EXPOSURE RISK TO *COXIELLA BURNETII* AT AGRICULTURAL FAIRS

Corresponding Author: Kelly M. Still Brooks, DVM, MPH, *Dipl*.ACVPM
Veterinary Diagnostic and Production Animal Medicine
College of Veterinary Medicine
Iowa State University
2424 Lloyd 1809 S. Riverside Dr.
Ames, IA 50011
kmstill@iastate.edu

Co-Authors: Wendy R. Stensland
Karen M. Harmon, PhD
Annette M. O'Connor, BVSc, MVSc, DVSc, FANZCVSc(epi)
Paul J. Plummer, DVM, PhD, *Dipl*.ACVIM-LA, *Dipl*.ECSRHM
Veterinary Diagnostic and Production Animal Medicine
College of Veterinary Medicine
Iowa State University
Ames, IA 50011

Risk of exposure to *Coxiella burnetii* from ruminant livestock exhibited at Iowa agricultural fairs.
Still Brooks, K.M, W.R. Stensland, K.M. Harmon, A.M. O'Connor, and P.J. Plummer

SUMMARY and KEYWORDS

Coxiella burnetii is a zoonotic pathogen typically associated with clinical and asymptomatic infection in ruminant livestock. A re-emerging pathogen of significant public health importance, *C. burnetii* has caused recent epidemics in the U.S. and Europe and public livestock exhibitions are increasingly scrutinized as a potential source of *C. burnetii* exposure. Although *C. burnetii* prevalence data among North American domestic ruminants is extremely limited, contemporary studies suggest that this pathogen is both geographically widespread and highly prevalent on a herd basis, especially in dairy cattle and goat populations. We utilized a real-time PCR assay to detect *Coxiella burnetii* fecal shedding by clinically normal, non-periparturient beef cattle, meat goats, and sheep exhibited at Iowa agricultural fairs. Individual fecal samples were collected from beef cattle, meat goats, and sheep exhibited at twelve Iowa county fairs during the summer of 2009. The sample pool was blocked by species and fair, ten samples from each block were randomly selected for the diagnostic assay; this test pool is considered sufficient to identify with 95% confidence a shedding animal in a population prevalence of 2.85% (cattle and sheep) and 6.25% (goats). Detection of *Coxiella burnetii* DNA was determined through use of a real time PCR assay validated for use in bovine, ovine, and caprine feces; threshold of detection is one DNA copy per PCR (sensitivity 95.8%, specificity 100%). All tested samples were negative for *Coxiella burnetii* DNA. We conclude that non-dairy, non-periparturient ruminants exhibited at Iowa fairs are unlikely to shed *Coxiella burnetii* in their feces and that this population should not be considered to be a significant exposure risk to other livestock or fair attendees.

Key words: Q fever, ruminant, livestock, zoonosis, veterinary medicine, public health

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- Although *Coxiella burnetii* is an emerging zoonosis associated with aerosol exposure to infected ruminant livestock operations, prevalence of infection in non-dairy domestic ruminant herds is unknown. Consequently, risk of exposure from exhibitions such as agricultural fairs cannot be estimated.
- This prevalence study utilized sensitive (95.8%) and specific (100%) rPCR to identify fecal shedding of *C. burnetii* from beef cattle, sheep, and meat goats exhibited at Iowa county fairs; all tested samples were negative.
- The dataset is sufficient to identify *Coxiella burnetii* in this population, with 95% confidence, if population prevalence meets or exceeds 2.85% (cattle and sheep) or 6.25% (goats), indicating that risk of exposure from these entries is very low.

INTRODUCTION

Coxiella burnetii, the causative agent of human Q fever and livestock coxiellosis, is a pathogen of emerging global importance and is frequently associated with infection in cattle, sheep, and goats (Porter et al., 2011). In the past decade, this gram-negative intracellular coccobacillus has caused major outbreaks of human disease associated with small ruminant abortion epizootics in the United States, the Netherlands, Australia (CDC, 2010; Van der Hoek et al., 2011; Bond et al., 2015). This organism is primarily spread through aerosols derived from excretions from infected animals and can be found in the inhalable air fraction from premises with prior or asymptomatic infections (Hogerwerf et al., 2012). As a result, veterinarians and public health practitioners are seeking to better characterize risk and appropriate control measures. One major obstacle is the lack of data to evaluate exposure risk from non-dairy ruminants, especially ruminant livestock exhibited at community events that could potentially serve as a significant source of infection for both the public and other herds. The agricultural fair system in Iowa is a major industry, bringing together over 70,000 livestock projects and 3.3 million visitors on an annual basis (Association of Iowa Fairs, 2015). Considering the animal and human health implications, the lack of knowledge of the occurrence of *Coxiella burnetii* in Iowa's beef, sheep, or meat goat populations at these fairs is a major gap that this project sought to provide.

