THE ROLE OF THE HOUSE FLY (MUSCA DOMESTICA L.) IN THE TRANSMISSION OF COXIELLA BURNETI

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Abstract. The flies which are fed a single rickettsia suspension keep their infectivity throughout their life and can contaminate environment as long as that. Under conditions of experiment their life span is 32 days. Coxiella burnetii survives in the faeces of flies as long as 80 days, in the dead flies as long as 90 days.

Flies attracted attention as vectors of a pathogenic agent, mainly in relation to bacteria causing infections of the digestive tract (Bulling et al. 1959, Lyzenko and Povolný 1961). Less frequent were the studies dealing with the relationship between the house fly and non-bacterial agent. The house fly was indicated as a potential vector of the viral enteritis in minks (Bouillant et al. 1965), of trachoma (Zardi 1964, Forsey and Darougar 1981). Philip (1948) described a laboratory infection of the house fly with C. burnetii through infectious faeces of the tick Dermacentor andersoni.

The objective of the present paper was to investigate some relationships between C. burnetii — the causative agent of Q-fever and the house fly as its possible vector.

MATERIAL AND METHODS

Separate experiments were directed at the solution of the following problems concerning the relationship between C. burnetii and flies: 1. Survival of C. burnetii in flies, including the body surface. 2. Survival of C. burnetii in flies whose body surface was disinfected. 3. Detection of C. burnetii on mouth organs, legs and digestive organs of flies. 4. Survival of C. burnetii in flies killed by low temperature after infection and previously kept at laboratory temperature. 5. Transmission of C. burnetii by flies. 6. Shedding of C. burnetii in the faeces of flies. 7. Survival of C. burnetii in the faeces of flies kept at laboratory temperature.

While solving the above problems also quantitative correlations of infection of flies with rickettsiae were observed. A series of investigations was completed by an attempt to transmit C. burnetii transovarily.

Flies. The flies (Musca domestica L.), strain VÚAgC, used in the experiments, came from the Research Institute of Agrochemical Technology affiliated to the J. Dimitrov Chemical Works in Bratislava. The flies were kept in cages made of steel wiring 4 mm in diameter and covered with silon netting. For the rearing of non-infected flies we used cages measuring 25 × 25 × 25 cm, for the experiments with infectious flies — cages measuring 12 × 12 × 20 cm.

Method of infection of flies with C. burnetii. The Nine Mile strain, at second passage on yolk sacs, in the amount 10^9 CFU/ml was used for the infection of flies. C. burnetii was suspended in milk and this infectious milk was fed to flies from a tampon of a single use in Petri dish for 10—12 hours. In addition, the flies were daily fed non-infectious milk.

Collection of samples. In each experiment 10 specimens of M. domestica were tasted daily. In experiments 1 and 2 samples were collected of 10 specimens respectively from day 0 at 6-day intervals until the death of flies. The body surface of flies in experiment 2 was disinfected by shaking them in a test tube with 70 % alcohol and by subsequent rinsing in distilled water. In experiment 3 mouth organs (proboscis), legs and digestive organs (gut) of flies were removed and examined on the presence of C. burnetii. In experiment 4 the flies were killed after infection by a 10 min. freezing at -20 °C, divided into batches of 10 specimens each in Petri dishes and stored in laboratory. In experiment 5 the
Petri dishes for feeding were daily changed and dishes corresponding to 5-day intervals were examined on the premises of C. burnetii. In experiment 6, apart from lids of dishes were placed in the cages and changed daily. The dishes contaminated by faces, corresponding to 5-day intervals, were examined on the presence of C. burnetii. In experiment 7 a cage measuring 25 x 25 cm was used. In order to collect the faces lids of Petri dishes were placed in the cage. The dishes with faces were stored in laboratory and examined at 10-day intervals.

