Apparent seroprevalence for *Coxiella burnetii* in the Danish horse population

**Authors**

JØRGEN S AGERHOLM*, CAMILLA A SVEJSTRUP†, ANNA-BODIL CHRISTOFFERSEN‡ and JENS F AGGER§

1PROFESSOR, SECTION FOR VETERINARY REPRODUCTION AND OBSTETRICS, DEPARTMENT OF LARGE ANIMAL SCIENCES, FACULTY OF HEALTH AND MEDICAL SCIENCES, UNIVERSITY OF COPENHAGEN, DYRLÆGEVEJ 68, DK-1870 FREDERIKSBERG C, DENMARK

2DVM, SECTION FOR VETERINARY REPRODUCTION AND OBSTETRICS, DEPARTMENT OF LARGE ANIMAL SCIENCES, FACULTY OF HEALTH AND MEDICAL SCIENCES, UNIVERSITY OF COPENHAGEN, DYRLÆGEVEJ 68, DK-1870 FREDERIKSBERG C, DENMARK

3DVM, NATIONAL VETERINARY INSTITUTE, TECHNICAL UNIVERSITY OF DENMARK, BÜLOWSVEJ 27, DK-1870 FREDERIKSBERG C, DENMARK

4ASSOCIATE PROFESSOR, SECTION FOR ANIMAL WELFARE AND DISEASE CONTROL, DEPARTMENT OF LARGE ANIMAL SCIENCES, FACULTY OF HEALTH AND MEDICAL SCIENCES, UNIVERSITY OF COPENHAGEN, GRØNNÉGÅRDSVEJ 8, DK-1870 FREDERIKSBERG C, DENMARK

*PRESENT ADDRESS: SANDDALEN 10, DK-8641 SORRING
†PRESENT ADDRESS: SKT. KLEMENSVEJ 64, DK-5260 ODENSE S, DENMARK
§CORRESPONDING AUTHOR: E-MAIL – JAGER@SUND.KU.DK

**Summary**

*Coxiella burnetii* is a zoonotic bacterium that can infect a wide range of animals, including horses. The observed seroprevalence in horses varies considerably among studies, but a recent meta-analysis found a mean seroprevalence of 15.8%. *C. burnetii* DNA has been detected in aborted equine fetuses but also in the placenta after uncomplicated foaling. The significance of the infection remains unknown.

We determined the apparent seroprevalence in a cross-sectional study based on horses admitted to two equine hospitals in Denmark. Blood samples were taken from 105 horses, mainly Danish Warmblood (n = 48) and Icelandic horses (n = 15), and their serum was tested by the complement fixation test for antibodies against *C. burnetii*. Seropositive animals were not found.

Based on the sample size of 105 horses out of the Danish population of approximately 175,000 equines, it can be estimated that the apparent prevalence of seropositive horses is less than 3%. Although the study population was a convenient sample that may not be representative for the Danish horse population in general, several breeds, gender and age groups were represented, so it is considered that the observed seroprevalence reflects a low seroprevalence in Danish horses in general.

---

**Sammendrag**

*Coxiella burnetii* er en zoonotisk bakterie, der er i stand til at inficere et bredt spektrum af dyr, inklusive heste. Undersøgelser har vist, at seroprævalensen hos heste varierer betydeligt mellem studier, men en nylig metaanalyse fandt en gennemsnitlig seroprævalens på 15,8%. *C. burnetii* DNA er påvist i aborterede fostre, men også i placenta efter normale folinger. Betydningen af infektion med *C. burnetii* hos heste er ikke kendt.

I dette tværsnitsstudie har vi undersøgt den tilsyneladende seroprævalens hos heste indsat på to hestehospita-ler. Blodprøver af 105 heste, især Dansk Varmblod (n = 48) og islandske heste (n = 15) blev udtaget, og serum blev testet for antistoffer mod *C. burnetii* vha. komplement-fikseringstesten. Seropositive dyr blev ikke påvist.

