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(Continued from page 645, vol. vi.)

MEDITERRANEAN FEVER IN GOATS, COWS, AND OTHER ANIMALS.

BY STAFF-SURGEON E. A. SHAW.

Royal Navy; Member of the Mediterranean Fever Commission.

I.—Goats.

A.—Experimental.

To determine experimentally to what degree goats, which are so numerous in Malta, are susceptible to Malta fever, I determined, in July, 1904, to inject cultures of Micrococcus melitensis subcutaneously into these animals. Thinking that possibly there might be a difference of susceptibility between the mature and immature animal, I began with experiments on a goat and a kid. On July 30th, 1904, a female goat, two years old, and a female kid, three months old, were purchased, their blood was examined for agglutination reaction to M. melitensis (which was found to
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be nil), and they were kept under observation for a week. They were then dealt with as follows:

Experiment 1.—Goat, 2. July 30th, 1904, no agglutination reaction; temperature normal, practically between 101° and 102° F., till August 8th, 1904. Injected subcutaneously at noon of that day into left flank an emulsion of the six-day growth of M. melitensis on six agar slopes, second generation, from spleen of human fatal case. This caused a rise of temperature of 3·8° F., from 102° to 105·8° F. on the 9th, and 107° on August 10th, then gradually falling back to normal by August 14th.

Agglutination.—Agglutination reaction was first present on August 13th, 1 in 30; rising to 1 in 200 on August 14th, and 1 in 1,800 on August 20th, on which date the goat received a second injection of the growth from four similar agar slopes of M. melitensis, which caused a similar rise of temperature for three days. On August 29th it received a third injection of emulsified growth from four more slopes. On August 30th the agglutination reaction was 1 in 2,200, and on September 5th, 1 in 3,200. All these were visible under 3-in. objective in fifteen minutes.

Urine.—This was plated daily, after having been drawn off into a sterile vessel by a sterile catheter, which I passed myself from August 25th to September 6th, ½ cc. of urine being distributed over the surface of glucose-nutrose-litmus-agar contained in Petri dishes, which were incubated for six days at 37° C. On September 6th, 1904, I went on three weeks' leave. On my return experiments were resumed by me on September 30th. A fourth subcutaneous injection of M. melitensis growth from six agar slopes as before was made on October 10th, with the idea of giving the goats' kidneys plenty of the parasite to excrete. The experiment was continued till October 31st, and was then given up, no M. melitensis having been at any time recovered from the urine of this animal.

Milk.—This was first plated for recovery of possible M. melitensis on August 28th, 1904, but on September 1st the plates were found to be completely overgrown with a Staphylococcus, and on centrifuging the milk from each udder and making film prepara-

1 [It should be stated that the first of these experiments was briefly described by the author in the manuscript of the Report by him published in the first part of the Reports of this Commission (March, 1905), but the paragraph was deleted at his request, as the observations were, in his opinion, still incomplete, though he recognised and stated that the "goats, which are extremely numerous in Malta, might possibly be instrumental in transmitting the infection of Malta fever."—Sec. R.S.]
E. A. Shaw

... from the deposit, I recognised pus in the milk from each udder. On August 28th, also, feeding experiments with the milk of this goat were commenced on a healthy monkey (No. 61), a stomach tube being passed and 1 oz. given on this date, 2 ozs. on August 29th, 4 ozs. on the 30th, and 4 ozs. on the 31st; but such severe diarrhoea was developed that no more milk was given after the last date. This monkey, unfortunately, succumbed on September 4th. The usual inoculations were made from its organs and heart's blood into broth, and on to agar slopes, but no *M. melitensis* was recovered. The milk of this goat was examined from time to time, but the pus persisted till April 25th, 1905, by which time the milk was "drying up." It may be noticed in passing that suppurative mastitis is by no means infrequent amongst goats in Malta, and causes, from time to time, outbreaks of illness amongst children (see Health Reports of Malta). In June, 1905, the secretion of milk had practically ceased and pus was no longer present in the altered secretion, now thick, ropy, brownish and gelatinous, which, on being plated on June 24th, yielded colonies of *M. melitensis* in abundance.¹

**Blood.**—The agglutination reaction continued to increase, being 1 in 3,200 on September 30th, 1904, and 1 in 4,500 on October 18th. It then began to diminish, being 1 in 3,000 on November 1st, 1904, 1 in 3,000 on January 3rd, 1905, 1 in 3,000 on February 27th, 1905, and after this stationary period going down to 1 in 2,000 on April 25th, 1905, and 1 in 1,500 on June 12th, 1905.

