Q FEVER, A RESPIRATORY HUMAN EPIDEMIC DISEASE IN THE MEDITERRANEAN AREA, DETERMINED A MILKBORNE INFECTION FROM GOATS AND SHEEP

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The Q fever originally observed in the region of Queensland, Australia, has been up to today known in Australia and in North America as a sporadic human disease, localized in agricultural areas because of its transmission by ticks.

It made its appearance for the first time during World War II as an epidemic bronchopneumonia among the German troops that had invaded Bulgaria and Greece in the spring of 1941. Outbreaks continued in winter and spring of each year among the German troops of occupation in southern Greece.

The Germans recognized early the nosological entity of this epidemic bronchopneumonia. Its peculiar clinical and laboratory characteristics permitted its differential diagnosis from the common bronchopneumonias. That is why German physicians called it "Balkan gripppe" although they could not succeed in isolating the agent of the disease, in spite of obstinate researches during 4 consecutive years.

Its peculiar etiology was later shown in the spring of 1944, when we succeeded in transmitting to guinea pigs at the Pasteur Institute of Athens an experimental infection which could be transferred in serial passages to new animals by inoculation of blood. At that time we established the infection in guinea pigs with five samples of blood and five samples of sputum from sick German soldiers, and with the blood and sputum from two Greek patients.

For reasons of economy of guinea pigs, only one strain, originating from the blood of a German soldier, was kept for serial passages. With this strain some properties of the etiological agents were studied: (1) The filtrability of the virus; (2) its resistance in vitro for a long time (for 2 years at least); (3) the transmission of inapparent infection to guinea pigs by nasal instillation; (4) the establishment of strong immunity after the disease, as well as after the inapparent one; and (5) the exacerbation of virulence accompanying the serial passages, manifested by the death of the animal as well as by the creation of apparent lesions in the lungs and the pericardium of the guinea pigs. In the smears of spleen numerous organisms in compact masses were observed.

We maintained this strain for 15 months and through the courtesy of Dr. Zarafonitis, member of the Typhus Commission in Athens,
succeeded in sending to Brigadier General Bayne-Jones, president of the American Typhus Commission, specimens of infected blood from the eighteenth and nineteenth passages.

Dr. John H. Dingle, director of the Laboratory of the Commission for Respiratory Diseases, who received the specimen for study, was able to establish the disease quickly in guinea pigs, isolate the virus in chick embryos, and prove, by further immunological studies, that the agent was a rickettsia similar to that of Q fever.

Researches made in Italy among Allied troops, after my work in Greece became known, proved that the outbreaks of a disease in Italy diagnosed as atypical pneumonia were really outbreaks of Q fever. Thus was Q fever for the first time recognized as a respiratory disease. Its geographical distribution today must be considered very large. The disease was recently described in Rumania (Combescu, 1947), and its presence may be considered as very probable in Asia Minor, according to our own recent researches.

**Recent Epidemiological Investigations**

The presence of Q fever in the form of a respiratory disease and its establishment in so large an area could not be explained by the known mode of transmission. The epidemics occurring in Greece in winter and spring excluded the possibility of tick transmission. Besides, its interruption during the hot season shows clearly that the inter-human transmission by spumon is not the exclusive mode. The evolution of the epidemic and its interruption, both occurring always at a certain season and in the same rhythm during so many consecutive outbreaks among German troops, could be explained only by the existence of a peculiar source of human infection.

To find out this source, we proceeded, beginning in the winter of 1946, to new epidemiological investigations; and this time we had the advantage of employing not only the experimental method but also the complement fixation test on sera.

Dr. Robert Huebner, of the National Institute of Health of the United States of America, was kind enough to perform this test on sera taken from men and animals. From the very beginning I gave my attention to goats and sheep, which must be considered as peculiar in the Mediterranean area, especially in Greece, Italy, the Balkan countries, and Asia Minor, where they are employed as domestic milk-giving animals.

Among the specimens of sera from goats in Greece and Asia Minor there were early found some that gave a positive test, but in a low titer. Later on, specimens of sera of goats from Athens and from various other districts of Greece and Asia Minor also yielded significant titters. But this test was shown to be useless for the diagnosis of the infection of goats and sheep, as it was found negative, with only one exception, on sera of goats and sheep which had exhibited a severe experimental or natural infection.

