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Evaluation of agreement of ELISA and complement fixation test in the diagnostics of Q fever in cattle

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Summary

Q fever is a worldwide zoonosis that is manifested as a reproductive failure in animals and by polymorphic, nonspecific symptoms in humans. The infection may be acquired through the respiratory or alimentary route or an arthropod bite. The diagnosis of Q fever relies mainly upon serology: indirect immunofluorescence assays (IFA), complement fixation (CF) tests or enzyme-linked immunosorbent assays (ELISA) are used to detect antibodies against Coxiella burnetii in sera of infected animals. The aim of our study was to evaluate the agreement of the commercially available ELISA kit and CF test using statistical methods (Kappa value). We used serum samples that were collected from 122 dairy cows from one herd in central Poland. The general health status of the herd was good, and the animals were clinically normal. Our results showed a low agreement (Kappa value = 0.376) between the commercially available ELISA and CF test.

Keywords: Q fever, CF test, Kappa value

Query (Q) fever is a worldwide zoonosis. Its agent, *Coxiella burnetii*, causes abortions in goats and, less frequently, sheep and causes reproductive problems in cattle (1, 4, 5). Structurally this is an obligate intracellular Gram-negative bacterium. *C. burnetii* grows in yolk sacks of chicken, cell cultures and laboratory animals. In cell culture, good growth may require incubation for several weeks. In culture, *C. burnetii* transforms from a virulent phase I type (resists macrophage killing and multiplies slowly) to an avirulent phase II. Phase II bacteria have altered expression of cell wall lipopolysaccharide, do not occur in nature and are killed by macrophages. Though *C. burnetii* replicates only intracellularly, it survives extracellulary within the hosts and due to its resistance to physical agents it is stable for long periods in the environment (4, 5). The reservoirs are only partially known and include several species of mammals, birds, and arthropods, mainly ticks, but also mites, fleas, lice and flies. While an important reservoir seems to be small wild rodents, the most commonly identified sources of human infection are farm animals such as cattle, goats, and sheep (1, 4, 5). Pets, including cats (4, 5, 7), rabbits, and dogs, have also been demonstrated to be potential sources of urban outbreaks. Cattle and small ruminant, when infected, shed the desiccation-resistant organisms in urine, feces, milk, and, especially, in material getting out during abortion or parturition (1, 4, 5). High concentrations of *C. burnetii* are found in the placentas of infected animals (4). Reactivation of latent infection occurs in female mammals during pregnancy.

The infection may be acquired by the respiratory or alimentary route or arthropod bite. The prevalence of *C. burnetii* infections is unknown and may be underestimated (5). Over the last few years, the apparent increase in the incidence of this disease in humans may be due to improved increased reporting or diagnosis (5, 7). The clinical presentation of Q fever in humans is polymorphic and nonspecific and may be acute, most often pneumonia or hepatitis, or chronic, mainly endocarditis. In animals, a vulnerable site of *C. burnetii* localization is the female reproductive system and infection may be accompanied by abortion or infertility. Inapparent and subclinical infections are common (1, 4, 5).

The diagnosis of Q fever relies mainly upon serology. Indirect immunofluorescence assay (IFA), complement fixation (CF) test or enzyme-linked immunosorbent assay (ELISA) are used to detect antibodies against *C. burnetii* in sera of infected animals (2, 3, 8, 9, 11). Commercially available tests allow the detection of anti-*C. burnetii* phase II antibodies. Titers of antibodies to phase I antigen in acutely infected people are low, while antibodies to phase II antigens show

a rapid fourfold increase. If the infection becomes chronic, levels of antibodies to phase I increase. The presence of specific IgG provides evidence of a recent *C. burnetii* infection or a past exposure.

In cell culture, chicken embryos and tissues of experimental animals, direct immunofluorescent staining will identify this agent (2, 3, 8, 9, 11). Polymerase chain reaction-based assays employing specific DNA primers are also used to detect infections with *C. burnetii* (10, 13).