Q fever and its causative agent, *Coxiella burnetii*, were first identified in the late 1930s nearly simultaneously in abattoir workers in Brisbane, Australia, and in a Montana rickettsial laboratory; since then, its presence has been confirmed worldwide with the exception of New Zealand (Maruin and Raoult, 1999). In the agricultural sector, *C. burnetii* is an important cause of enzootic abortion outbreaks in goats and sheep and can cause infertility, abortion, and low birth weights in cattle; however, most livestock infections are asymptomatic. Historically, *C. burnetii* has been challenging to study due to difficulties in culturing the bacteria and poor sensitivity of the classic complement fixation serologic tests (Porter et al, 2011). Development of IFA, ELISA, and molecular diagnostic technologies are changing our understanding of *C. burnetii* prevalence, distribution, and shedding (Kim et al., 2005; Rousset et al., 2008; Guatteo et al., 2012). Compared to early estimates, contemporary data from other North American locations indicates that the prevalence of both seroconversion and bacterial shedding is much higher than previously identified data. The only reported prevalence data for *C. burnetii* in Iowa livestock was reported by Braun (1962) and indicated a very low prevalence of seroconversion in dairy cattle (0.7%); no epidemiologic data exists for Iowa's beef, sheep, and goat populations. In contrast, a 2010 review of published *C. burnetii* prevalence data reported that animal level seroprevalence in continental North America for cattle, sheep, and goats ranged from 24-82%, 0-40%, and 3.5-24% respectively and that herd-level serologic or shedding prevalence in cattle, sheep, and goats ranged from 37.7-100%, 21-

89%, and 20-26% respectively (Guatteo et al., 2011). This review includes U.S ruminant populations, confirming that *C. burnetii* infection is more common than those reported in the 1960's. Similarly, McQuiston et. al. (2005) demonstrated that 92% of bulk tank samples from U.S. veterinary school bovine dairy herds were seropositive for *C. burnetii* and Kim et. al. (2005) identified *C. burnetii* organisms in over 94% of bovine bulk tank milk samples submitted for routine milk quality diagnostics.

Molecular diagnostic techniques have also yielded novel data concerning shedding patterns in dairy cattle and dairy goats, indicating that *C. burnetii* infection may function differently in these species than previously assumed. Guatteo et. al. (2006 & 2007) identified variable major shedding routes in naturally infected dairy cattle, including feces, milk, and vaginal mucous, and identified the presence of heavy-shedder cows within positive herds while Kim et. al. (2005) demonstrated that shedding prevalence in positive herds and among positive animals was static over a three year and over a four year period, respectively. Similarly, Rousset et. al. (2008) found that there was no difference in *C. burnetii* shedding in vaginal mucous, feces, or milk between aborting and non-aborting members of dairy goat herds experiencing natural *Coxiella* abortion storms. Additionally, this study identified that at least 24% of seronegative non-aborting herd members were actively shedding organism, and *C. burnetii* has also been identified in excreta from seronegative cattle and sheep (Rousset et al., 2008; Berri et al., 2001; Guatteo, Joy, and Beaudeau, 2012). Unfortunately, the published literature centers on dairy animals or foreign herds and there is an absence of baseline data for Iowa beef, meat goat, and sheep populations.

Our understanding of the epidemiology of *C. burnetii* in Iowa is insufficient because of outdated data. This study determined the prevalence of *C. burnetii* fecal shedding among domestic non-dairy ruminants exhibited at Iowa agricultural fairs, thereby improving the context for assessing the clinical risk of *C. burnetii* exposure to the other livestock operations and the visiting public. The primary impact of the study findings is to facilitate future outbreak investigations and control recommendations through an accurate assessment of risk from *C. burnetii* shedding at livestock exhibitions centers. Given that *C. burnetii* is widely distributed, commonly identified in dairy cattle and goat operations in the U.S. and Europe, and is frequently asymptomatic, we hypothesized that *Coxiella burnetii* would be in the feces of a significant proportion of clinically normal beef cattle, sheep, and meat goats exhibited in Iowa. The prevalence of *Coxiella burnetii* fecal shedding in Iowa cattle, sheep, and goats was evaluated through an observational study.

