EUROPEAN Q FEVER COMMITTEE

Which samples should be taken in case of abortion in small ruminants suspected of Q FEVER/coxiellosis?

In case of serial abortions in small ruminants, *Coxiella burnetii* is one of the possible infectious agents, along with *Chlamydia abortus* and *Toxoplasma gondii*. Recent investigations have frequently demonstrated the presence of *C. burnetii* in abortion materials, in many cases in mixed infections with other pathogens [1–3]. Hence, it is important to apply proper sampling schemes in diagnosing the cause of small ruminant abortions.

PURPOSE OF THIS FACTSHEET

To review the diagnostic procedures for Q Fever/coxiellosis in the event of serial abortions in small ruminants, and to answer the following questions: Which animals? What kind of samples? What kind of tests? Which interpretation of the results?

GEORGE VALIAKOS

DVM, MSc, PhD, ASSOCIATE PROFESSOR OF BACTERIOLOGY, CO-CHAIR OF THE EU Q FEVER COMMITTEE.

"The combination of implementing PCR and ELISA has revolutionised Q Fever diagnostics in small ruminants, offering a rapid, cost-effective, and accessible means to detect and manage this economically significant disease accurately."

PIET VELLEMA

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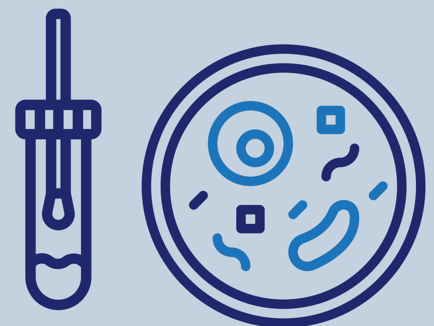
"Abortion outbreaks in small ruminants have consequences for animal health, animal welfare, and are an important cause of economic loss. Because many agents that can induce abortion, like Coxiella burnetii, Chlamydia abortus *and* Toxoplasma gondii, *can pose a zoonotic risk, every serious outbreak should be investigated."*



THE MOST SUITABLE SAMPLES ARE ABORTED FOETUSES AND PLACENTAS.

INFORMATION FOR **VETERINARY PRACTITIONERS**

PCR AND ANTIBODY TESTS ARE COMPLEMENTARY

In case of a *Coxiella burnetii* suspicion in small ruminants, direct (such as PCR) and indirect diagnostic methods (such as antibody tests like ELISA) are complementary. Research shows that:

- Aborting female(s) may still be seronegative although excreting at the time of abortion [4,5], as shown in Figure 1;
- Seroprevalence within farms on which animals have aborted is high (>50%) [6] suggesting the relevance of estimating the within-herd seroprevalence at the time of abortion;
- Given the frequently asymptomatic nature of infection by *C. burnetii*, females that have not aborted may also excrete [5,7], and/or re-excrete during subsequent (normal) parturition [8].

These facts support combining PCR and serological testing (ELISA) in case of a *C. burnetii* suspicion, especially when abortion product samples from all recently aborted animals are not available.