*M. melitensis* was recovered from the blood (5 cc.) of the jugular vein on November 7th, 1904, and again from the blood of the same vein on June 27th, 1905.

**Experiment 2.**—Goat, juv. The kid of three months, purchased at the same time as the goat, received injections of *M. melitensis* on the same dates as the goats, but only in half the quantities.

Its urine was plated on the same days above detailed for the goat, but it never yielded any *M. melitensis*. Its agglutination reaction was similarly examined with the following results:—

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Date</th>
<th>React.</th>
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</thead>
<tbody>
<tr>
<td>1904</td>
<td>July</td>
<td>30</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>14</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>...</td>
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<td></td>
<td></td>
<td>30</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>...</td>
</tr>
</tbody>
</table>

¹ [Major Horrocks had previously recovered *M. melitensis* from apparently normal goats, and had shown the plates to Staff-Surgeon Shaw.—Sec. R.S.].
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1904—October

11 1—5,500
18 1—5,000
25 1—2,000

November 1 1—3,000

1905—

January 3 1—2,000
February 27 1—1,500
April 25 1—1,500

These figures follow closely those obtained from the goat, but rise somewhat higher in degree. *M. melitensis* was recovered from the blood of the kid in November, 1904, and in June, 1905.

From an inspection of the accompanying charts it will be seen that the rise of temperature following each injection is only temporary, lasting but three or four days, thus decidedly differing from the prolonged wave of fever produced in monkeys by a similar procedure, and suggesting a sort of racial tolerance of this infection on the part of the Maltese goat. It was thus proved by the development of a high agglutination reaction in August, 1904, and by the recovery of living *M. melitensis* from the blood in November, 1904, that the goat is at least experimentally susceptible to Mediterranean fever.

B. Natural.

Dr. Zammit, in June, 1905, found that the blood of four goats (out of six purchased for further experimentation) reacted to *M. melitensis* at the time of purchase. This observation having been confirmed by Major Horrocks and myself, and the micro-organism having also been recovered from the milk of one of these animals, the question of the agency of the goat in diffusing the *M. melitensis* was brought from the domain of speculative experiment into the range of everyday life. And after discussing the matter with Deputy Inspector-General Bentham, of the Naval Hospital at Bighi, we decided that I should at once begin an examination of all the goats supplying milk to this hospital.

The method of procedure adopted was as follows: The goats were taken in batches of 12 to 16 at a time. The milk contractor's son took down in Maltese the name and description of each goat.

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1 [Major Horrocks states that Dr. Zammit and he found that five of the normal goats reacted, and that he recovered *M. melitensis* from the milk of all of them but one. He remarks, further, that "the fact that the milk of infected goats causes agglutination of the *M. melitensis* was first shown by Zammit, and in the combined paper by Kennedy and myself the reaction is called 'Zammit's test.'"—S.C. R.S.]
which was numbered. A little blood was taken from the goat’s ear in a capillary tube for examination for agglutination, and at the same time 40 to 50 cc. of milk were drawn off into a sterile test-tube; to both of these the same number was attached, the intention being to subsequently eliminate all the goats which might be found infected. After examination of the blood for agglutination reaction, the milk of each goat which reacted was centrifugalised, and the centrifuged portion was plated on nutrose-litmus-agar, $\frac{1}{4}$ cc. being distributed over the surface of each Petri dish. These were incubated at 37° C., and were then examined for \textit{M. melitensis} colonies in the usual way. Four to six plates were used for each milk thus treated.

The examination was begun on June 29th. Surgeon Whiteside, R.N., was so good as to collect the necessary material from the goats at times when I was unable to attend, and he also helped with the agglutination reactions. These were done in two stages, the first to eliminate the non-agglutinating bloods (or milks), the second to determine highest dilution giving agglutination of the remainder. All went well for a few days, until the goat-herds, who had all along looked unhappy over the pricking of their goats’ ears for blood, broke out into open rebellion, and henceforth we had to be content with only milk. This necessitated either plating every milk or ascertaining the existence of the agglutination reaction with it. I have already mentioned the excretion of agglutinins in the urine (Part iii. of these Reports). It seemed not unlikely that they would be found to be present in the milk of infected animals. I accordingly put up specimens of such milk, centrifuged and uncentrifuged, diluted and undiluted, with freshly-prepared emulsion of \textit{M. melitensis}, in drops on slides in a moist chamber, with controls of normal milk, and left them for an hour. I then examined them under the microscope and found distinct agglutinations with the infected milks, most palpably in the uncentrifuged series, the appearance in the centrifuged series being somewhat masked by \textit{débris} of various sorts. When the milk was allowed to stand in the sterile test-tubes for a couple of hours a considerable layer of cream came to the top and a deposit of \textit{débris} gathered at the bottom. By passing a pipette down to the middle of the column, aspirating milk from there, withdrawing the pipette and then breaking off the capillary end of the pipette well above the adherent cream, I could obtain a specimen of milk almost free from \textit{débris} and with relatively few oil globules, in which the presence or absence of agglutination was fairly easily determinable.
This method was, therefore, perforce adopted for the ascertaining of agglutination reaction in the batches of goats examined on July 13th and 14th, a twenty-four-hour limit for contact of diluted milk and emulsion being adopted for determination of highest dilution giving agglutination.