MATERIALS AND METHODS

Livestock were sampled at several Iowa county fairs during 2009 and real time PCR was used to identify *Coxiella burnetii* shed in fecal samples collected from beef cattle, sheep, and meat goats. The source population for the samples were beef cattle, meat goats, and sheep exhibited at twelve Iowa county fairs during the summer of 2009; two fairs from each of the six state fair districts were included in the study based on scheduled dates with the consent of the participant fair boards. Up to thirty individual freshly voided fecal samples per species per fair were collected on a convenience basis (not more than one sample per pen), yielding a sample bank of 295 cattle, 338 sheep, and 112 goat origin samples. Samples were identified only by date and species, then banked at -20 C. The banked samples were blocked by species and fair, and ten samples per block were randomly selected by the diagnosticians for PCR analysis. Less than ten goats were exhibited at some locations, in that case all available samples in the block were tested. An IACUC review of the protocol was conducted prior to implementation.

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Quantitative multiplex real-time PCR (rPCR) based on the *icd*, *com1* and IS*1111* sequence targets is a highly sensitive and specific diagnostic tool for *Coxiella burnetii*, assuming the animal is actively shedding organism at the time of sample collection. DeBruin (2011) reports that this technique is highly efficient (98%) with a level of detection of 10 copies per reaction; we were able to consistently achieve a LOD of 5 copies per reaction during internal PCR validation on a linearized plasmid containing the target (unpublished data). Inclusion of a *Bacillus thuringiensis* internal positive control is a reliable indicator of both successful DNA extraction and unintended inhibition of the PCR amplification process.

Fecal samples were extracted using the MagMAX® Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Foster City, CA) and a Kingfisher96 Magnetic Particle Processor (Thermo Fisher Scientific, Foster City, CA) using the manufacture's recommendations. The High Volume extraction program for the Kingfisher instrument, available from Thermo Fisher Scientific, was used. Following extraction, real-time PCR (rPCR) was conducted with the VetMAX®-Plus qPCR MasterMix using *Coxiella* specific primers and probe for target IS*1111*, described by de Bruin, as well as primers and probe for target *cry1b* for the internal positive control (IPC) *Bacillus thuringiensis. B. thuringiensis* spores were added to the samples prior to extraction at a level of 3.6 x 10^4 CFU/reaction. PCR was performed on an Applied Biosystems® 7500 Fast instrument (Thermo Fisher Scientific, Foster City, CA) using cycling conditions of 10 min at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Analysis was performed using the 7500 Fast software with automatic baseline settings, and threshold settings of 0.1 for Coxiella and 10% of the maximum fluorescence for the IPC. Samples were considered positive if the Coxiella target exhibited a Ct<40. Samples in which neither the IPC nor the Coxiella target were detected were considered inconclusive. These samples were diluted 1:2 and 1:4 with PBS, after which the extraction and PCR were repeated. The status of these samples remained inconclusive if the IPC still failed to be detected after sample dilution.

The FreeCalc2 epidemiological calculator (http://epitools.ausvet.com.au) was used for post-collection freedom testing analysis to determine the threshold prevalence at which at least one positive sample would be expected (α=0.05) if *Coxiella burnetii* was being shed in fecal samples in this fair population. While the total number of non-dairy ruminants exhibited at Iowa county agricultural fairs in 2009 is not known, an estimate was extrapolated from observed populations at the sampled fairs. Calculations were performed with a modified hypergeometric extract assuming sample independence at test sensitivity (96%) and specificity (100%).

RESULTS

Samples were collected from 295 cattle, 338 sheep, and 112 goat origin samples; 108 beef cattle samples, 49 meat goat samples and 109 sheep samples were randomly selected for testing. All were test negative for *Coxiella burnetii*. An additional 47 fecal samples (12 cattle, 11 sheep, and 24 goats) were tested and excluded due to repeated internal positive control failure of that test iteration. The minimum detection threshold of this sample set was 2.85% (cattle and sheep) or 6.25% (goats).

