Q Fever

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SUMMARY

Q fever is a zoonosis that occurs in most countries. Humans acquire infection from animal reservoirs, especially from ruminants. Q fever is a contagious disease, which is due to the proliferation in the body of a rickettsia, *Coxiella burnetii*, a small bacterium measuring 0.3 μm x 1 μm. It can be stained by Stamp’s method. It occurs in two forms: the pathogenic phase I, recovered from animals or humans, and the less pathogenic phase II, obtained by repeated passages in eggs or in cell cultures. As an obligate intracellular bacterium, *C. burnetii* can be grown only in embryonated eggs or cell cultures.

In humans, Q fever occurs in either an acute form (pneumonia, hepatitis, influenza-like symptoms, headache) or a severe chronic form (endocarditis) following an early infection, which may have passed unnoticed. The first form resolves quickly after appropriate antibiotic therapy, but the chronic form requires antibiotic therapy for 2 years or more, coupled with constant serological monitoring. In some countries, including Australia, a vaccine is available for laboratory and abattoir workers.

In animals, cattle develop a subacute form with abortion, retained placentitis, endometritis, infertility and low birth weight. In small ruminants, Q fever is often associated with an acute form inducing outbreaks of abortions followed by recovery without complications. Recurrences over many successive years are quite common. Domestic animals, such as cats, rabbits, etc., can present the same type of disease, and should be regarded as sources of infection for humans.

Laboratory personnel handling *C. burnetii* must remember that this microorganism is extremely hazardous, and isolation of *C. burnetii* must be done only in biosafety level 3 (PC3) laboratories.

Identification of the agent

For laboratory diagnosis, samples can be taken from the placenta, vaginal discharges, stomach contents of the aborted fetuses and, when necessary, from milk and colostrum (although *C. burnetii* is irregularly present in these fluids).

The organism can be seen by microscopy with an oil-immersion objective lens, applied to placental smears stained with 2% basic fuchsin and counterstained with methylene blue, where it appears as a red-stained body (Stamp’s staining). This finding, coupled with serological tests and clinical findings, is sufficient to establish a diagnosis at the flock or herd level.

*C. burnetii* can be isolated by inoculation of specimens into conventional cell cultures or into embryonated hen yolk sacs or laboratory animals. Inoculation of laboratory animals (guinea-pig, mouse, hamster) is helpful in cases requiring isolation from tissues contaminated with various bacteria or in order to obtain phase I *Coxiella* antigens from phase II cells. Demonstration of the agent by the polymerase chain reaction can be done in suitably equipped laboratories.

Serological tests

A number of tests can be used, particularly the indirect immunofluorescence test, the enzyme-linked immunosorbent assay, and the complement fixation test.

Status of Australia and New Zealand

Infection with *C. burnetii* does not occur in New Zealand. In Australia, Q fever is endemic in the northern States where it is maintained in a tick-wildlife (marsupial) cycle with infection frequently being present in domestic and feral ruminants. Human infection occurs in certain occupational groups, particularly abattoir workers, veterinarians and laboratory staff.
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Whereas infection is not endemic in southern States, there is still a zoonotic risk due to the transport of ruminants, including feral goats, for slaughter and processing in southern abattoirs. A vaccine is available and recommended for personnel who are at risk.
Introduction

Q fever is a serious zoonosis and the common modes of infection for humans are inhalation of aerosols or contaminated dust from infected placentae, fetuses, fetal fluids, urine, urinary tract tissue, milk, mammary gland and faeces. Suspect specimens and any culturing should be handled in a Class II Biohazard, cabinet using proper microbiological techniques by trained staff. Serological procedures use inactivated antigens and do not require the same level of biosecurity.

Aetiology

The aetiological agent, Coxiella burnetii, belongs to the family Rickettsiaceae, although it has distinct genetic characteristics and modes of transmission. Unlike other rickettsias, C burnetii produces a small, dense, highly resistant spore-like form, whose stability in the environment is important for transmission.1, 2

Epidemiology

Serological evidence of C burnetii has been found in most countries in Europe, Asia, Oceania and the Americas. The organism is transmitted in nature by one of two cycles: a tick-host wildlife cycle or a domestic animal cycle. A wide variety of ticks have been found to be naturally infected and transmit the agent to various wildlife species occasionally spilling over to infect domestic cattle, sheep or goats. In domestic cattle, sheep and goats direct or indirect transmission from animal to animal occurs. Humans usually become infected through the inhalation of infected aerosols via the domestic animal cycle. Farmyard chickens and dogs can be infected. Aerosols can be generated during parturition in infected animals. Because the organism is resistant to heat, drying and sunlight, soil contaminated with birth fluids, blood, faeces or urine from infected animals can produce C burnetii-rich dust which spreads via aerosol or as formites. Herd infection is probably maintained by inhalation of infected dust and aerosols although low levels of infection are found in udders and milk and the organism may be transferred by the milking process.