The details of these examinations of the goats supplying milk to Bighi Hospital in June and July, 1905, are subjoined.

June 29th.—Twelve goats examined. Agglutination reaction found in blood of three: No. 4, in a dilution of 1 in 30; No. 8, 1 in 100; No. 10, 1 in 60. Milks of all three centrifugalised, plated and incubated. *M. melitensis* recovered and verified from No. 8 only (49 colonies).

July 3rd.—Second batch of 12 goats examined. Agglutination reaction found in blood of five: No. 4, 1 in 100; No. 5, 1 in 60; No. 6, 1 in 60; No. 8, 1 in 30; No. 9, 1 in 30. Milks of all these five centrifugalised, plated and incubated. *M. melitensis* recovered and verified from No. 5 (38 colonies), and No. 6 (728 colonies).

July 6th.—Third batch of 16 goats examined. Agglutination reaction found in blood of three: No. 1, 1 in 100; No. 3, 1 in 60; No. 14, 1 in 160. Milks of these three centrifugalised, plated and incubated. *M. melitensis* recovered and verified from No. 3 (six colonies).

July 7th.—Fourth batch of 12 goats examined. Agglutination reaction found in blood of three: No. 5, 1 in 30; No. 7, 1 in 30; No. 10, 1 in 30. Milks of these three centrifugalised, plated and incubated. No *M. melitensis* recovered from any.

July 10th.—Fifth batch of 12 goats examined. Agglutination reaction found in blood of six: No. 1, 1 in 60; No. 5, 1 in 200; No. 7, 1 in 200; No. 8, 1 in 60; No. 9, 1 in 60. Milks of these six centrifugalised, plated and incubated. *M. melitensis* recovered and verified from No. 1 (10 colonies) and from No. 7 (seven colonies).

July 13th.—Sixth batch of 15 goats examined. Agglutination reaction found in milk of four: No. 2, 1 in 100 after twenty-four hours' contact in moist chamber; No. 11, 1 in 60; No. 14, 1 in 30; and No. 15, 1 in 30, all under the same conditions. Milks of these four centrifugalised, plated and incubated. *M. melitensis* recovered from No. 2 (15 colonies) and No. 11 (five colonies).

July 14th.—Seventh batch of 12 goats examined. Agglutination reaction found in milk of six: No. 1, 1 in 150 after twenty-four hours' contact in moist chamber; No. 2, 1 in 160; No. 4, 1 in 30; No. 7, 1 in 30; No. 9, 1 in 30; No. 12, 1 in 30. *M. melitensis* was recovered and verified only from No. 4 (two colonies).
For convenience of reference these results may be arranged in tabular form, thus:

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of goats examined</th>
<th>Number presenting agglutination reaction</th>
<th>Distinguishing No. of the goats of each batch whose milk yielded <em>M. melitensis</em></th>
<th>Agglutination limit of latter</th>
<th>Number of <em>M. melitensis</em> colonies recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 29</td>
<td>12</td>
<td>3</td>
<td>No. 8.</td>
<td>1 in 100</td>
<td>49</td>
</tr>
<tr>
<td>July 3</td>
<td>12</td>
<td>5</td>
<td>No. 5.</td>
<td>1 in 60</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>No. 6.</td>
<td>1 in 60</td>
<td>728</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>No. 3.</td>
<td>1 in 60</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>No. 1.</td>
<td>1 in 60</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>No. 7.</td>
<td>1 in 300</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>No. 2.</td>
<td>1 in 100</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>No. 11.</td>
<td>1 in 60</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>No. 4.</td>
<td>1 in 300</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>91</td>
<td>30</td>
<td>9</td>
<td></td>
<td></td>
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</table>

Thus 91 goats were examined, of these, 30 presented the agglutinating reaction to *M. melitensis*, and the milk of these 30 was examined culturally for the parasite, this organism being recovered from the milk of nine of them. The implicated animals were eliminated from the herds supplying the Naval Hospital, and the most stringent measures were taken to ensure that all milk entering the hospital gates was forthwith boiled. It will be interesting to see whether any alteration takes place in the future incidence of cases of fever developing in this hospital.