Routine diagnosis of Q fever in aborted ruminants is generally performed by the detection of bacteria in smears or impressions of placentas stained by the Stamp, Gimenez or Machiavello methods and combined with the serological investigation of at least ten sera samples from the herd (2, 4, 5, 9). The CF test is still the OIE reference method, however it is weakly sensitive and the antigen used in this test frequently fails to detect antibodies in some animals (2, 3, 8, 9). Moreover many ruminants may be seropositive in the CF test without any clinical signs (1). So also other serological tests are now being used in the Q fever diagnostics, especially the ELISA (2, 3, 8, 9). However, little is known about the correlation of the results of different serological methods. So, the purpose if this study was to compare the results of the CF test and a commercially available ELISA obtained with sera from random selected animals in one cattle herd.

Materials and methods

Serum samples. Serum samples were collected from 122 dairy cows from one herd in central Poland. The general health status of the herd was good, and the animals were clinically normal. The sera were kept in the freezer (–20°C) until the time of the examination.

Complement fixation test. This method was performed in the National Institute of Veterinary Hygiene as described previously (8). The starting dilution of the examined serum sample was 1 : 5, the ending dilution was 1 : 80. The results were expressed as (–) full haemolisys, (+) slight erythrocyte sediment, (++), significant erythrocyte sediment, (+++) weak haemolysis or (++++) no haemolysis. A serum was considered as positive when a partial inhibition of haemolysis (++) was observed in the dilution 1 : 10.

ELISA. The ELISA was performed in 96-well flat bottom microtiter plates from the commercially available kit (ELISA Q Fever serum screening, Institute Pourquier, France). All the wells were coated with *Coxiella burnetii* phase I and II antigens. Sera to be tested were incubated in the wells for 1 hour at 21°C. Any immunoglobulin specific to *C. burnetii* present in the serum formed an antigen-antibody complex and remained bound in the wells. After threefold washing an anti-ruminant IgG conjugate (coupled to peroxidase) was added to the wells and incubated to bind to the immune-complexes. After threefold washing the enzyme substrate TMB was added to the wells, forming a blue compound with the conjugate, which became yellow after blocking. The optical density (OD) of the wells was read in the photometer at 450 nm. The intensity of the colour was a measure of the rate of anti-*C. burnetii* antibodies present in the serum tested.

Tab. 1. Calculation of the Kappa value
Kappa = 2 × (a × d – b × c)/[(a + c) × (c + d) + (a + b) × (b + d)]
where:

Results of test X	Results of test Y		Total
	positive	negative	
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	n = a + b + c + d

The limit of positivity was defined by using a positive control serum supplied with the kit. The positive control had a minimal mean OD of 0,350. The final result of the examination was calculated according to the formula:

$$\frac{\text{S/P \} = (\text{OD of the sample} - \text{OD of the negative control})}{(\text{mean OD of the positive control} - \text{OD of the negative control}) \times 100}$$

where S/P% is the percentage value of the examined sample.

The obtained percentage value of the examined sample was considered as follows: up to 40% – negative result, 40-50% doubtful result, 50-80% moderately positive, over 80% strongly positive result.

Statistical analyses. In order to evaluate the agreement of the results of ELISA and CF test, collections of sera which included positive samples in both tests, negative samples in both assays and positive samples only in one test were used. The evaluation of the agreement was performed by calculating the Kappa value using the programme Win Episcope 2.0 (free shareware) (12). Only the results which were positive or negative in both tests were considered for calculation of the Kappa value (tab. 1).

Tab. 2. Evaluation of tests agreement using the Kappa value statistical analysis (12)

Kappa value	Degree of test agreement
< 0.10	no
0.11-0.20	very low
0.21-0.40	low
0.41-0.60	medium
0.61-0.80	high
0.81-1.00	very high

Obtained Kappa results are values between 0 and 1.0. The degree of agreement is proportional to the obtained Kappa value, as given in tab. 2.

Results and discussion

The results of the comparison of the complement fixation (CF) test and ELISA are presented in tab. 3.

To evaluate the agreement of the results of the CF test and ELISA the Kappa value was calculated only from sera positive and negative in both tests. The results which were considered in this calculation are shown in tab. 4.

