June 18 about 5 c.c. of blood was taken from Goat No. 6 and distributed in six broth-tubes. On June 25 passages from the broth-tubes were made on to agar slopes, and the *M. melitensis* recovered in pure culture. This micro-organism was also recovered from the blood of Goat No. 5. Blood was also taken from Goats Nos. 1, 2, and 3, but so far the *M. melitensis* has not been recovered.

Material from Abattoir.—Dr. Caruana Scicluna having suggested that possibly infected goats might be met with in the abattoir, I have examined 46 spleens, and have recovered the *M. melitensis* from one. The blood of seven of these goats gave a positive reaction to the agglutination test.

Preliminary Note on Goats as a Means of Propagation of Mediterranean Fever.

By Major W. H. Horrocks, R.A.M.C., Member of the Mediterranean Fever Commission.

(Communicated by Colonel Bruce, C.B., F.R.S.—Received July, 1905.)

With the object of ascertaining, by experimental inoculation, whether goats could be infected by the *M. melitensis*, six goats were bought on June 12, 1905, from two different herds, and placed in the lazaretto. On June 14 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 15 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 15 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 15 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 15 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°, 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 18 milk was withdrawn, and 1 c.c. centrifugalised; the deposit was then carefully spread over 10 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 22 the milk was again examined and the *M. melitensis* recovered once more.

On June 23 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 24 and 26 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 27, 28, 29, and 30, and on July 1, 3, 4, 5, 7, 8, 9, and 10, 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 22, 1 c.c. 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 24 and 26 the milk was again examined and similar results were obtained.

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

On July 1, 10 c.c. 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 22, 1 c.c. was centrifugalised and the deposit plated. After four days' incubation about 30 colonies appeared in every plate. On June 24

and 26 the milk was again examined, and colonies of the *M. melitensis* were recovered on both occasions.

The urine was examined on June 23, 26, 27, 28, 29, and 30, and on July 1, 3, and 6, 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 22 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 24 and 26 the milk was again examined and similar results were again obtained.

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

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 22 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 24 and 26 the milk was again examined, and densely crowded plates were obtained as before.

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

On July 1 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 appeared very desirable to examine the herds which were actually supplying milk to Valetta, Sliema, and the various hospitals.

I therefore asked Captain Kennedy, R.A.M.C., to visit the various herds, and, with the owner's consent, take blood from the ears, and test the action of

the sera on the *M. melitensis*. The results he obtained are given in Part VIII; 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 centrifugalised, and the deposit plated on July 4, 5, 6, 7, 8, and 10, 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 4, and 2 c.c. centrifugalised; 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 5 and 6 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.

Density at 15° C.	1030
Fat	4·3 per cent.
Total solids.....	13·18 „
Solids, non-fat	8·8 „
Ash.....	0·51 „

Goat No. 43.—The milk of this goat was examined on July 4, 5, 6, 7, 8, 9, and 10, 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 Valetta 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 29 and 30 milk was centrifugalised and plated in the usual manner, but the *M. melitensis* was not detected.

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

On July 3 10 c.c. 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 6 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 29 and 30, and on July 1, 3, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Examination of a Small Herd Supplying Milk to Valetta.

This herd assembled in St. John's ditch, and 17 out of 25 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 6, 1 c.c. of the milk was centrifugalised and the deposit 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 5 milk was withdrawn and 1 c.c. 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 crowded with colonies of *M. melitensis* that a reliable count could not be made.

On July 6 and 8 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:—

Specific gravity at 15° C..... 1031

Total solids, 14·0 per cent.; fat, 3·6 per cent.; ash, 0·73 per cent.

Examination of a Herd Supplying Milk to Sliema.

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

Goat No. 15.—On July 5 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 c.c. centrifugalised; the deposit was then plated in the usual manner. On July 9 the plates were found covered with small colonies of the *M. melitensis.*

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

The urine was withdrawn by a catheter and plated on July 5, 6, 7, 8, 9, and 10, 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:—

Total solids, 13·5 per cent.; fat, 4·1 per cent.; ash, 0·75 per cent.

Goat No. 16.—This goat was taken from the same herd as No. 15. On July 4 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 4, 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 characters 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.