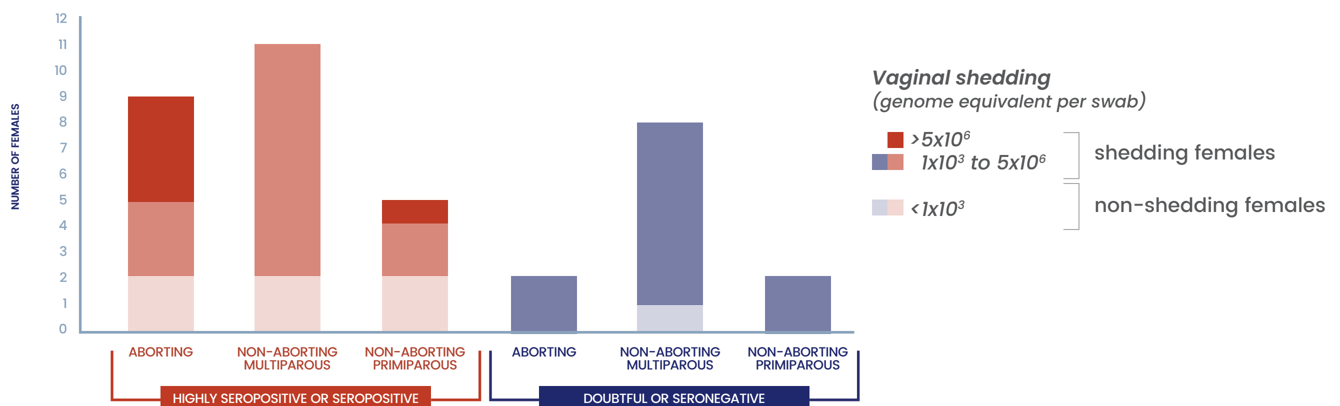


Fig 1. Number of ewes shedding *C. burnetii* in their vaginal mucus during the first month post-lambing or post-abortion, according to their serological status and their aborting status and parity[5].

WHAT ARE THE MOST SUITABLE SAMPLES TO SEND TO THE LAB?

The most suitable samples are aborted foetuses and placentas. If these are no longer available or if the laboratory does not perform analyses on these matrices, vaginal swabs from dams that aborted are **the sample(s) of choice for PCR**[9], if taken as soon as possible after abortion (ideally within 48 hours and no later than eight days). After eight days, it is too late to be able to reach a reliable diagnosis by PCR testing[10]. In all cases, samples must be transported in a cold chain (4°C), preferably within 24 hours. However, if vaginal samples are taken for PCR using a swab with a liquid-based sample and transportation device, they can be sent by mail.

Serological testing of females that have aborted or are presenting other reproductive problems (e.g. stillbirths) is always complementary to PCR. To reach the recommended number of samples (see below), females within three weeks following lambing can also be tested. For the detection of anti-C. *burnetii* antibodies, ELISA is the most commonly used test, but other tests like complement fixation test (CFT)[11] and indirect fluorescent antibody test (IFAT)[12] are also possible.

NOTE: PCR testing of bulk tank milk (BTM) is of minor interest in the diagnosis of abortion, as there is no concomitant excretion between the vaginal route and milk. A PCR positive BTM indicates an active circulation of *C. burnetii* in the flock/herd, but is not enough to ascribe a concurrent abortion to *C. burnetii*.
However, in the Netherlands, all dairy sheep and dairy goat farms are PCR BTM tested every month. With such intensive monitoring, a PCR positive BTM result during the lambing/kidding period on a previously PCR negative farm, is an indication of abortion caused by *C. burnetii*.

HOW MANY ANIMALS SHOULD BE SAMPLED?

In the event of an abortive episode, a group diagnosis should be based on a set of results. If aborted foetuses and placentas are no longer available, **vaginal swabs from as many aborted females as possible (abortions within the last 7 days) should be tested by PCR.** Pooling of samples is possible, but this results in a reduction in information. **Serological testing of at least five (and ideally ten) females that have aborted** or are presenting other reproductive problems, is also possible. If there are not enough females in this situation, blood samples from females that are within three weeks after lambing can be collected, to reach the appropriate sampling number. Collecting blood samples earlier than 15 days after abortion/lambing may give false negative results.

THE CONTRIBUTION OF QUANTITATIVE PCR

The diagnostic approach aims at identifying a massive colonisation of the reproductive tract by *C. burnetii*, and animals that have aborted present more often higher loads than asymptomatic animals (Figure 1). This highlights the use of a quantitative PCR (qPCR), and 10^4 bacteria per gram of placenta or per vaginal swab (individual analysis) or of 10^3 in the case of pooled samples, indicate that *C. burnetii* is the main abortifacient agent. However, lower qPCR positive yields to not exclude this possibility (see below), and further herd/flock investigations are needed in future abortion cases, in combination with investigating more abortive agents.

In case of a result obtained on aborted foetuses, especially when obtained from stomach content, the mere presence of *C. burnetii* is an indication to suspect its involvement.

HOW TO INTERPRET RESULTS?

The European Food Safety Authority (EFSA) has produced a flowchart (Figure 2) to assist in the interpretation of PCR and serological results. This flowchart should not be used as a substitute for an in-depth analysis of serious abortion outbreaks. It is also important to consider the conditions under which the samples were taken and transported, especially when PCR values are close to the threshold.