DISCUSSION

The major conclusion of this study is that fecal shedding of *Coxiella burnetii* from ruminant livestock exhibited at Iowa fairs is unlikely to occur at significant levels. We demonstrated that *C. burnetii* was not present in any fecal samples submitted for a highly sensitive and specific real time PCR. The tested sample set is sufficient to identify a positive animal even if the prevalence of fecal shedding in the exhibition livestock population is much lower than reported for North America. Since *Coxiella*

burnetii serostatus and shedding are poorly correlated, fecal PCR is the best indicator of shedding and potential for environmental aerosol exposure for nulliparous animals.

The general prevalence estimates for *Coxiella burnetii* in the North American cattle, sheep, and goat populations are presented in a contemporary literature review by Guatteo et. al. (2011). North American animal-level prevalence estimates are based on five seroprevalence studies in cattle, five seroprevalence studies in sheep, and five seroprevalence studies in goats; most of these studies were performed on Canadian livestock and none of the studies were performed on a representative, random population. Median reported animal-level seroprevalence was 28.0% (23.8-82.0%), 6.7% (0.0-41.0%), and 19% (3.5-35.0%) for cattle, sheep, and goats respectively. North American herd prevalence estimates in cattle were based on three seroprevalence, four bulk tank milk serology, and one bulk tank milk antigen detection (nPCR) tests largely performed on U.S. dairy herds with a median value of 74.5% (37.7-100.0%). In contrast, herd prevalence was based on two seroprevalence studies each in sheep (Canadian) and goats (Canada and California) and best suggests a herd prevalence of 21% and 20%, respectively. More recently, Meadows et. al. (2015a and 2015b) reported that the 2010-2012 seroprevalence among Ontario sheep and goat farms was much higher than previously described, with at least one seropositive animal identified in 48% (sheep) and 63% (goat) of randomly sampled herds. Average within-farm seroprevalence (14.7% sheep; 32.5% goats) in these two studies was comparable to upper estimates from previous publications. In both species, *Coxiella* seroprevalence was significantly greater for dairy than meat operations.

Contrary to our findings, one published case study describes a multi-herd caprine abortion epizootic following exposure to *Coxiella burnetii* at the Canadian Royal Winter fair (Sanford, Josephson, and MacDonald, 1994). In this report, the five affected herds experienced clinical abortions from 21 to 76 days after exposure at the fair and the diagnosis was confirmed by identification of typical placental lesions in conjunction with tissue-associated organism. One key aspect of this case study is that the source of exposure was from does that kidded prematurely at the fair and were housed in the same barn as the affected herds. This exposure did result in one human case linked to contact with the periparturient goats at the fair and five additional cases associated with subsequent exposure to the affected herds (Gallant et. al. 1992). Despite the paucity of such examples in the refereed literature this case is widely cited to indicate that fairs present a risk of *Coxiella burnetii* exposure. With the exception of livestock birthing centers, late-gestation livestock are a small proportion of animals exhibited at fairs.

In this study we determine that the risk of exposure to *Coxiella burnetii* via fecal shedding from the prototypical Iowa beef, sheep, or meat goat exhibit is substantially lower than expected based on broader prevalence studies. This finding fills a critical knowledge gap regarding exposure risks for veterinarians and public health practitioners and will better inform future recommendations for disease control measures that could impact a fair structure with an annual economic impact exceeding $275 million and its 3 million visitors (Association of Iowa Fairs, 2015).

One caveat to these findings is that they are narrowly applicable to the sampled populations – specifically beef, sheep, and meat goat exhibits. When present, dam/offspring pairs and breeding stock exhibits were included in the sample pool, but the vast majority of exhibits were younger, nulliparous market animals. Specifically, dairy animals and parturient birthing exhibits were not included in this study. There are several reasons why shedding risk in those populations may be higher including history of parturition and inclusion of birth products or vaginal discharge and milk as additional routes of bacterial shedding. Furthermore, it is not known whether there are significant differences in prevalence between the dairy and meat animal industries.

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With the elevation of *Coxiella burnetii* in veterinary and public health discourse, it is imperative that exposure risk and control measures are grounded on a solid basis of evidence. Furthermore, livestock fairs and exhibitions are increasingly scrutinized as a potentially high-risk setting for several zoonotic concerns. Over 70,000 livestock projects are exhibited annually at Iowa's local fairs, and the vast majority of the ruminant entries are market or young breeding stock beef, sheep, and meat goats (Association of Iowa Fairs, 2015). Our study, which is the first study to systematically examine the prevalence of *Coxiella burnetii* in exhibition settings, indicates that the risk of exposure from these entries is very low.

The authors have no conflicts of interest to report.

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