SEROLOGICAL STUDY OF EXPERIMENTAL AND NATURAL Q FEVER IN CATTLE AND SHEEP

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ABSTRACT
The objective of the work has been to investigate the serological response in cattle and sheep against Coxiella burnetii after experimental or natural infection. Two cows and four sheep were intravenously inoculated with 1st phase C. burnetii isolated from sheep in yolk sacs of chicken embryos. The serological examination was carried out in a complement fixation test (CFT) with 1st – and 2nd phase. Henserling strain antigens. It was found that CF-antibodies in 2nd phase appeared in experimentally infected cows or sheep in 1-2 weeks after the infection. The maximal titers were found between 4th and 6th weeks. The titers in low levels were found up to 18 month (observation term). The 1st phase antibodies appeared considerably later (5-7th week) and the maximum titers were established in 3rd or 4th month. In sera of naturally infected cattle and sheep 1st phase antibodies were found in low titers up to one and a half-two years, and 2nd phase antibodies in low titers kept themselves to five years.

Introduction
By reason of its high specificity, the Complement Fixation Test (CFT) is one of the most applied methods of laboratory diagnostics of the Q-fever now (9, 11, 12, 14, 15). We used 2nd –phase antigens because when the animals are infected a rapid formation and long lasting retention (up to a few years) of the complement fixing (CF) antibodies to the second-phase antigens begins (2, 3, 5).

There are no any reports devoted to the formation and the preservation of the CF-antibodies to the first-phase Coxiella burnetii antigen in the domestic animals. It was found out that in guinea pigs they appear between the 30th and 60th day and that they hold back in low titres just for a few months after the infection (8, 10).

We set ourselves the task of studying the dynamics of the 1st – phase CF – antibodies in cattle and sheep. The purpose was to explain their importance to the diagnosis as well as to the early laboratorial detection of the Q-fever in the animals, because in cases of a positive serological reaction, it is not always considered that this is a center of an active rickettsial infection.

Material and Methods
Antigens – We used 1st – and 2nd – phase Henserling strain antigens made in the Republican Antiepidemiological Station in Sofia. Two antigenic units were used in the main experiment.

Sera – We examined the blood sera of two infected cows and four sheep which were previously controlled by means of CFT of Q-fever and results were negative. An intravenous inoculation with 1st – phase C. burnetii isolated from sheep /4/ at a dose of 10 ml suspension of infected yolk sacs (YS) of chick embryos (CE) was applied. A dilution of 10^-8 in order to infect the cows and a dilution of 10^-4 for the sheep. The serological investigation began on the fourth day after the inoculation, then it continued at one-week intervals and so in
Biotechnol. & Biotechnol. Eq. 19/2005/1 166

Weeks post inoculation

Fig. 1. Dynamics of 1st - phase (2) and 2nd - phase (1) complement fixing antibodies of two experimentally infected with Coxiella burnetii cows; 3 - changes of the body temperature.

the course of eighteen months as a whole. Also were examined 19 blood sera (12 of cattle and 7 of sheep) which were taken from two farms where active foci of Q-fever were revealed.

The sera were taken from aborted cattle and sheep, from cattle suffering of mastitis and from healthy animals of the same herds as well.

**Complement fixation test.** It was applied by the method of Zdrodovskii and Golinevich (1). The sera which showed retention of the complement at dilution of 1:10 were further examined at ascending dilutions. For the titer of the CF-antibodies was taken the highest serum dilution showing a retention of the hemolysis +++ or +++. The positive sera were also checked-up for brucellosis, vibriosis, salmonelosis and Chlamydia.

**Results and Discussion**

The results of the examination of the blood sera with 1st-phase and 2nd-phase C. burnetii strain BP antigens (the strain was isolated from sheep) show that the experimental infection with 1st-phase C. burnetii BP strain in cattle and sheep gives a considerably different response of the CF-antibodies (Figs. 1 and 2).

Fig. 1 shows that the examined by CFT bovine blood sera give a negative reaction to the 1st-phase antigen in the starting 35-40 days after the infection. The CFT with 2nd-phase antigen gives a positive reaction in increasing titers between 1:10 and 1:160 in the same period of time. Maximum rise in the titer /1:40 respectively 1:20/ of the 1st-phase CF-antibodies was found out between the 50th and 60th day and later on it remained the same level up to the end of the experiment (18 months).

A similar dynamics of the titers is noted in experimenting with sheep (Fig. 2). Applying 2nd-phase antigen, the CF-antibodies appeared in the blood of the four of the sheep quite early /to the 7th day post inoculation/. Between the 7th and the 15th day the titers of all sheep were at their maximum, reaching to 1:80 – 1:320. They remained in a comparatively high level /1:40 – 1:60/ up to the 40th – 50th day after the inoculation. In the examination with 1st – phase antigen, the CF-antibodies appeared in the blood of the four sheep considerably later – between the 40th and the 60th day of their inoculations in comparatively lower titers – from 1:10 to 1:80. The highest limits of the titers /1:40 respectively 1:80/ were found out in sheep № 2 –
Fig. 2. Dynamics of 1st - phase (2) and 2nd - phase (1) complement fixing antibodies of four experimentally infected with Coxiella burnetii sheep; 3 - increase of the body temperature.

towards the 3rd month and in the sheep № 3 – towards the 4th month of the infection.

