

Short Report: First Genetic Detection of *Coxiella burnetii* in Zambian Livestock

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Abstract. Q fever is a widespread zoonosis caused by *Coxiella burnetii*, an obligate intracellular gram-negative bacterium. The investigation of *C. burnetii* infection in Zambian livestock was carried out using molecular detection techniques. A total of 489 cattle and 53 goat blood samples were collected from Chama, Chongwe, Monze, and Petauke districts in Zambia. Molecular screening by polymerase chain reaction was performed using *C. burnetii*-species-specific primers. In total, 38 cattle and 4 goat samples were positive. The prevalence of *C. burnetii* differed among the four sites, with Chama (Eastern province) recording the highest, although Monze (Southern province) did not record any case of the bacteria. This study reports the first genetic detection of *C. burnetii* in Zambia.

INTRODUCTION

Coxiella burnetii, an obligate intracellular gram-negative bacterium, is the causative agent of Q fever in humans and animals. It causes a variety of symptoms such as acute flu-like symptoms, pneumonia, hepatitis, and chronic endocarditis in humans.^{1,2} It also causes abortion or infertility in animals.^{1,2} The disease is a ubiquitous zoonosis with worldwide distribution.¹ From spring 2007–2011, a Q fever outbreak of unprecedented scale occurred in the Netherlands, involving 4,108 notified human cases including 24 fatal cases.³ The life cycle of *C. burnetii* is not fully understood, but humans are considered incidental hosts. The reservoir of Q fever is a wide range of domestic and wild animals and arthropods such as ticks⁴; of note, domestic ruminants including cattle, goats, and sheep are often infected and serve as main sources of human infections.^{1,5}

The sero-prevalence of Q fever in humans has been reported from several African countries,^{6,7} including Zambia.^{8,9} In addition, *C. burnetii* DNA was detected from febrile patients in the malaria-endemic area in Senegal.¹⁰ These data may suggest widespread distribution of Q fever in African countries. Discrimination between malaria, other endemic febrile diseases, and Q fever in affected regions including Africa, is important for disease management and control strategies. Therefore, epidemiological surveillance of *C. burnetii*, and elucidation of the transmission routes are necessary in these areas.

The objective of this study was to investigate the prevalence of *C. burnetii* in domestic animals in Zambia and to extrapolate the potential infection route to humans. From 2008 to 2010, blood samples were collected from the cattle (Angoni breed) in Chama ($N = 295$, $11^{\circ}21'S$, $33^{\circ}16'E$), Chongwe ($N = 50$, $15^{\circ}33'S$, $28^{\circ}69'E$), Monze ($N = 80$, $16^{\circ}28'S$, $27^{\circ}49'E$), and Petauke ($N = 64$, $14^{\circ}24'S$, $31^{\circ}32'E$) districts in Zambia (Figure 1). Blood samples were also obtained from goats (Boer breed) in Chama district. In each district, sampling was conducted at 2–7 different sites where the pastured cattle and goats were gathered by the owners. A total of 489 cattle and 53 goat blood samples were collected, from which genomic DNA was extracted using the DNA Isolation Kit for Mammalian Blood (Roche Molecular Biochemical, Boehringer, Germany). The