For this reason, in our subsequent investigation as to the relation of goats and sheep with the human disease, we employed exclusively the experimental method. By this method we have been able to prove: (1) That sheep and goats are very susceptible to the virus of Q fever; (2) that they serve as a source of infection because of a peculiar characteristic of their disease, that is to say, that the virus appears in their milk, and remains long after the end of the disease; and (3) that the infection of goats and of sheep is transmitted, above all, through the respiratory tract.

Among British troops in Athens, although diminished in number, numerous cases of the disease occurred during the winter and spring of the year 1946-47. Cases among Greeks were also numerous. At the British Military Hospital in Athens we studied 40 severe cases, and among Greek soldiers, 8 at the Rimini Hospital (Dr. Kalitzantzis and Dr. Papanicolaou), 4 at the Air Forces Hospital (Dr. Trivizas and Dr. Corombilis), and 12 among civilians (Dr. Tsangridis, etc.).

The virus was recovered from the blood of 24 cases and from the sputum of 12 cases by injection into guinea pigs.

In nearly all of these cases the complement fixation test was performed and the result was found to be positive. The test was also found positive among persons (physicians and nurses) who were in contact with patients, and among persons who lived with these patients before the manifestation of their illness.

Thirteen cases of bronchopneumonia were observed among British troops in Salonica. The complement fixation tests performed on four specimens were positive.

The outbreak among British troops began in January and ended in June (January, 5 cases; February, 7; March, 9; April, 13; and May, 6).

British units were camped around Athens in small buildings, and the soldiers lived in contact with Greek people. Dogs wandered frequently about, and sheep and goats were herded in the vicinity. It is remarkable that sheep and goats were herded in nearby pastures in the Grottaglie Air Base, Italy, where American soldiers were affected by the disease (Epidemiological Studies, American Journal of Hygiene, July 1946, p. 89).

1 Although the complement fixation test proved to be of no use in these investigations, yet we were able to try it in any case in man or animal. The results will be published in collaboration with Dr. Huebner of the National Institute of Health, who performed the test from the very beginning of our investigation and who was kept aware of our research work on goats and sheep.

2 Five strains were sent to Drs. Topping and Huebner, of the National Institute of Health, United States of America.

3 The clinical study of these cases will be published in a separate paper. We think it is interesting to note that in many chest X-rays, lung lesions gave the impression of a primary tuberculosis infiltration. It is worth while also to mention a case of paraplegia of the posterior spinal nerves of the spinal cord of a Greek soldier who completely recovered within 2 months (Dr. Kalitzantzis). A quite similar paraplegia followed the experimental infection in a sheep (ram), which died after 6 months.
Susceptibility of Goats and Sheep to the Virus of Q Fever

In these experiments, animals from various districts of Athens and suburbs and a small number recently imported from Asia Minor were employed.

Experiment 1. (22 April 1947).—One kid and one lamb were inoculated into the lung with infected human blood, one kid with sputum in the same way, and one kid and one lamb by nasal instillation of blood after narcosis with ether. Four young dogs and two young pigs were inoculated with blood into the lungs, and one young pig and two young dogs by nasal instillation of blood. For each series two guinea pigs served as controls. All kids and sheep exhibited, after an incubation period of 6-10 days, a high fever that lasted 7-12 days. Animals that had been inoculated into the lung, or had been infected by nasal instillation, presented symptoms of bronchopneumonia. The blood of infected lambs injected into guinea pigs proved to be infectious during the whole febrile period.

Experiment 2. (4 May 1947).—Five kids of almost 40 days of age, born and bred in the garden of a colleague (Dr. Minas, Paleon Faliron, near the seashore) were employed. Three of them were inoculated subcutaneously with infected blood of guinea pigs; while the other two were inoculated subcutaneously with blood of the two kids of the first experiment. All kids presented, after 8-10 days, a severe infection. The fever rose frequently to 42°C and was accompanied by a strong shivering (chills).

At the place of injection there occurred an extensive inflammation. This inflammation subsided with the fever, which always fell by lysis. In smears from skin tissues of local inflammation numerous rickettsias were found.

Heart puncture, made on two kids for taking blood, revealed pericardial fluid in great quantity (20-30 cubic centimeters). The blood of these kids proved to be infectious to the guinea pig.

A kid died 2 days after the fall of the fever. In its autopsy the spleen was found to be very much enlarged, with marked adhesions, and the pericardium contained increased fluid. In spleen smears were found numerous intracellular and extracellular rickettsias.

Simultaneously with the inoculation of these kids, we proceeded to the subcutaneous inoculation of two milk-giving goats. Both developed a severe infection, which evolved exactly in the same way as with the kids, and their blood also was shown infectious to guinea pigs.