It will be noticed that in these naturally-infected goats the agglutination limit is low, the highest found being 1 in 200, whereas in the experimentally infected animal it was found as high as 1 in 4,500. No indication has been observed of any relation between agglutination value and the number of colonies of *M. melitensis* yielded by the milk.

The number of organisms other than *M. melitensis* found in the milk from these 30 goats varied enormously, though the milk was collected under precisely similar conditions from all. In some cases 1 cc. of milk would contain but two or three organisms, in others they would be present by the thousand. Time did not admit of a detailed examination being made of these.

The infectivity of the milks obtained from the goats which were the subjects of the experiments here detailed was investigated as follows:

A monkey received from Genoa on July 12th was kept under observation for a week; its temperature was found to be normal
during this period, and its blood did not react to *M. melitensis*. On July 20th the colonies of this micro-organism obtained from No. 2 goat’s milk, plated July 13th, were emulsified in a little normal saline solution. The monkey being held on its back, three drops of this emulsion were dropped down each nostril with a capillary pipette. The animal developed a typical attack of Mediterranean fever, its blood gave agglutination reaction to *M. melitensis* first on August 3rd; fourteen days after infection in a dilution of 1 in 30, running up to 1 in 320 on August 6th, and 1 in 960 on August 10th; *M. melitensis* was recovered from its blood during life on August 22nd, and from its lymphatic glands after death on October 8th.

II.—Cows.

As it seemed by no means impossible that cows also might be found to be infected with Mediterranean fever, I determined to investigate this question. Not many milch cows are to be seen in the island of Malta, there being no pasturage for them. Their owners keep them shut up, some of their stables being most scrupulously clean, while others are much the reverse. The cows seldom get outside. There is a considerable demand for their milk, especially on the part of the resident English population, many of whom dislike the taste of goat’s milk, while others object to receive milk from an animal which has just previously been lying down in the street with its udders and teats in close contact with the excreta, liquid and solid, of the various animals, higher and lower.

To Mr. A. M. Macfarlane, M.R.C.V.S., Veterinary Surgeon to the Malta Government, who helped me to procure the necessary materials for bacteriological examination, my warmest thanks are due for the kindness with which he took me round to the various farms, used his influence with the owners of the cattle, and personally assisted in collecting the necessary material for examination.

The method of investigation determined on was as follows: At each of the various farms visited blood was taken from the cows, each animal being assigned a number, which was cut deep in Roman numerals into the hair of its back. The samples of blood were correspondingly numbered, and were subsequently examined for agglutination reaction to *M. melitensis*. The numbers of the cows at each farm giving this reaction, were sent with a daily supply of sterilised test tubes to Mr. Macfarlane, who undertook the collection of a daily sample of milk from each of the cows speci-
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These milks were received at the laboratory about an hour after they were drawn. They were at once centrifuged, and the deposit was plated on nutrose-litmus-agar in Petri dishes, three plates to each sample. These were incubated five days at 37° C., and were examined in the usual way for colonies of the micro-organism. The milks were thus treated daily from August 1st to August 24th, 1905, inclusive, with the following results:

G. F. of Tarxien.—Nine cows, of which three presented agglutination reaction to *M. melitensis* as follows: No. 3, 1 in 30; this being a heifer, no milk was attainable, and no other form of examination was permitted. No. 4 agglutinated *M. melitensis* in a dilution of 1 in 30, and No. 7 in a dilution of 1 in 60. The milks of the two latter were daily plated for twenty-four consecutive days. Cow No. 7 never yielded any colonies of parasite, but these were found in the milk of Cow No. 4 as follows:

Plates of 7th August 5 colonies of *M. melitensis*:

8th   7
12th  7
16th  3
19th  40
20th  3
21st  39
24th  19

none being found on the other days.

