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INFLUENCE OF *COXIELLA BURNETII* INFECTION OF MALE MICE ON THEIR OFFSPRING

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Summary. - The dramatic spread of Q fever in Poland among cattle kept in isolation from natural environment (ticks, wild animals) has suggested the possibility that the infection may also be transmitted sexually. To test this hypothesis series of experiments have been performed in controlled laboratory conditions. Male mice infected with *C. burnetii* were allowed to mate with healthy female mice. On day 18 of pregnancy serum IgM antibodies to *C. burnetii* antigens and bacteria in spleen, liver and placenta were detected. The influence of *C. burnetii* transmission between parents of their offspring was investigated. It has been found that *C. burnetii* infection in males diminish the number of fertilized females. Their litters are fewer in number and the number of dead embryos is increased.

Key words: Coxiella burnetii; sexual transmission; mice

Introduction

During the last few years in Poland we have observed mass infections of cattle bred in isolation, excluding any possibility of contacts with exterior environment, which suggested that there might exist other ways of transmitting *Coxiella burnetii* infection than those known so far.

The principal ways of transmission of *C. burnetii* are associated with the inhalation of infected dust and aerosols or consumption of infected meat or milk (Baca and Paretsky, 1983; Aitken *et al.*, 1987).

This paper aimed at discussing the possibility of sexual transmission of *C. burnetii* and evaluation of the influence of such infection on male mice and their offspring.

Materials and Methods

Rickettsiae. Suspension of phase I Henzerling strain of *C. burnetii* prepared in saline.

Animals. The experimental group consisted of Sffis/pzh inbred mice of both sexes, males and virgin females (8-10 weeks old, sexually mature), who were seronegative for *C. burnetii* antigens.

The fertility of each male was tested by mating with 5 healthy female mice one week before the experiment. Only mice which fertilized all female mice were considered fertile. Female mice were mated with male in oestrus phase, as revealed by cytological methods. At 1, 2, 3, 4, 5, 6, and 7 weeks post-infection (p.i.) with $2.1x10^{10}$ rickettsiae which had been injected intraperitoneally (i.p.), each infected male mice was mated overnight with 5 healthy female mice, then the mice were separated.

Serological examination. Indirect immunofluorescence asay (IFA) was performed with acetone-fixed tissue homogenates and spermatozoa as antigens.

Dotblot assay was carried out to detect the presence of antibodies in serum samples or antigens in the sperm. *C. burnetii* antigens (Manufacture of Sera and Vaccines, Kraków) and spermatozoa were dotted on strips of nitrocellulose paper (Bio-Rad Lab.). Peroxidase-conjugated immunoglobulins against mouse, rabbit or human IgG (Dakopatts A/S) were applied to develop the reaction. Chloronaphthol served as substrate.

Bacterial isolation. Amniotic fluid and homogenized spleen, placenta from female mice suspected of being infected were inoculated into guinea pigs. Later on, samples of guinea pig spleens were passed to the Vero cells cultures. Isolated *C. burnetii* cells when killed with formalin served as vaccine for rabbit immunization. Statistical evaluation of differences between treatment and control groups was performed by X^2 test and Student's t-test.

Results and Discussion

Spermatozoa were obtained from the cauda of epididymis as well as the impression preparations were prepared from the spleen and liver of infected male mice; both showed the presence of *C. burnetii* organisms, as revealed with Gimenez staining and IFA (Fig. 1). All serum samples from the male showed the presence of anti-*C. burnetii* antibodies in the dot-blot test.

One day before the delivery of female mice, *C. burnetii* antigens were detected in the amniotic fluid, liver, spleen, and placenta using IFA. Rickettsiae were isolated from Vero cells inoculated with the homogenized organs. Class IgM and IgG antibodies to phase I and phase II *C. burnetii* antigens were detected by dot-blot assay in the serum of all mated female mice. Antibodies were present in the sera of both pregnant and non-pregnant mice regardless of

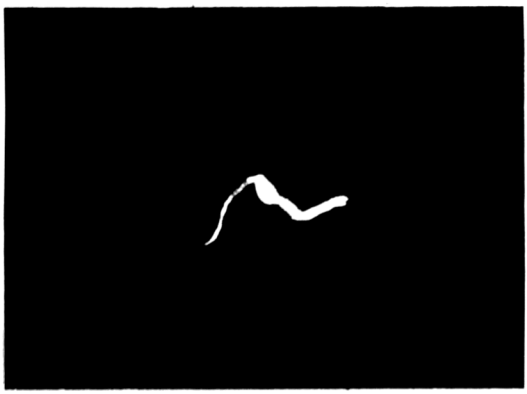

Fig. 1
Immunofluorescence of spermatozoa isolated from *C. burnetii* infected mice