Baseret på en stikprøvestørrelse på 105 heste ud af ca.175.000 heste i Danmark kan seroprævalensen i bestanden estimeres til under 3 %. Ugtaget at de undersøgte heste er en bekvemmelighedstikprøve, som muligvis ikke er repræsentativ for den danske hestebestand, så var flere racer, køn og aldersgrupper repræsenterede. Det vurderes derfor, at resultatet kan tages som et generelt udtryk for, at den tilsyneladende seroprævalens er lav hos heste i Danmark.
Background

Coxiella burnetii is a Gram negative bacterium that causes Q fever in humans. Human Q fever usually has a subclinical course or a mild course characterized by transient flu-like symptoms. Rarely, more severe conditions such as pneumonia, endocarditis, hepatitis and miscarriage may develop. C. burnetii can infect a wide range of animals and Q fever is a known abortifacient in domestic ruminants (1). The infection may cause abortion, premature delivery and stillbirth, particularly in goats and sheep while such incidents seem to be rare in cattle (2). The infection is highly prevalent in Danish cattle, where 60–80% of dairy herds had antibodies in the bulk tank milk (3,4) and 4.1% of slaughtered beef cattle were seropositive (5). Except for reproductive failure, clinical signs of spontaneous Q fever haven’t been reported in domestic animals.

Due to the wide range of hosts for C. burnetii, horses may also become infected although the significance of the infection needs to be further investigated. Two older experimental studies performed in non-pregnant horses have shown that horses may develop fever, conjunctivitis, respiratory and gastrointestinal symptoms after inoculation (6,7). In a recent meta-analysis, Marenzoni et al. (8) investigated the seroprevalence for C. burnetii in horses and estimated a mean seroprevalence of 15.8% (95% confidence interval (CI): 9.6 – 23.0%). However, studies varied in design, test methods, sampling population, geographical region, etc., so exact comparison of studies is difficult. Seroprevalences consequently ranged from 0 (9) to 52.5% (10).

As C. burnetii is a known cause of abortion in ruminants, some studies have focused on this aspect in horses. Low levels of C. burnetii DNA have been detected in aborted equine fetuses (11,12) but also in placentas from horses without a known abortion history (13). Presence of C. burnetii DNA has not been associated with fetal or placental lesions and in some cases, concomitant infection with well-known causes of abortion such as equine herpesvirus type 1 have been present (11,12).

To obtain preliminary knowledge on the occurrence of C. burnetii infection in Danish horses, we performed a seropreva-
lence study based on horses admitted to two major equine hospitals located in eastern and western parts of Denmark, respectively.

Materials and methods

Study design

The design was a cross-sectional study performed during September and October 2012. The sample size (n) was calculated as \( n = \frac{Z^2pq}{l^2} \) with an assumed prevalence of infected (\( p = 0.50 \)), the proportion not infected (\( q = 1 - p = 0.50 \)) and an allowable error on the estimate (\( l = 0.10 \)) at the 95% confidence level (Z=1.96). The chosen prevalence of 0.50 maximizes the sample size estimate. Thus, the necessary sample was estimated to 96 horses. Due to logistics the study was based on convenience sampling by including horses that were referred to two large animal hospitals.

Study population

Horses (n = 105) admitted to either the Large Animal Teaching Hospital, University of Copenhagen, located outside Copenhagen in eastern Denmark (n = 44) or Hammel Animal Hospital located in western Denmark (n = 61) were included in the study. All horses admitted to the hospitals were included if blood sampling was done for other reasons. Animal experiment permission was not required for this study. Ethical approval was given by the Large Animal Teaching Hospital.

Serology

Blood was sampled from the jugular vein by using plain Vacutainer© tubes. The blood was centrifuged at 3000 round per minute (rpm) for 10 min after a clotting period for up to 24 h. Serum was isolated and stored in glass tubes at -18 °C until analysis.

Detection of antibodies was done by an enzyme-linked immunosorbent test (ELISA) using veronal buffer (VBD) were made, i.e. (Stelling 611U96). Two-fold serial dilutions in glass tubes at -18 °C until analysis. Serum was isolated and stored up to 24 h. Serum was isolated and stored (rpm) for 10 min after a clotting period for run in parallel.

Cut-off values according to the OIE Terrestrial Manual 2010 (14) were used, i.e. titers ≥10 to ≤40 were considered indicative for a latent infection and titers ≥80 were indicative for an active infection when using two-fold serial dilutions.