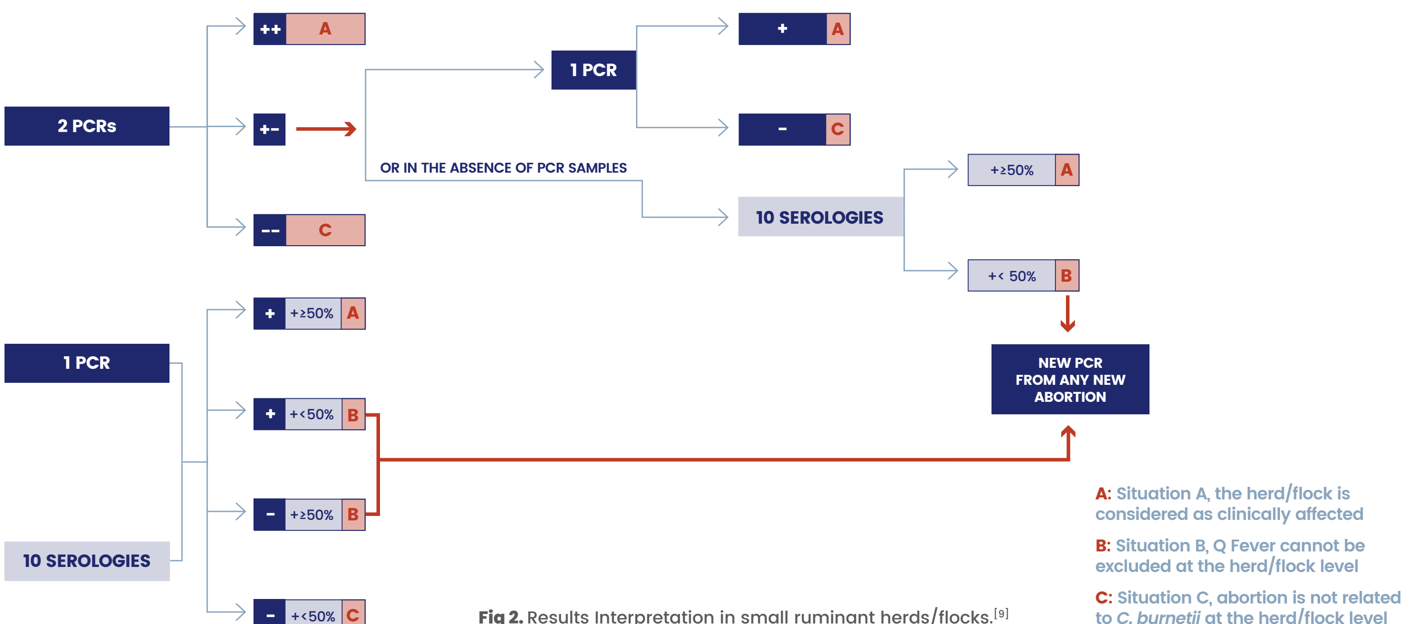
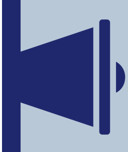


Fig 2. Results Interpretation in small ruminant herds/flocks.[9]



REMINDER: Compliance with the diagnostic protocol (for PCR as for serology) is fundamental! Any deviation will possibly influence sensitivity and specificity of the tests used. In all cases, it is the combination of tests (PCR and ELISA), or even their repetition, that consolidates a diagnosis.

WHAT ABOUT Q FEVER IN SMALL RUMINANTS?

Further details are provided in our factsheet: *"When to suspect Q FEVER* (Coxiella burnetii *infection) in small ruminants?".*

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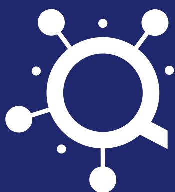

EUROPEAN Q FEVER COMMITTEE

The Q FEVER COMMITTEE, co-chaired by Professors Raphaël Guatteo and George Valiakos, was created in July 2024 with the support of Ceva Santé Animale.

Discover who we are, our mission, and the latest expertise on Q Fever - Simply scan the QR code or visit the link below.

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