EXPERIMENTAL Q FEVER IN SHEEP

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The aim of the present investigation is the complex study of experimental infection in pregnant ewes by means of clinical, serological, biological, histological and electron microscopy methods. Four ewes, pregnant from the 2nd to 5th month, were infected by intravenous (in one case by intraperitoneal) routes with a C. burnetii strain at 10^9 ID 50/ml. The clinical illness in all of the animals was characterized by fever and two-phase temperature reaction on the 5th and 12th days. The clinical symptoms were as follows: torpidity, reduced appetite, thirst, conjunctivitis, rhinitis, rapid breathing. As a result of the developed latent infection, after the acute stage, the animals gave birth to three unviable lambs who died within 24h. Another lamb was still-born. The lambs showed cachexia, arthritis, ataxia, wrinkled skin. The highest CF-titers (1:256-1:512) were reached on the 40th day, but serum antibodies (1:8-1:32) first appeared on the 8th day. The titers began to decrease on the 60th day. The pathomorphological changes testify to a latent infection characterized by placentitis, lymphocellular proliferation of the lamb's parenchymal organs and lymph nodes, multiple thromboses, interstitial pneumonia and plural proliferative changes. The EM exam showed rickettsiae in placentas mainly in the form of inclusions in cytoplasm of leukocytes and epithelial cells.

INTRODUCTION

Experimentally produced Q fever in sheep and lambs has been described by some authors (1, 3, 5). The aim of the present work is the complex study of experimental infection by C. burnetii using clinical, serological, biological, histological and electron microscopic (EM) methods.

MATERIALS AND METHODS

Experimental animals. Four pregnant ewes were selected: one was at the beginning of the 4th month of pregnancy; two, at the beginning of the 3rd month, and one at the 45th day of pregnancy. The animals were seronegative for Q fever, chlamydiosis, brucellosis, and toxoplasmosis. The infection of the ewes was preceded by a 3-day oral treatment with Biofurasolidon for inhibition of banal microflora, followed by a 7-day period for elimination of the drug. Four pregnant rabbits, sero-negative for C. burnetii and C. psittaci, were also selected. The rabbits were treated orally with tetracycllilne for 3 days to inhibit possible latent infections.

Strains. For infecting, the Bulgarian strain Tchilnov isolated from an aborted sheep was used. The strain had well-expressed pathogenic properties for 6-7-day-old chicken embryos (CE) with yolk sac (YS) inoculation and for guinea pigs and mice with peritoneal (IP) inoculation. The infectious suspension with a titre of 10^9 ID 50/ml was prepared from the 125th passage of the strain in YS of CE.

Infecting of animals. Three ewes were injected intravenously (I.V.) with 10 ml of the suspension, and one ewe was injected I.P. with the same dose. The pregnant rabbits serving as a biological probe were inoculated I.P., between the 10th and 22nd day of pregnancy, twice in a two-day interval with 3 ml (1st injection), and 6 ml blood from

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the experimental ewes, collected during the temperature rise.

Serology. Complement fixation test (CFT) with C. burnetii 2nd phase antigen was used.

Histological investigations. Routine methods were used to examine parenchymal organs of sheep, fetuses, and placentae.

E.M. The above materials were used for preparation of ultrathin sections which were examined by EM JEM 100C at accelerating voltage 100 kV and instrumental magnification from 5000 to 100,000 according to methods described earlier (4).

RESULTS

Clinical data. Readings of the temperature had been taken two days before inoculation, on the day of inoculation, and daily thereafter up to the 20th day after infection. The animals manifested signs of illness on the 5-7th day after the inoculation. The temperature rose to 40.9°C. The fever continued for 2-3 days. A new temperature rise of 0.6-0.7°C was registered on the 12-13th day, followed by normalization on the 14-15th day. Besides the two-phase temperature reaction, the ewes showed torpidity, reduced appetite and thirst. These symptoms were most evident during the febrile period. Lacrimal discharge, conjunctivitis, serous-mucous rhinitis, and rapid breathing were present. Two of the ewes bore unviable lambs (No. 1 on the 25th post-infection day; No. 3 on the 60th post-infection day). The third ewe had twins on the 65th post infection day: one was unviable and one was still-born. Several days before lambing a certain change for the worse in the ewes’ general condition was observed: difficulty in standing or holding the head erect and reduced appetite. The lambs were normal without retention of placentae. The lambs showed cachexia, wrinkled skin, arthritis and ataxia. They died within 24h. Two of the ewes infected I.V. were killed 3 to 5 days after lambing, and the third - at the 48th h. The fourth ewe infected I.P. was killed on the 7th day after infection at 4 and a half months of pregnancy. All of the inoculated rabbits developed illness accompanied by elevated temperature up to 41.2°C and torpidity, and they aborted 6-10 days after infection.

Serological data. CF-antibodies against C. burnetii with titers 1:8-1-32 appeared on the 8-10th day after the infection. The maximal titers (1:256-1-512) were established on the 40th day (ewe No. 2 and 4) or on the 50th day (ewe No. 3). These titers remained stable up to the 60th day, and then began to drop. The CF-antibody dynamics were evaluated up to the 70th day after infection when the experiment was discontinued. That period was shorter with ewe No. 1 that lambed on the 25th day. The infected rabbits had CF-antibodies with titers from 1:64 to 1:256.

Pathomorphological data. Characteristic changes in the infected sheep were thromboses in mesenterial lymph nodes, interstitial pneumonia, and proliferative processes in the liver. Macroscopically, placentae were edematous, thickened, and hyperemic. Cotyledons were swollen, hyperemic, and covered in some places with whitish coatings. The chorion was thickened, folded and had a dirty-red colour. Histologically hyperemia, desquamation, and necrosis of chorionic epithelium, lympholeukocytic infiltration, single rickettsiae or groups of them were observed. In the unviable and still-born lambs moderate perivascular proliferation in interstitial tissue, lympholeukocytic knots in the liver, necrobiosis of hepatocytes, lightly expressed interstitial pneumonia, and lymphadenitis of mesenterial lymph nodes were noted (Figs. 1, 2). There were vascular and degenerative processes in the kidneys and adrenals. Inflammatory changes of various degrees were observed in all internal organs.

Electron microscopy. The concentration of rickettsiae in ultrathin sections of placentae and