Treatment and examination of samples. The samples from experiments 1, 2, 4 were processed to suspension and incubated in 30% of white mice intraperitoneally (ip). In experiment 3 the proboscides and tibiae of infected flies were cut off with a pair of fine scissors and processed to suspension. The guts of infected flies were partly processed to suspension and partly used as smears for microscopic examination. Haemotoxul stained. In experiment 5 the tamps from Petri dishes were rinsed in saline solution and the elution inoculated into white mice i.p. In experiments 6, 7 the Petri dishes with dilutions were prepared from the samples originating from day 0, day 15 and the last days of C. burnetii demonstration in the samples and inoculated into white mice i.p.

While studying the possibilities of transovarial transmission of C. burnetii in flies, all developmental stages, i.e. egg, larve, pupae, progeny were processed to suspensions and inoculated into white mice i.p. 21 days after inoculation the blood was collected from sinuses orbitalis of the experimental animals by Pasteur pipette for serological tests. The serum obtained by centrifugation of blood at 2500 rev./min. was tested by microagglutination method after Freist et al. (1969). Agglutination reaction diluted 1: 8 and higher was considered as positive.

RESULTS

Under laboratory conditions the house fly can become infected with C. burnetii from infectious food, in our experiments from infectious milk. The results of experiments (probuscides, legs, gut) show, that there may be the surface infection as well as internal infection, i.e. infection of the digestive tract. The flies maintain their infectivity throughout their life span — for 32 days. C. burnetii probably does not multiply in the flies. This fact may be deduced from the lower titres of C. burnetii depending on time intervals, and from the negative detection of the pathogen in epithelial cells of the digestive tract. C. burnetii persisted for 90 days in the flies killed by low temperature. The infectious flies can contaminate the environment throughout their life — for 32 days. The contamination may be caused by legs, proboscides, or by hair cover of body, as well as by shedding rickettisia in faces. It was observed that rickettisia were shed in faces till day 15 p.i. Under our conditions C. burnetii survived in the faces for 80 days (Table 1).

The samples collected in different experiments were also examined quantitatively, in order to find out to what extent and in what number the flies can become infected from the food offered (day 0) and what is the amount of rickettisea which survive in the flies during their life (day 15) and at the end of their life, or in other samples on the last day of the demonstration of C. burnetii presence in them. We ascertained that on day 0 the amount of rickettisia in samples ranged from 10^{2-3} to 10^{4-5} ID_{50}/ml, on day 15 from 10^{0-1} to 10^{0-2} ID_{50}/ml and on the last day of C. burnetii demonstration in samples from 10^{0-2} to 10^{0-9} ID_{50}/ml (Table 2).

Experiments on transovarial transmission of the pathogen we failed to demonstrate the transfer of C. burnetii from one developmental stage to another, or multiplication of rickettisia in individual stages. Only the eggs were found to be infected, probably due to surface contamination.

DISCUSSION

Infectious dose for experimental flies was chosen so that it corresponded to the infection to which flies are exposed under natural conditions, i.e. when rickettisia are shed in milk or when the infected cows are calving. Several authors reported
The importance of flies as carriers of infection is limited by the fact that *C. burnetii* probably does not persist in the fly population by transovarial transmission. A restricted survival of the pathogen in the digestive organs of flies is evidenced by the results of experiments with faeces during gradual sampling and detection of rickettsiae from the fly gut. The flies were daily offered a new Petri dish with uninfected milk so that it may be presumed that their digestive tract was rinsed out after about 10-15 days, or that the infection was eliminated to a certain degree during the process of digestion.

The results obtained showed that the house fly may play a role in the transmission and dissemination of *C. burnetii*, mainly as a mechanical carrier in that it occurs in the contaminated environment, primarily during epizootics, when it comes into contact with infected food (milk), or other sources of infection (placenta, amniotic fluid).

The importance of flies as possible vectors of *C. burnetii* consists in the fact that they maintain the infectivity throughout their life, that they can contaminate environment, as well as in the fact that the pathogen can survive for a long time (80 days) in faeces of flies and in the dead flies (90 days). The infected dead flies may become a component of dust and when inhaled indoors may become a source of infection, this being a characteristic mode of infection of man or animals.