F. G. of Hamrun.—Nine cows, of which five presented agglutination reaction as follows:

<p>| | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>agglutinated 1 in 30.</td>
<td></td>
</tr>
<tr>
<td>&quot; 4 &quot;</td>
<td>1 in 800.</td>
<td></td>
</tr>
<tr>
<td>&quot; 8 &quot;</td>
<td>1 in 200.</td>
<td></td>
</tr>
<tr>
<td>&quot; 9 &quot;</td>
<td>1 in 30.</td>
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</tbody>
</table>

Of these, Cow No. 9 was ailing and not yielding any milk. The milks of the others were plated daily. Nos. 1 and 4 never yielded any colonies of *M. melitensis*, which, however, were found in the milk of Cow No. 8, as follows:

Plates of 11th August, 63 colonies of *M. melitensis*.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>12th</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>13th</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>16th</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>18th</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>19th</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>20th</td>
<td>231</td>
<td></td>
</tr>
</tbody>
</table>

none being found on the other days.
S. G. of Hamrun.—Six cows, none of which presented any agglutination reaction.

C. G. of Hamrun.—Three cows, of which only one presented an agglutination reaction in a dilution of 1 in 30. The milk of this animal was daily examined, but never presented any *M. melitensis*.

C. C. of St. Julian’s.—Four cows, of which No. 2 presented a high agglutination reaction, this being present in a dilution of 1 in 1,000. Unfortunately this animal happened to be a heifer, so again no further material for bacteriological examination was procurable.

S. M. of Imsiel.—Two cows, neither of which presented any agglutination reaction to *M. melitensis*.

Result.—Thirty-three cows examined. Ten of these presented an agglutination reaction to *M. melitensis*, varying from 1 in 30 to 1 in 1,000. From the milk of two of these cows, *M. melitensis* was isolated.

III.—Other Animals.

During the months of July and August, 1905, I examined specimens of blood kindly procured for me by Mr. Macfarlane, M.R.C.V.S., from thirty-one bullocks which were ailing in a vague indefinite sort of way, and which he thought might possibly be infected with Malta fever. Of these, five presented a very faint agglutination reaction. None of these animals had been in the island over three months.

I also examined several times the blood of two dogs similarly suspected. Neither of these presented any reaction to *M. melitensis*.

Remarks.

The manner in which animals become infected with the virus of Mediterranean fever is a matter of considerable interest and importance. Up to the present all the evidence available points to their food as being the main vehicle of infection. The feeding experiments carried on by Major Horrocks and myself show conclusively that monkeys and goats may be thus infected. Besides the very obvious way of infection of the young through their mother's milk, the successful result of various feeding experiments with food soiled, directly and indirectly, with the urine of two ambulatory cases of Mediterranean fever which I discovered working in the dockyard, and in whose urine living *M. melitensis* was being excreted, indicates another way in which these animals may be infected while feeding. Goats may be seen any day in the streets of the chief city of the Island of Malta, feeding on filth and
rubbish of every possible variety, some of it visibly saturated with urine, animal and human. Among the lower class Maltese, as above stated, workmen have been found who void living *M. melitensis* in their urine, as do a certain number of the infected goats. Thus the path of this manner of infection becomes clear. Having satisfied their hunger in this manner, the goats lie down in the streets to digest their meal, with their teats and udders often in contact with the ordure of the gutters and roads, till they are kicked up by the goat-herd to be milked into the vessel brought to the doors of the adjacent houses by their occupants. It is hence not to be wondered at that these animals frequently suffer also from suppurative mastitis, and give milk containing pus. In the Health Reports of the Malta Government may be seen reports of outbreaks of illness amongst children directly traced to this cause by their medical officers.

With regard to cows, the evidence is not quite so clear. Kept shut up in "shippens," and seldom allowed outside, they have their food brought to them, but as this food is composed of vegetable and other refuse collected from every possible source and situation, it is easy to understand that they can hardly escape from receiving infected food from time to time.

**SUMMARY.**

1. The susceptibility of goats to experimental infection by *M. melitensis* was ascertained by me in the summer of 1904, and is here further demonstrated.

2. The persistence of living micrococci in the blood of a goat for seven months has been proved. The bearing of this observation on the preparation of a therapeutic serum is obvious.

3. Of ninety-one goats in full milk, thirty were found to have become infected with Mediterranean fever at some time or other, as shown by their agglutinating power on *M. melitensis*. Living examples of the micro-organism were recovered from the milk of nine of these, and its infectivity was demonstrated on a monkey.

4. Of thirty-three cows examined, ten were found to have become infected with Mediterranean fever, and living *M. melitensis* was recovered from the milk of two of these.

5. Of thirty-one bullocks examined, five were found to show a faint agglutination reaction, which may indicate that they had become infected with Malta fever.

6. Of two ailing dogs, thought to be suffering from this fever, neither was found to be infected.