PRELIMINARY NOTE ON GOATS AS A MEANS OF PROPAGATION OF MEDITERRANEAN FEVER.

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With the object of ascertaining, by experimental inoculation, whether goats could be infected by the M. melitensis, six goats were bought on June 12th, 1905, from two different herds, and placed in the lazaretto. On June 14th Dr. Zammit, as a preliminary step to our experimental work, took blood from each of these goats, and proceeded to test the action of the serum on the M. melitensis. He found, to his great surprise, that the serum of five of the goats, when considerably diluted, caused agglutination of this microbe. On June 15th similar results being again obtained, Dr. Zammit brought specimens of the bloods to the Public Health Laboratory, and asked me to confirm his observations. I obtained the following results:--

Goat No. 1.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the M. melitensis, visible to the naked eye. When diluted 1 to 100, however, the serum gave no reaction.

Goat No. 2.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the M. melitensis. A dilution of 1 to 100 produced a complete reaction after fifteen minutes.

Goat No. 3.—Blood serum diluted 1 to 10, 1 to 40, and 1 to 100, caused immediate agglutination of the M. melitensis, but in the case of the dilution 1 to 100, the clumps were not visible to the naked eye until after fifteen minutes.

Goat No. 4.—The blood serum produced no reaction with the M. melitensis.

Goat No. 5.—The blood serum diluted 1 to 10 caused immediate agglutination, but dilutions of 1 to 40 and 1 to 100 did not produce a complete reaction until after fifteen minutes.

Goat No. 6.—Blood serum diluted 1 to 300 caused complete agglutination of the M. melitensis, visible at once with the naked eye.

The reactions thus obtained, and especially that of Goat No. 6, suggested that possibly five of the goats were suffering from
Mediterranean fever, acquired under natural conditions. The goats were stated to be healthy, but were sold cheaply, as they had given very little milk for some time. They were bought from pens in the neighbourhood of Birchircara and St. Julians, and taken straight to the lazaretto, where they were placed in clean stalls, which had never been used for any experimental work with the *M. melitensis*.

Dr. Zammit and I then arranged to make a complete study of these animals; Dr. Zammit undertook the investigation of the blood, and I made myself responsible for the bacteriological examination of the milk and urine.

**Bacteriological Examination of Milk and Urine Obtained from Naturally Infected Goats.**

*Goat No. 6.*—I commenced work with this goat, as its blood serum, when diluted 1 to 300, caused immediate agglutination of the *M. melitensis*. The animal did not appear well, and had a very poor coat. The udders were flaccid, but the milk exuded appeared normal in character. The temperature was taken morning and evening, and compared with that of a healthy goat. The evening temperature never rose above 103°F., and as this temperature is often recorded in the case of perfectly normal goats, a febrile temperature could not be said to be present.

On June 18th milk was withdrawn, and 1 cc. centrifugalised; the deposit was then carefully spread over ten litmus-nutrose-agar plates. After four days' incubation at 37°C., colonies of the *M. melitensis* appeared in every plate. The colonies were at once tested with a dilute (1 to 100) specific serum obtained from an inoculated rabbit. The micrococci were found to agglutinate at once, the clumps being visible to the naked eye. Some of the colonies were then planted out on agar slopes, and the resulting growths, when subjected to the usual confirmatory tests, showed that the *M. melitensis* was undoubtedly being excreted in the milk of this goat.

On June 22nd the milk was again examined and the *M. melitensis* recovered once more.

On June 23rd examination of the urine was commenced. The vagina was washed out with an antiseptic solution and a catheter, previously sterilised in boiling water, passed into the bladder. The urine so obtained was plated on litmus-nutrose-agar, but after four days' incubation at 37°C., in spite of the precautions taken,
the plates were found densely crowded with saprophytic organisms, and the *M. melitensis* could not be detected.

On June 24th and 26th the urine was again plated, the same precautions being used, but the plates were densely crowded with foreign organisms and the *M. melitensis* could not be seen.

On June 27th, 28th, 29th and 30th, and on July 1st, 3rd, 4th, 5th, 7th, 8th, 9th and 10th, the urine was also examined, but up to the present the *M. melitensis* has not been recovered.

The milk was plated again in June and July, and the *M. melitensis* was found on each occasion.

*Result.*—The *M. melitensis* appears to be steadily excreted in the apparently normal milk of this goat, but up to the present it has not been found in the urine.

*Goat No. 1.*—This animal appeared healthy, but the udders were flaccid, and the milk exuded had a thin serous appearance. The temperature was taken regularly, but no indications of fever were observed.

On June 22nd, 1 cc. of the milk was centrifugalised and the deposit plated. After four days' incubation at 37° C., the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24th and 26th the milk was again examined and similar results were obtained.