Experiment 3. (9 June 1947).—Four lambs were inoculated subcutaneously with blood from two kids of the preceding experiment. At the same time, two horses and two mules were inoculated via the conjunctiva of the upper eyelid; six young dogs, four subcutaneously and two into the conjunctiva; eight kittens, four subcutaneously and four into the conjunctiva. The lambs exhibited a severe infection after an incubation period of 6-10 days, and on the site of the injection there developed an extensive inflammation. The horses and mules exhibited an intensive inflammation of the conjunctiva accompanied by a high fever of 6-10 days duration.

The dogs showed a short febrile reaction without any local inflammation, while the kittens presented neither fever nor local inflammation.

The blood of the lambs was infectious for guinea pigs at the beginning and the end of the fever. The same occurred with the blood of the horses and the mules. On the contrary, dog and cat blood injected into guinea pigs did not produce any infection.

The complement fixation test of dogs and cats was found to be positive, while the results on sera of horses and mules were negative.

Experimental Infeciton of Goats and Sheep in the Form of Broncho-Pneumonia by Nasal Instillation of the Virus

Experiment 4.—For this experiment the following animals were employed: six sheep, one ram, two lambs, two kids, three goats (one barren, just imported from Asia Minor (P)); one milk-giving goat (G) and another milk-giving goat from Athens (D); two male goats (one bred at the Pasteur Institute in contact with the infected guinea pigs, whose complement fixation test before the inoculation was found positive). The two kids and the two lambs had already been inoculated in the first experiment.

All these animals were tested by a direct nasal instillation of the virus. Some of these were also inoculated into the conjunctiva of the upper eyelid.

With the exception of the two kids and the two lambs which were reinoculated, and the male goat with positive complement fixation test, all animals, after an incubation of 6-9 days, showed a severe infection accompanied by cough and dyspnoea. In two goats coarse râles were heard and in radiograms dense consolidations of the lungs were seen.

The animals inoculated into the conjunctiva exhibited an intense conjunctivitis. The eye lesions, however, disappeared after 2 months without important sequelae.

The blood of the ram, which developed a severe infection, served for a nasal instillation to the milk-giving goat (ED). Simultaneously we injected blood of the goat (G) into another young milk-giving goat (A) from Old Phaleron. The latter was found immune. But in the first a severe bronchopneumonia and conjunctivitis developed.

We must add that in all the cases of bronchopneumonia the cough was always dry. Certain goats and sheep presented a nasal catarrh, more productive on the sheep. Injection of nasal secretions of the goat (E) into the guinea pig was positive at the height of the disease.

The fever remained high for 10 days. Marked weakness appeared later, and after 2 months the animal presented a paraplegia of the posterior limbs, in consequence of which it died after 6 months.
and negative in a few days after the fall of the fever. In smears of the conjunctiva and the cornea were found numerous intercellular masses of rickettsia. The injection of urines of these animals into guinea pigs, repeated in the course of the disease as well as after its end, was always negative.

The goat milk, however, proved to be infectious to the guinea pig very early in the course of the disease, and it remained so during the whole lactation period. This fact, in connection with the transmission of the infection to goats and sheep by nasal instillation, is of great importance, as it explains the presence and establishment of the Q fever in the form of a respiratory disease.

Presence and Maintenance of the Virus in the Goat-Milk After Experimental Infection

Experiment 5.—We had the opportunity to inject into guinea pigs the milk of a goat (G) of the fourth experiment, on the eighth day of fever (30 July 1947). The injected guinea pigs developed, after incubation for a few days, a typical infection.

This experiment was repeated with milk from the same goat (G) on 12 August 1947, 2 days after the end of the illness, and at the same time with milk from the goat (ED) in which 3 days previously the illness had started. In both cases the result was positive.

In a third experiment (19 August 1947), 9 days after the disease of the first goat and on the tenth day of fever in the second goat, the result was also positive.

In a fourth experiment (29 August 1947) we injected the milk of three goats, (G), (E), and (D). The results for all three were positive.

In a fifth experiment (18 September 1947) and in a sixth (6 October 1947) performed with the milk of the three goats (ED, G, D), the results were again positive.

The above experiments show definitely that the virus is present in the milk from the first days of the disease until at least 3 months after the end of the disease.

These experiments assume greater importance from the following observation concerning a milk-giving goat which presented a bronchopneumonia after her cohabitation, for a few days, with the above goats at the Pasteur Institute: Her milk was found infectious to the guinea pig toward the end of the disease, and remained so till the end of the experimental work.