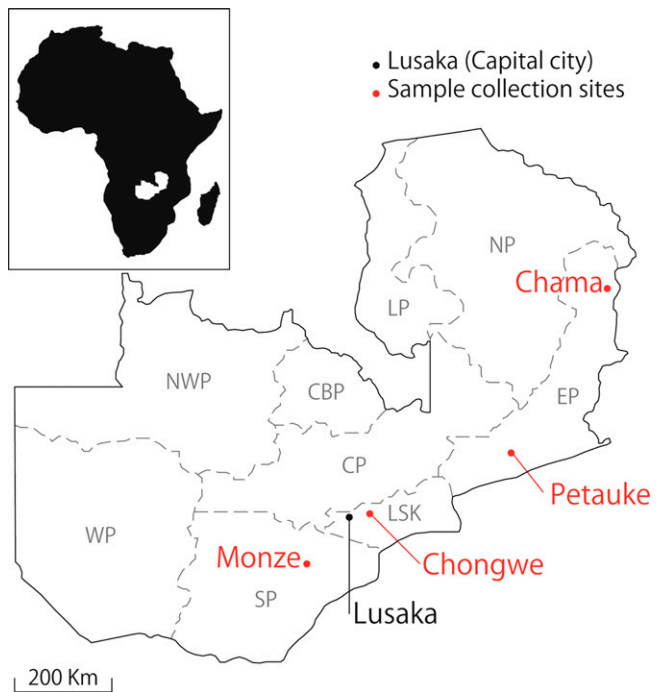


FIGURE 1. Map of Zambia showing its nine provinces: Northern (NP), Eastern (EP), Luapula (LP), Central (CP), Copperbelt (CBP), Lusaka (LSK), Southern (SP), Western (WP), and North-western (NWP) provinces. The *Coxiella burnetii* samples used in this study were obtained from Monze (SP), Chongwe (LSP), Petauke (EP), and Chama (EP) districts.

DNA was extracted from 1 mL of EDTA-treated blood and was eluted in a final volume of 200 μ L according to the manufacturer's instructions. For the screening of *C. burnetii* infection, polymerase chain reaction (PCR) was performed with primers designed based on a repetitive transposon-like element of *C. burnetii* (Trans-PCR).¹¹ The sensitivity and specificity of the assay have been well evaluated.^{12,13} The PCR reaction was conducted in a final volume of 10 μ L, containing 5 μ L of KAPA Blood PCR Mix B (Kapa Biosystems, Boston, MA), 0.125 μ L of each primer, and 1 μ L of template DNA. The PCR conditions were initiated with a 5-min denaturation step at 95°C followed by 40 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min, and final extension step at 72°C for 2 min. The resulting PCR products were electrophoresed

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TABLE 1
Prevalence of *Coxiella burnetii* DNA in Zambian cattle and goats

Animal	District	Prevalence*
Goat	Chama	4/53 (7.5%)
Cattle	Chama	33/295 (11.2%)
	Chongwe	3/50 (6.0%)
	Monze	0/80 (0%)
	Petauke	2/64 (3.1%)
	Sub-total	38/489 (7.8%)

*No. of positives/no. of tested samples.

on 1% agarose gel, stained with Gel-Red (Biotium, Hayward, CA), and visualized with a UV trans-illuminator.

Out of 489 cattle samples, Trans-PCR detected 38 (7.8%) *C. burnetii*-positive samples (Table 1). The prevalence of *C. burnetii* differed among the four sampling sites, with the highest prevalence observed in Chama (33 of 295, 11.2%), followed by Chongwe (3 of 50, 6.0%), and Petauke (2 of 64, 3.1%). Furthermore, out of the 53 goat samples from Chama, only 4 (7.5%) were positive for *C. burnetii* (Table 1). On the other hand, all the samples from Monze were negative for *C. burnetii*.

The major infection route of *C. burnetii* to humans is through the inhalation of aerosol following parturition of an infected animal and the ingestion of contaminated raw milk or milk products.¹⁴ This may partially explain why the sero-prevalence of *C. burnetii* in humans in Zambia was reported to be higher in extensive cattle-breeding areas (Eastern and Western provinces) than less breeding areas (Northern province).⁹ In our study, the highest prevalence of *C. burnetii* was obtained in the samples collected from Chama district (Eastern province). Collectively, these data may imply that Eastern province is an endemic area for *C. burnetii* infection in Zambia. Future studies should be expanded to include the other sampling areas and specimen such as vaginal swab, placenta, and milk, which are likely to contain a higher concentration of *C. burnetii* than blood samples, and to elucidate the genotypes of *C. burnetii* circulating in Zambia.⁶

In conclusion, this is the first report on the genetic detection of *C. burnetii* in Zambia. People should be aware of the infection of *C. burnetii* as a cause of non-malarial illness. Further studies should be conducted to assess the potential risk of Q fever in humans.

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