On June 26th, 29th and 30th the urine was examined, but no signs of the *M. melitensis* could be discovered.

On July 1st, 10 cc. of the urine were centrifugalised and the deposit plated; four days later every plate was found studded with colonies of the specific microbe. The colonies were fished, planted on agar slopes, and the resulting growths tested in the usual manner.

*Result.*—The *M. melitensis* is excreted in very large numbers in the serous-looking milk of this goat. It is also excreted in the urine.

*Goat No. 2.*—This goat appeared quite well, and the milk exuded from the udders had a normal appearance. There were no indications of fever.

On June 22nd, 1 cc. was centrifugalised and the deposit plated. After four days' incubation about thirty colonies appeared in every plate.

On June 24th and 26th the milk was again examined, and colonies of the *M. melitensis* were recovered on both occasions. The urine was examined on June 23rd, 26th, 27th, 28th, 29th
and 30th, and on July 1st, 3rd and 6th, but the *M. melitensis* could not be detected.

**Result.**—The *M. melitensis* appears to be excreted in small quantity in the normal-looking milk of this goat. It has not yet been detected in the urine.

**Goat No. 3.**—This goat looked healthy and had no fever, but its milk was thin and serous. On June 22nd the milk was examined, one loopful of the serous milk being spread over each plate. After four days' incubation all the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24th and 26th the milk was again examined and similar results were again obtained.

The urine was examined on June 23rd, 26th, 28th and 30th, and on July 1st, 3rd, 6th, 8th, 9th, 10th and 11th, but no signs of the *M. melitensis* could be recovered.

**Result.**—The *M. melitensis* appears to be present in enormous quantities in the thin serous-looking milk of this goat, but it has not yet been found in the urine.

**Goat No. 5.**—This goat was in poor condition, the udders were flaccid, and the milk exuded had a thick jelly-like appearance.

On June 22nd the milk was examined, one loopful of the jelly-like material being spread over each plate. After four days' incubation all the plates were covered with minute colonies of the *M. melitensis*. On June 24th and 26th the milk was again examined, and densely crowded plates were obtained as before.

On June 25th and 30th the urine was examined, but no colonies of the *M. melitensis* were detected.

On July 1st the urine was again plated, and four days later every plate was found to contain numerous transparent colonies strongly resembling those of the *M. melitensis*. Some of the colonies were fished and planted out on agar slopes. The resulting growths were then subjected to the usual confirmatory tests, and the *M. melitensis* proved to be undoubtedly present.

The five goats just examined being considered by their owners to be "out of milk," would not be likely to be employed for milking purposes, though in the case of Goats Nos. 2 and 6, the milk might easily have been used without any fear of suspicion arising as to its being abnormal. Consequently it appears very desirable to examine the herds which were actually supplying milk to Valletta, Sliema, and the various hospitals.

I therefore asked Captain Kennedy, R.A.M.C., to visit the various
herds, and, with the owners' consent, take blood from the ears, and test the action of the sera on the *M. melitensis*. The results he obtained are given in Report VIII. of the Mediterranean Fever Commission, Part III.; it will be seen that, out of 161 goats examined, 84 gave a reaction, corresponding to a percentage of 52, probably infected with Mediterranean fever. I then obtained samples of milk from some of the apparently infected animals, and proceeded to plate them on litmus-nutrose-agar. The following results have been obtained up to the present time:

EXAMINATION OF THE GOATS SUPPLYING MILK TO FORREST HOSPITAL.

I visited this herd, which assembles outside the hospital gate every morning, and selected Goats Nos. 38, 48, 37 and 43 from Captain Kennedy's list.

*Goat No. 38.*—The milk from this animal was centrifuged, and the deposit plated on July 4th, 5th, 6th, 7th, 8th and 10th, but up to the present the *M. melitensis* has not been isolated.

*Goat No. 48.*—The milk was examined on the same dates as Goat No. 38, but so far the *M. melitensis* has not been isolated.

*Goat No. 37.*—The milk of this animal was taken on July 4th, and 2 cc. centrifuged; the deposit was then plated. After four days' incubation every plate was found densely crowded with small colonies of the *M. melitensis*; the colonies were so numerous that it was impossible to make an accurate count. The colonies were fished and planted on agar; the growths resulting responded to all the tests characteristic of the *M. melitensis*.

On July 5th and 6th the milk was again plated, and similar results were obtained.

As this goat was in full milk, there cannot be any doubt that the *M. melitensis* was being excreted in large numbers. A pint of the milk was then collected, and Dr. Zammit very kindly made a chemical examination of the sample. The result given below shows that the milk was of good quality.