Specimens of infectious milk, after having been kept in the Frigidaire at least 3 months, remained infectious; on the contrary, after souring, the milk was no longer infectious.

Recovery of Virus From Milk of Goats (Malta Race) Bred in Small Flocks in the Area of Athens

On 22 August 1947 we visited a flock at the Delta, Old Phaleron, near camps of British troops, and obtained specimens of milk from 16 Malta goats. Ten guinea pigs were inoculated with these specimens. The milk of six goats was mixed in three couples and injected into three guinea pigs. The other specimens were injected into four guinea pigs. Two guinea pigs of the first series and two of the second one developed a fever after incubation of 15–20 days. The virus was recovered from four goats.

On 28 August 1947 we took specimens of milk from eight goats of another flock (Eden, Old Phaleron) and with these, mixed two by two, four guinea pigs were inoculated. One of these guinea pigs fell ill after an incubation of 9 days.

On 14 January 1948 we tested again the infectivity of milk of goats and sheep, of this flock, which had just given birth. Out of six goats whose milk was injected into guinea pigs, three were found carriers of the virus. The milk of one sheep, which had developed a week before a severe bronchopneumonia, was also found infectious.

On 2 February 1948 we examined the milk of two other sheep of the same flock, which had also presented a severe bronchopneumonia, and in both the virus was recovered from their milk.

All these sheep had recently been imported from the island of Chio. In goats experimentally infected, udder lesions were never seen. On the contrary, inflammation of the udder was observed in sheep and goats, of this last flock, in whose milk the virus was present. It is also worth mentioning that in the autopsy of a sheep following death due to bronchopneumonia we observed large lung consolidations, much pleural fluid, and a markedly enlarged spleen. This sheep was in a late stage of pregnancy with two embryos. An emulsion prepared from the spleen and liver of these two embryos was injected into guinea pigs, which developed no signs of illness.

In another case also, that of the goat (ED), which was experimentally infected and in which the virus persisted in the milk until pregnancy, we had the opportunity to proceed to the autopsy of a dead embryo. Spleen and liver were injected into guinea pigs, with negative results.

Maintenance of the Virus in the Milk After Pregnancy and Infection of New-born

Later on, we observed that a kid of the same goat (ED) presented on the eighth day after birth a high fever, which lasted 12 days without any other symptom. Injection of its blood, made twice during the fever, transmitted a typical infection to guinea pigs. An indirect proof of the nature of this kid's infection was obtained by the positive
result of the tested milk of the parent goat. The same results occurred with the five kids of the experimentally infected goats G and D.

Nine kids and five lambs of naturally infected sheep and goats, of the said flock, were found with fever, and their blood was also infectious to guinea pigs. These observations point out that the infection of newborn lambs and kids is transmitted by milk; so milk is the source of infection to these animals.

It remains to examine the role that ticks play in the transmission of the infection to goats and sheep.

It is to be remarked that ticks are not found in domestic goats and sheep in the suburbs of Athens but only on those of rural districts.

**SUMMARY**

The seasonal incidence of Q fever in Greece, manifested as a respiratory disease, occurs during the period from December to July.

The presence of virus in human blood and sputum is shown by the experimental infection of guinea pigs.

The great susceptibility of goats and sheep is demonstrated by inoculation of the virus.

The experimental infection of goats and sheep, in the form of bronchopneumonia, may be accomplished by nasal installations of virus.

A natural infection, in the same form, occurs in goats and sheep.

The virus is present in the milk of goats and sheep, experimentally or naturally infected, during the whole milk period.

After pregnancy the virus reappears in the milk of infected animals and may be transmitted directly to new-borns.

Milk appears to be the source of infection in men.

The interhuman infection by sputum cannot be the main mode of transmission, because of the interruption of the disease during the hot season.

The outbreaks of the human disease are concurrent with the milking period of goats and sheep.

The manifestation of the disease in the form of bronchopneumonia may be attributed to the susceptibility of the respiratory system of both man and animal.

**ACKNOWLEDGMENTS**

We must acknowledge our indebtedness to Dr. G. Vernon, Dr. G. Smith, pathologist of the British Hospital in Athens, Dr. E. Kendall, medical specialist, Dr. Catto, and Dr. Skaffly, radiologist, for the aid they offered us in our research work.

*All these facts concerning the infection of goats and sheep and the role played by their milk were reported to Dr. Rolla Dyer, Director of the National Institute of Health, in a letter of 31 January 1948.*