Animals may remain infected for prolonged periods even though they possess detectable serum antibody. Serology is therefore useful for detecting potential carrier animals as well as infected herds and flocks.

Clinical signs

In humans the infection has two forms: acute and chronic. The acute form is manifested by pneumonia, prolonged fever, granulomatous hepatitis and, rarely, meningoencephalitis. The main clinical manifestation of chronic Q fever is endocarditis.

In cows, ewes and goats, Q fever can induce abortion and reproductive disorders. Domestic animals, such as cats and rabbits can present the same type of disease, and should be regarded as sources of infection for humans. In animals in Australia, infection is usually inapparent.

Diagnostic Tests

Diagnosis of Q fever in humans depends on demonstration of a four-fold or greater increase in complement-fixing antibody between acute and convalescent serum samples or the presence of IgM antibody in indirect immunofluorescence assays.

Diagnosis of Q fever abortion in ruminants is difficult and often depends on herd serological sampling and / or detection of the organism in affected placenta. It must be remembered that detection of specific antibody or the presence of C burnetii in a placenta is not definitive as the organism has been recovered from placenta after normal parturition.

The major requirement for Q fever serology in veterinary laboratories in Australia is for herd/ flock certification on Q fever status for livestock export requirements.

Appropriate biosecurity precautions to protect staff should be taken in handling specimens from suspect cases.

In suspect abortion investigations, samples should be collected from the fetus, placenta and vaginal discharges soon after abortion for attempted detection of C burnetii. Milk or colostrum samples can also be taken.

Plain blood should be collected for serological testing from a number of aborted animals and from in-contact animals that have had normal births.

Identification of the agent

All laboratory procedures involving potentially infectious material should be conducted using...
strict biosecurity technique, in a Class II Biohazard cabinet, by appropriately trained staff.

Smears on microscope slides of placental tissue from a case of abortion that is suspected of being due to a rickettsial infection are stained with a rapid staining procedure (Stamp). The procedure uses a 2% basic fuchsin solution followed by rapid decolouration with 3% acetic acid solution, and counter-staining with 1% methylene blue or malachite green solution. The smears are examined microscopically (× 500). Many thin, small (0.3 – 1.5 µm long and 0.25 µm wide). pink-staining bacillary bodies will be seen against a blue or green background. Their small size is compensated for by their large numbers, which appear as red masses against the blue or green background.

Microscopically, Coxiella can be confused with Chlamydia and Brucella. However, by the same staining procedure, Chlamydia have sharper outlines, are round, small and may resemble globules. Brucella organisms are larger (0.6 – 1.5 µm long and 0.5 – 0.7 µm wide), more clearly defined and stain more intensely. It is preferable that known positive slides of Coxiella, Chlamydia and Brucella are available for comparison.

Diagnosis can be made on the basis of microscopy, coupled with positive serological results in cases of abortion with histological evidence of placental damage.

For specific laboratory investigations, it may be necessary to isolate the agent in culture. Where microscopical examination has revealed large numbers of Coxiella and there appears to be only a low rate of contamination with other bacteria, direct isolation by inoculation of embryonated chicken eggs may be attempted. For this method, a portion of placenta is homogenized in phosphate-buffered saline containing streptomycin and penicillin. After low-speed centrifugation, dilutions of the supernatant fluid are inoculated into five-day-old embryonated chicken eggs via the yolk sac. Any embryos that die during the first 5 days after inoculation are discarded. The yolk sacs are harvested after 10 – 15 days of incubation. Stained smears of the yolk sac wall are examined to ensure the absence of bacterial contamination and to determine the presence of Coxiella. Further passages may be required to obtain an isolate in pure culture. Microscopical observation of rickettsial bodies is adequate for confirmation of diagnosis.

In the case of a more heavily contaminated sample, such as a placenta, or where there is only a small number of Coxiella in a body fluid, such as in milk, the inoculation of laboratory animals may be necessary. Mice and guinea pigs are best for this purpose. Guinea pigs are used in preference because they are about 10-fold more susceptible than mice.

Following intraperitoneal inoculation, body temperature and antibody status are monitored. If pyrexia develops, the animals is killed and the spleen removed for recovery of the agent by inoculation into embryonated chicken eggs. This method should always be performed in conjunction with serological tests on other guinea pigs, which have been inoculated with the same samples. These sera are collected 21 days after inoculation. Any positive results will confirm a diagnosis of C burnetii.

Serological tests

The most commonly used method for Q fever serology is the complement fixation test (Appendix 1). This is routinely used for serological testing of herd or flock status for export of livestock. Indirect immunofluorescence using IgM-specific conjugates is used in human serology. Other serological procedures used overseas include a micro-agglutination technique, a capillary agglutination test and an ELISA. An allergic delayed-type hypersensitivity skin test has also been used in the past.