Results

The study population represented 22 breeds. Most horses were Danish Warmblood (n = 48) followed by Icelandic horses (n = 15). Sex distribution was 43% mares, 7% stallions and 50% geldings. The age varied from less than one year to 25 years (Fig. 1) with a mean age of 8.5 years (95% CI: 7.5–9.5).

None of the 105 horses tested positive for neither phase I nor phase II C. burnetii antibodies.

The horses were admitted to the hospitals for a range of diseases and causes with general health check (39.8%), digestion/colic (11.7%) and lameness (10.7%) as the most common. Around 50% of the animals were admitted for other reasons than disease, i.e. health check, vaccination, pregnancy check and castration (Fig. 2).

Discussion

Based on the sample size of 105 horses out of the Danish population of approximately 175,000 equines, it can be estimated that the apparent prevalence of seropositive horses is less than 3%.1 However, this estimate is based on a number of assumptions that are not fulfilled in the present study such as 100% sensitivity and specificity of the test and representative sampling.

Serological testing for C. burnetii in animals has been done by the enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescent assay (IFA) and CFT (14). The ELISA is the preferred method due to its high sensitivity and because it is easy to perform, but C. burnetii ELISAs are only available for use in cattle, sheep and goats. The IFA is mainly used in human diagnostics, while the CFT is used by many veterinary laboratories for serological testing of non-ruminants. The CFT was therefore used in the present study. However, the sensitivity and specificity of the test in equines is unknown although the specificity is considered high (14). The sensitivity of the used CFT has been compared to an ELISA in cattle, sheep and goats (15). The sensitivity and specificity of the CFT in aborting goats compared to ELISA have been determined to 89% (95% CI: 80–95)

1 Max. prevalence calculated as \( [(1-(1-0.95)/105)]/175,000 = 0.03 = 3\% \)
Figure 2. Reasons for admitting participating horses to the equine hospitals.

- Health check
- Castration
- Vaccination
- Pregnancy scanning
- Digestion/colic
- Lameness
- Trauma
- Circulation
- Other

and 82% (95% CI: 62; 95), respectively (16). As we only found CFT-negative animals, the main question is to which extent false-negative samples occurred in the CFT rather than false-positives. Emery et al. (15) tested 555 CFT-negative ruminant sera by ELISA and found 1.1% positive. These findings indicate that false-negative samples are of limited significance if this observation applies to horses as well.

The study population, i.e. horses admitted to two hospitals during a 2-month-period, is not a random sample of the Danish horse population. However, two geographic locations were selected to account somewhat for a possible geographical variation. Infection with *C. burnetii* in cattle occurs at an equal level across Denmark as indicated by the prevalence of bulk tank milk positive dairy herds (3,4), although the cattle density differs across Denmark. The Danish horse population size is approximately 10% of the cattle population and probably has a different distribution. Cattle are obviously more prevalent in rural areas while horses generally are more common near larger cities, so the risk of exposure to infected cattle is probably higher in some regions than in others although it is unknown, if *C. burnetii* cattle strains can infect horses. Age, gender, breed and cause of hospitalization may also have affected the observed prevalence, although these factors varied in the sample population. It is remarkable that even old horses were seronegative. Thus, the 14 oldest horses have had a risk of exposure to *C. burnetii* for up to 16-25 years and still tested negative. As the present study is a preliminary investigation into *C. burnetii* infection in Danish horses, these non-representative distributed parameters are not considered severely to compromise the results indicating that the apparent seroprevalence is low.

**Conclusion**

Although the seroprevalence for *C. burnetii* couldn’t be determined precisely due to limitations in test performance and sampling strategy, the results indicate that the apparent seroprevalence is low in Danish horses.

**Acknowledgement**

Ceva Animal Health, Denmark is acknowledged for financing Camilla A Svejstrup’s veterinary master study that founded the basis for this article. The owners of the horses are thanked for giving us access to their animals and the staff at the Large Animal Teaching Hospital, University of Copenhagen and Hammel Animal Hospital is thanked for assistance.

**References**