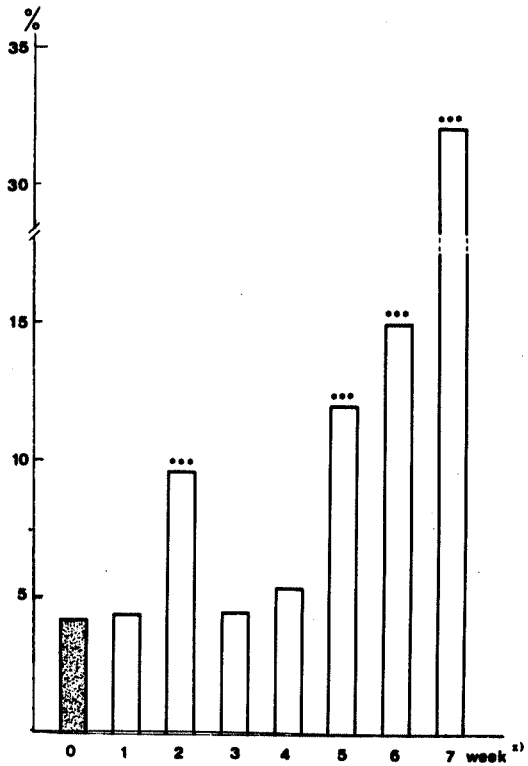

Fig. 2
Percentage (%) of dead embryos from females (black columns) mated with an infected male; females (empty columns) mated with healthy males
* males infected 1 to 7 weeks before mating with the tested female mice
*** $p \leq 0.005$

the duration of infection in males. The range of serum titres was from 20 to 40, both for phase I as well as for phase II *C. burnetii.*

In order to eliminate the possibility of non-sexual transmission of the infection due to overnight contact with urine or faeces of infected partners, one infected male mouse was housed overnight together with 5 healthy male mice. Neither antibodies to *C. burnetii* in the serum nor rickettsiae in organs of healthy male mice were detected.

We conclude that *C. burnetii* infection in mice can be sexually transmitted to females, most probably due to the ability of rickettsiae to adhere to spermatozoa.

The influence of *C. burnetii* infection on the offspring of mice infected by sexual route was investigated. The number of embryos and their foetal mortality were evaluated.

Significant decrease in number of embryos from females mated with male mice in the 6 and 7 week of infection was observed. This was accompanied by

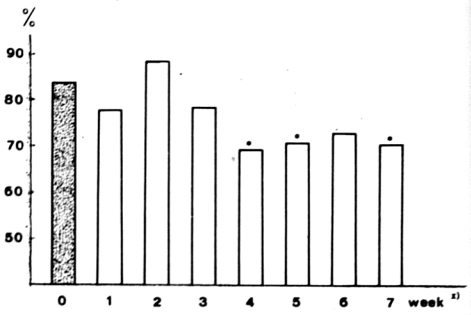

Fig. 3
Percentage (%) of pregnant mouse females (black columns) mated with an infected male; females (empty columns) mated with a healthy male
* males infected 1 to 7 weeks before mating with the tested female mice
* $p \leq 0.5$

a significant increase in the number of underdeveloped, dead embryos from females mated with males in the 2nd and later by 5th to 7th weeks p.i. (Fig. 2). Significant decrease in the percentage of pregnant mice in the group fertilized by males on the 4th to 7th week of infection was also found (Fig. 3). The infection transmitted by sexual route results in the decrease in fertilizing capabilities of the male followed by decrease in the number of offspring and in increase of the number of dead embryos.

It is well documented that *C. burnetii* infection leads to abortions (Baca and Paretsky, 1983; Aitken *et al.*, 1987). Aborted embryos, amniotic fluid and placenta contain enormous number of rickettsiae. The mechanism of this phenomenon is unknown: the rickettsiae themselves are strongly angiotropic and toxic substances produced by them can damage the foetus and cause abortion. Possibly rickettsiae or their metabolic products can affect spermatozoa, but this does not eliminate the possibility to transmit the infection from a male mouse to a female mouse with infected semen.

References

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