References

5. Behymer D., Ruppaner R., Brooks D., Williams J. Enzyme immunoassay for

Appendix 1

Complement fixation test for Q fever

The complement fixation test (CFT) for Q fever has been conducted as a cold complement fixation procedure, especially in human serology, and a warm fixation procedure more commonly used in veterinary serology. Either procedure is acceptable providing equivalent titres are obtained with a panel of reference sera available from the Australian National Quality Assurance Program.

The procedures for preparation of standardised erythrocyte suspension, haemolysin titration, complement preparation and complement titration are followed exactly as for the bovine brucellosis CFT. As the Q fever antigen used is prepared by chicken embryo yolk sac inoculation, it is necessary to ensure that CF reactions are not associated with anti-yolk sac antigens so reactive sera should also be tested against yolk sac control antigen.

The haemolysin used for the test is glycerinated haemolysin (Cat No. HEM 3805) from the Institute of Medical and Veterinary Science, Frome Road, Adelaide, and guinea pig complement is supplied by GIBCO, BRL, Grand Island, NY, USA, or is prepared as an in-house reagent.

The antigen recommended is Q Fever Complement Fixation Antigen Phase 2 "Nine Mile" Strain from Dade Behring (Brisbane), Coxifix (Rhone-Merieux, Lyon, France), Virion Q fever antigen (Cat No. 1123-Virion International, Cham, Switzerland). However some antigens have not given consistent results with reference positive control sera and it is essential that the antigen used in the CFT produces a titre within the consensus range for the panel of reference sera.

All sera used in the test should be inactivated by heating at 58°C for 30 minutes before testing. Nonspecific and anticomplementary activity, often detected in sheep serum, can be removed by heat inactivating serum at 60°C for 45 minutes. Standard positive and negative sera are used in the antigen titrations and as controls in the test proper.

Standardisation of antigen

The quantity of antigen required for use in the test is determined from the following procedure:

Antigen dilutions
a) Set up 6 tubes and add 0.3 mL of diluent (Veronal buffer, pH7.2) to each tube.
b) Add 0.3 mL of antigen to tube 1 and sequentially transfer 0.3 mL from tube 1 through to tube 6.

Antiserum dilutions
a) Set up 6 tubes and add 0.3 mL of diluent to tubes 2 through to tube 6.
b) Prepare a 1:4 dilution of antiserum and add 0.3 mL to tube 1, and 0.3 mL to tube 2. Sequentially transfer 0.3 mL from tube 2 through to tube 6.

Block titration
a) Add 25µL of the appropriate antigen dilutions to all wells of the horizontal rows A to F.
b) Add 25µL of the appropriate antiserum dilutions to all wells of the vertical columns 1 to 6.
c) Add 25µL of diluent to row G and column 7. These are control rows.
d) Add 25µL of complement to all wells.
e) Mix thoroughly and incubate at 37°C for 30 min.
f) Add 25µL of sensitised 1.5% red blood cells, mix and incubate at 37°C for 30 min.
g) Centrifuge the plate and read the results.
To read the plate

Read the highest antigen-antiserum dilution combination that is not subject to anticomplementary action of either component. This gives the dilution equivalent to one unit of antigen. In the test proper, antigen is used at a dilution equivalent to 2 units.

Complement fixation test

Each serum should be tested with both positive antigen and negative control antigen. These antigens are usually tested in separate plates. Columns 1 - 10 are for test sera, column 11 is for a known positive control serum and column 12 is for a known negative control serum.

a) Add 25 μL of diluent to all rows.

b) Add 25μL of heat inactivated serum, diluted 1:4 to rows A and B

c) Serially dilute from row B to row H (25 μL transferred).

d) Add 25 μL of complement fixing antigen to rows B through to row H (plate 1).

e) Add 25 μL of control antigen to rows B through to row H (plate 2).

f) Add 25 μL of diluted complement to all rows.

 g) Mix thoroughly, incubate at 37°C for 30 min.

h) Add 25 μL of 1.5% sensitised red blood cells to all rows.

i) Mix thoroughly, incubate at 37°C for 30 min, mixing at 15 min.

j) Centrifuge trays or let stand, for one to two hours to allow unlysed cells to settle and read the results. The titre of the serum is taken as the highest dilution in which 50% or less lysis occurs and can be expressed as a dilution or as the reciprocal of the dilution.

Interpretation

A true positive is read when a serum reacts with the positive antigen and not with the control antigen. Non-specific reactions may be seen in some sera that react with both antigen preparations. A serum titre of 1:8 is considered positive if there is no reaction with the negative control antigen and no anti-complement activity shown by the serum. Some of Australia’s trading partners consider that any complement fixing activity in ruminants at a 1:8 serum dilution is suspicious for Q fever and their import protocols reflect this.