The CF-antibodies of these sheep showed a rapid decrease then, and towards the 15th month of their infection they disappeared completely.

In five of all six examined with 2nd-phase antigen animals (1 cow and 2 sheep) the positive titers remained so during 18 month, which coincides with the data of the other authors (12, 13).

The next of our tasks was to investigate the duration of the preservation of the CF-antibodies in cattle and sheep infected by natural way in a long lasting period of time (Table).

The table shows that in the examination with 1st-phase antigen, the rate of the positive tests made a year and a half after the infection, is 47.4%. Later on, in two years it decreased to 10.5%. The titer of the CF-antibodies was varying between 1:10 and 1:20 up to the end of one year of the infection; it was 1:10 in two years and then we did not find out any positive reactions. When the serological examination of the samples was made by 2nd-phase antigen, the rate of the reacting animals varied in an wide scale between 31.3% and 100%. We noted a higher number of reacting animals when the same examination was made in one year /100%/ and in two years /89.5%/.

It was the lowest one in five years /31.3%/. The titer of the CF-antibodies remained in comparatively higher limits (between 1:20 and 1:80) up to 5 years of the infection.
TABLE

Results from the examination by CFT with 1st – and 2nd – phase Coxiella burnetii antigens made on 19 blood sera of naturally infected cattle and sheep

<table>
<thead>
<tr>
<th>Term of the examination after the infection</th>
<th>Total No of samples</th>
<th>Complement fixation test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No/% 1:10 20 40 80</td>
<td>2nd phase antigen</td>
</tr>
<tr>
<td>12 months</td>
<td>19/100 2 7 6 4</td>
<td>94/7.4 8 1 - -</td>
</tr>
<tr>
<td>18 months</td>
<td>17/89.5 2 8 4 3</td>
<td>63/1.6 5 1 - -</td>
</tr>
<tr>
<td>2 years</td>
<td>14/73.7 3 5 5 1</td>
<td>2/10.5 2 - -</td>
</tr>
<tr>
<td>3 years</td>
<td>10/52.6 4 4 2 -</td>
<td>- - - -</td>
</tr>
<tr>
<td>4 years</td>
<td>7/38.9 4 2 1 -</td>
<td>- - - -</td>
</tr>
<tr>
<td>5 years</td>
<td>3/31.3 3 2 -</td>
<td>- - - -</td>
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</tbody>
</table>

There is no publications dealing with the diagnostic value of CFT in parallel testing of the animal sera with 1st-phase antigen and 2nd-phase antigen. Our investigations in this direction show that the comparative examination by using both antigens could be applied in the laboratorian practice for finding out of the diseased animal in the acute stage of the infection or shortly afterwards. In our study, like in human Q-fever cases (7), when animals were serologically examined, positive results with 1st-phase antigen with sera not reacting with 2nd-phase were not found.

All the 19 sera taken from naturally infected cattle and sheep gave negative results when examined for brucellosis, vibriosis, salmonelosis and chlamydiosis.

Conclusions
1. The complement fixing antibodies to Coxiella burnetii in 2nd phase in experimentally infected sheep with titers 1:10 – 1:20 appeared on the 7-8th day after the infection. The maximal titers (1:160 – 1:320) were found on the 30th – 40th day. They remain stable to the 50th day and then began to drop but in a low level (1:10) they were found to the 74th week after the infection. The maximum titers (1:40 – 1:80) were found in the 3rd or 4th months in a halt of the animals, and then rapidly decreased to 1:20 – 1:10 – 1:10. In the other half of the animals the titers never exceed 1:20.

2. The experimentally infected with C. burnetii cows accumulated CF – antibodies with expressed dynamics which is similar to that of sheep: earliear appearance (2nd week) of 2nd phase antibodies with higher maximum titers (1:80 – 1:160) towards the 1st phase antigens (5th – 6th weeks; 1:20 – 1:40). Both types antibodies remained in the blood serum with low titers (1:10 – 1:20) to 18th month (observation term).

3. The experimentally infected with C. burnetii cows accumulated CF – antibodies with expressed dynamics which is similar to that of sheep: earliear appearance (2nd week) of 2nd phase antibodies with higher maximum titers (1:80 – 1:160) towards the 1st phase antigens (5th – 6th weeks; 1:20 – 1:40). Both types antibodies remained in the blood serum with low titers (1:10 – 1:20) to 18th month (observation term).

4. The examination by CFT on sera of naturally infected cattle and sheep indicates that 1st phase antibodies against C. burnetii can be found in low titers up to one and a halt-two years. The 2nd phase antibodies in low titers keep themselves up to five years.

REFERENCES
6. Fedorova N., Djuisalieva R. (1963) GMEI, 6, 68-