8th day. The autopsies showed fluid in the peritoneal cavity, a congestion of most of the internal organs, hypertrophy of the spleen and the suprarenal glands. Up to March 5, inoculations of this virus had been made into 56 animals: 40 guinea pigs, 13 white rats, 2 rabbits, and one *Macacus rhesus*. The virus was introduced via the peritoneum, through the anterior chamber of the eye, and by subcutaneous injection. In the early passages there were abundant endothelial cells of the peritoneum containing *Rickettsiae*; a scrotal reaction in the males; and reaction of the ovaries, tubes, and uterus in the females. Those guinea pigs inoculated with urine showed no reaction nor did they prove to be immune upon injection of virulent blood after an interval of 50 days. The blood cultures on this patient also proved sterile, and agglutinations with *B. proteus* and the typhoid group were negative. This strain of virus killed about 98 per cent of the guinea pigs inoculated and invariably killed the other laboratory animals used. The patient from whom the virus was obtained died on the 6th day of the disease.

3. On January 2, 2 guinea pigs were inoculated intraperitoneally at the National Institute of Hygiene with 2 cc. each of the blood of Gerardo Olaya, who was in the third day of the

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disease, with a temperature of 40.5°, a generalized rash, and acute delirium. The blood had been collected three hours previously in Villeta by Drs. J. M. Montoya and N. Bernal. Six white mice were inoculated intracerebrally with 0.03 cc. of the serum from the same bleeding. The guinea pigs died on the 9th and 12th day respectively after inoculation, having shown a febrile reaction. Autopsy disclosed petechiae on the feet and hairless areas; subcutaneous edema; a bloody, serous fluid in the peritoneal cavity; a congestion of the mesentery, tubes, lungs, and brain. There was hypertrophy of the spleen, suprarenal glands, and kidneys. In one of the guinea pigs there was an abundance of endothelial cells containing *Rickettsiae*. This strain of the virus is being carried along in the laboratory at the time of writing. Thirty-four passages in a total of 97 animals have given a mortality of 98 per cent. The white mice showed no response to the inoculations. Blood cultures of this patient were negative, as were also the agglutinations. The patient died on the 5th day and an autopsy was performed.

4. On January 2, 2 guinea pigs were inoculated intraperitoneally with 2 cc. of blood from Obdulia María Díaz. Six white mice were given 0.03 cc. of her serum intracerebrally. The patient had been bled about three hours previously in the Hospital at Villeta by Drs. Montoya and Bernal. She was in the fourth day of illness, with a temperature of 39.8°, a beginning eruption, and nervous symptoms. Both guinea pigs died late in the fifth day. This virus was carried for six passages through guinea pigs, only one of the 9 animals used having survived. The characteristic findings of orchitis, and endothelial cells containing *Rickettsiae* were observed in only the first passages. No reaction in the white mice was noted. Blood cultures and agglutinations with the patient's serum were negative. The patient died on the 7th day, and an autopsy was performed.

5. On January 5, blood was obtained from Rosa Rodriguez who was visited on her farm and was in her second day of illness. Two guinea pigs were inoculated with 2 cc. each of this blood. The first guinea pig died on the 3rd day and the second on the 9th day after inoculation. Autopsies showed orchitis and intense con-

gestion of the internal organs. The virus was passed through guinea pigs 3 times and one of the 6 animals used recovered. The patient was removed to the Hospital of San Juan de Dios in Bogotá, where she was under the observation of Dr. Alfonso Rueda, director of the clinical laboratory there. Dr. Rueda's findings were as follows: blood cultures, negative; 2 grams of albumin per liter of urine; febrile reaction in guinea pigs, all of which died from the 7th to the 9th day after inoculation. The autopsies on the guinea pigs revealed orchitis with effusion in the tunica; peritoneal effusion; congestion of the spleen, suprarenal glands, and meninges. A study of the sections showed congestion of the liver and spleen, with beginning fatty degeneration in the former. The kidneys showed a subacute nephritis, and there were areas of emphysema in the lungs. Sections of the testicles showed a vascular congestion, with hyperplasia of the glandular epithelium. The ovaries showed a congestion of a subacute inflammation. The agglutinations with the serum from this patient were all negative. She died on the 7th day after the onset of the disease, and an autopsy was performed.

6. On January 8, during the autopsy of Obdulia María Díaz, 5 head lice (*Pediculus capitis*) were collected. They were washed with dilute alcohol and salt solution and then triturated for injection into a female guinea pig via the peritoneum. The animal died on the 11th day, after a marked febrile reaction. The autopsy showed peritoneal effusion and congestion of the tubes, spleen, suprarenals, and cerebrum. The virus was passed three times through guinea pigs, none of which survived. This strain of virus was abandoned because of a shortage of guinea pigs.

7. On January 12, 2 guinea pigs were inoculated intraperitoneally with a suspension obtained from the trituration of lice (*Pediculus corporis*) taken from healthy persons in the area of Tobia. These animals were observed for 2 weeks and showed no reaction.

8. On April 25, some bed-bugs (*Cimex hemipterus*) were collected in the bed of Marcos Morera, a convalescent, and were ground up in saline and inoculated into a guinea pig. The animal showed no response for 60 days, at the end of which period he was reinoculated with the virus and died.

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9. On the same day and in the same way, some ticks (*Rhipicephalus sanguineus*) from a dog belonging to Dominga and Julia Olaya, 2 convalescents, were injected into a guinea pig. The guinea pig having shown no response for 60 days was reinoculated with the virus and died.

10. Using the same technique, and on the same day, a guinea pig was inoculated with an emulsion of horse ticks (*Amblyoma cajennense*) collected on one of the farms. The pig showed no response for 60 days and showed no immunity when reinoculated with the virus.