**ANALYSIS OF MILK FROM GOAT NO. 37.**

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<th>Component</th>
<th>Amount</th>
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<tr>
<td>Density at 15° C</td>
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<tr>
<td>Fat</td>
<td>4.3%</td>
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<tr>
<td>Total solids</td>
<td>13.18%</td>
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<tr>
<td>Solids, non-fat</td>
<td>8.8%</td>
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<tr>
<td>Ash</td>
<td>0.51%</td>
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Goat No. 43.—The milk of this goat was examined on July 4th, 5th, 6th, 7th, 8th, 9th and 10th, but up to the present the M. melitensis has not been isolated.

A reference to Captain Kennedy’s list shows that, while Goat No. 37 reacted in a dilution of 1 to 60, Goats Nos. 38, 48 and 43 only reacted in a dilution of 1 to 20, and were probably in an early stage of the disease.

EXAMINATION OF A SMALL HERD SUPPLYING MILK TO VALLETTA STATION HOSPITAL.

Goats Nos. 27, 30 and 32 were selected from this herd. The goats were kept at Casal Curmi, and brought every morning to the Station Hospital.

Goat No. 30.—On June 29th and 30th milk was centrifugalised and plated in the usual manner, but the M. melitensis was not detected.

On July 1st plates were again made, and a few typical colonies appeared.

On July 3rd 10 cc. of the milk were centrifugalised, and the deposit plated; four days later every plate was found densely crowded with colonies of the M. melitensis.

On July 6th similar results were obtained.

A sample of the milk was then analysed by Dr. Zammit, and found to have an average chemical composition.

Goats Nos. 27 and 32.—The milk from these goats was examined on June 29th and 30th, and on July 1st, 3rd, 7th, 8th and 10th, but up to the present the M. melitensis has not been isolated.

EXAMINATION OF A SMALL HERD SUPPLYING MILK TO VALLETTA.

This herd assembled in St. John’s ditch, and seventeen out of twenty-five animals showed a blood reaction with the M. melitensis, and six of them reacted when the serum was diluted 1 to 100. Goats Nos. 50 and 52 were selected from Captain Kennedy’s list.

Goat No. 50.—On July 6th, 1 cc. of the milk was centrifugalised and the deposits spread over the usual plates. Four days later all the plates were found densely crowded with small colonies of M. melitensis.

The confirmatory tests were applied in the usual manner. This animal was considered one of the best milkers in the herd, and its
owner valued it at £5, whereas the ordinary price for a goat in milk varies from £3 to £4.

Goat No. 52.—This animal appeared in good health, and its udders were full of milk. It was purchased and placed in the lazaretto.

On July 5th milk was withdrawn and 1 cc. centrifugalised; the deposit was then spread over nutrose-agar plates in the usual manner. After four days' incubation at 37° C., all the plates were found so densely crowded with colonies of *M. melitensis* that a reliable count could not be made.

On July 6th the milk was again examined and similar results were obtained.

A sample of the milk was submitted to Dr. Zammit for chemical analysis; he obtained the following results:

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<td>Specific gravity at 15° C.</td>
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<td>Total solids</td>
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<td>Fat</td>
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**EXAMINATION OF A HERD SUPPLYING MILK TO SLIEMA.**

Two goats were brought from this herd and placed in the lazaretto. The pens were in the neighbourhood of Misida.

Goat No. 15.—On July 5th the blood was examined and the serum, diluted 1 to 50, was found to cause complete agglutination of the *M. melitensis* visible to the naked eye. The goat appeared to be in good health, and the udders were full of milk. Some milk was withdrawn and 2 cc. centrifugalised; the deposit was then plated in the usual manner. On July 9th the plates were found covered with small colonies of the *M. melitensis*.

On July 6th the milk was again examined, and the deposit from 1 cc. produced as before an immense number of colonies of *M. melitensis*.

The urine was withdrawn by a catheter and plated on July 5th, 6th, 7th, 8th, 9th and 10th, but up to the present the *M. melitensis* has not been isolated.

A chemical analysis of the milk was made by Dr. Micalef, with the following results:

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Goat No. 16.—This goat was taken from the same herd as No. 15. On July 4th the blood was examined, and the serum,
diluted 1 to 60, was found to cause immediate clumping of the *M. melitensis*. The milk and urine have been examined daily since July 4th, but up to the present the *M. melitensis* has not been isolated from either source.

**Conclusions.**—The results obtained show that some of the goats in every herd examined are suffering from Mediterranean fever. The *M. melitensis* is exuded in the milk in enormous numbers when the disease has been present sufficiently long to cause a change in the physical character of the fluid. It is also excreted in considerable numbers even when the animals are in “full milk,” and no changes have occurred in either the physical or chemical characters of the milk.

The *M. melitensis* is also excreted in the urine of goats suffering from Mediterranean fever, but up to the present it has only been found when the disease has existed for some time and physical changes have occurred in the milk.