The Kappa value of test agreement calculated on these results was 0.376. This means, that the degree of the agreement between the ELISA test and the CF test was low.

There are only few reports comparing the value of CF test and the ELISA in the detection of anti-*C. burnetii* antibody in humans and animals (2, 3, 9, 11). In these reports the ELISA demonstrated high sensitivity and specificity, comparing with the CF test, which showed high specificity, but low sensitivity. However,

Tab. 3. Results of the comparison of the complement fixation (CF) test and ELISA for anti-*C. burnetii* antibodies

Result	ELISA		CF test	
	number	percentage	number	percentage
Positive samples	43	36%	72	60%
Negative samples	60	49%	50	40%
Doubtful samples	19	15%	0	0%
Total	122	100%	122	100%

Tab. 4. Agreement of CF test and ELISA for anti-*C. burnetii* antibody in cattle

ELISA results	CF test results		Total
	positive	negative	
Positive	35	8	43
Negative	25	35	60
Total	60	43	103

if the ELISA was used as a commercially available kit, its sensitivity was much lower, even lower than that obtained in the CF test (2). The results of these two tests usually demonstrated a high agreement, but sometimes discrepant result were obtained (2). In our study we got also partially discrepant results using the CF test and a commercially available ELISA for anti-*C. burnetii* antibody in a cattle herd (tab. 3). In the CF test 60% of samples were positive compared to 36% in ELISA. However, we cannot asses if the CF test was more sensitive than the ELISA, because it is possible that the specificity of CF test was low.

ELISA and the IFA test are considered to be more sensitive than the CF test for detection of anti-*C. burnetii* antibody in animals or humans with an acute Q fever, but not in humans with chronic infection or in aborting cows (1). ELISA and IFA detect antibody in acute-phase sera, as they are quite efficient in binding IgM (these antibodies predominate in the acute stage), in contrast to the CF test. A recent *C. burnetii* infection is difficult to demonstrate serologically by the CF test, because antibodies detected by this assay can persist longer than the acute stage (1). Similarly, chronic and persistent *C. burnetii* infections are difficult to diagnose serologically by the ELISA, because this test is more accurate in the acute stage of Q fever, when the IgM predominate in the response. The cows used in our study were clinically healthy. It is therefore probable, that all or most of seropositive animals had serum antibodies as a result of an earlier (that means chronic) infection. This would explain the discrepant result obtained using ELISA and CF test, as well as the low agreement of the results of these two tests.

In our study we demonstrated a relative high prevalence of anti-*C. burnetii* antibodies in healthy cows on this farm (36% using ELISA and 60% by the use of CF test). A screening for anti-*C. burnetii* antibody in milk showed that over 90% of US dairy herds were positive based on bulk tank milk testing over a period of 3 years (6). This high prevalence showed little temporal or regional variation, suggesting that *C. burnetii* infections in dairy herds are common throughout the United States. The study demonstrated, that the number of positive bulk tank milk samples was between 90.0% to 95.8% of all samples depending on the state. In the European countries the prevalence of anti-*C. burnetii* antibody seems to be not so high as in the USA. For example, in 1998 there were 13 to 20% seropositive cattle herds in Italy (1). Among sheep flocks in 1999 57% were found to be seropositive in Germany and 10% in Turkey (1). In Germany 30.4% of dairy cattle herds were seropositive in 1986, 32.8% in 2001 and 43% in 2003. Besides, *C. burnetii* was found by PCR in common available raw milk products (7). It should be stressed, that the fact that an animal is seropositive does not mean, that it is shedding *C. burnetii* in its milk and that it is dangerous for human's health (1).

Both ELISA and the CF test allow testing of a great number of animals and flocks, but positive results of such an examination do not allow to consider an animal as an infection source shedding *C. burnetii* in milk or feces. There is no true relationship between the seropositivity and excretion of the agent; even though most animals shedding this agent in the vaginal mucus, feces or milk are seropositive (1). On the other hand some animals can excrete *C. burnetii* and remain seronegative (1). Therefore, there are no indications to remove seropositive animals from a dairy herd.

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