DISCUSSION

An epidemic disease of very high mortality has been discovered in a rural region of Colombia. It has been confined in its ravages to a strictly limited area. It is a febrile disease of the typhus group and is characterized by an exanthematic eruption. However, because of its epidemic characteristics, its clinical manifestations, and its behavior in experimental animals, it must be considered as distinct from the exanthematic typhus (3) which appears from time to time in Bogotá and in other cities of the Andean plateau. In some respects the disease resembles the Rocky Mountain spotted fever of North America (4, 5) and the typhus fever of São Paulo, Brazil (6), but the resemblance is not complete. Blood cultures taken from the second to the seventh day of the disease give uniformly negative results. The agglutinations of sera with the typhoid and paratyphoid group of organisms, as well as with proteus X19, XK, and XO, performed at the National Institute of Hygiene, were also negative in all dilutions, in 2 cases as long as 2 months after the onset. The 43 specimens of serum tested for protective antibodies against yellow fever virus were also negative. Naturally these were chiefly from contacts and not from the patients themselves. All attempts to recover the virus of yellow fever through intracerebral inoculation of mice were negative. There were 2 complete human autopsies, one man and one woman; these showed congestion of the spleen, kidneys, and suprarenal glands. The congestion was very intense in the meninges. In the case of the man, practically

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all of the left cerebral hemisphere was covered by a sanguinous coagulum. The woman showed intense congestion of the genital organs.

The fever is transmitted from patients to animals by the injection of blood, and the virus may be conserved indefinitely by passages in guinea pigs and other laboratory animals. Injection was followed by death in white rats, rabbits, and *Macacus rhesus*. Over 96 per cent of the guinea pigs infected with blood or emulsions of the internal organs succumbed. The reaction in animals is manifested by high fever, congestion of various organs, and death between the 6th and 9th day of fever.

The period of incubation in guinea pigs inoculated with blood is ordinarily 4 days. If they are injected with blood from a guinea pig in its first day of fever, the incubation period is as short as 2 days, while if an emulsion of organs from an infected guinea pig is used, 3 days usually elapse before symptoms are shown. On the average there is a fever for 4 days, although in a few instances this extended to 2 weeks. In the earlier cases studied the male guinea pigs showed a characteristic scrotal reaction, with hemorrhagic spots in the skin, and edema and inflammation of the testicle, with congestion and inflammation of the seminal vesicles. The females showed congestion and inflammation of the external genital organs and the breasts as well as the internal organs.

Using the method of Laveran and Giemsa, it was possible to demonstrate the presence of bodies of the type of the *Rickettsiae* in the endothelial cells taken from peritoneal scrapings. The reaction in the genital organs and the presence of *Rickettsiae* became much less marked after the first passages through animals. The most outstanding findings at autopsy in the animals were: edema of the subcutaneous tissues; hypertrophy of the axillary and inguinal glands; serous effusions (sometimes blood tinged), hyperemia, and punctate hemorrhages of the mesentery. The spleen, kidneys, and suprarenal glands generally showed an increase of volume and congestion. A less frequent finding was congestion of the lung and cerebral centers. Histologically the liver showed pronounced congestion of the veins, chronic diffuse

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hepatitis, and beginning fatty degeneration; the kidneys showed subacute and chronic nephritis; the lungs, areas of emphysema; the testicle, vascular congestion and hyperplasia of glandular epithelium; the ovary, subacute and chronic inflammation.

A *Macacus rhesus* inoculated with 2 cc. of blood from a guinea pig in the second day of fever (5 days after inoculation) showed a high temperature from the 4th day and died on the 8th. The inoculation was made through the peritoneum. In the period of fever it showed a rash on the face and pelvic region, and at autopsy lesions characteristic of congestion.

White rats were treated by x-ray irradiation and by the injection of benzol until their white cells were below 1000 per cubic millimeter (7). When these were inoculated with blood or emulsions of organs they invariably died between the 5th and 7th day, showing intense congestion and hemorrhage. From some of these there were intra- and extracellular organisms in the cells scraped from the peritoneum.

It is a strange coincidence that in the narrow little valley of Tobia there exists the insect vector of several of the typhus group of diseases. For example: *Amblyoma cajennense*, the transmitting agent of the typhus fever of São Paulo (8, 9); *Rhypicephalus sanguineus*, the vector of "fièvre boutonneuse" of the Mediterranean; larvae of the genus *Trombicula*, which genus has been incriminated as the vector of tsutsugamushi fever in Japan (10). Nevertheless, various epidemiological considerations lead us to think that perhaps the vector involved in the present epidemic might well be found in a parasite of the beds and that among these the one to be considered first of all should be the *Ornithodoros*.

Various methods of treatment were tried out on the few patients who were under the care of physicians, but without encouraging results. Of the 11 patients admitted to hospitals, 8 died and 3 survived. The 54 patients of whom we have records who remained on the farms all died.

In giving this paper its title, we did not wish to imply that the disease is limited to the community of Tobia, but simply to make note of the fact that up to the present time this is the only spot in the country in which this fever is known to exist.

CONCLUSIONS

1. There exists in Colombia a high fatal febrile disease characterized by an eruption. It is undoubtedly one of the "Rickettsioses" and has up to the present not been described in this part of South America.
2. It is a distinctly rural disease and, as far as is known, is restricted to a single narrow valley.
3. It differs from classical typhus fever.
4. It can be transmitted experimentally from the human being to laboratory animals by the inoculation of blood.
5. The disease has a case fatality of about 95 per cent in human beings and is experimentally fatal for 96 per cent of the laboratory animals used.
6. Head lice collected from a cadaver were found to be infected.
7. The spotted fever of Tobia constitutes an interesting new problem in public health.

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