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Serological and molecular evidence of Q fever among small ruminant flocks in Algeria

H. Khaled[a,*], K. Sidi-Boumedine[b], S. Merdja[a], P. Dufour[b], A. Dahmani[a], R. Thiéry[b], E. Rousset[b], A. Bouyoucef[a]

[a] LBRA, Institute of Veterinary Sciences, University Blida 1, Algeria
[b] ANSES, Laboratory of Sophia-Antipolis, Animal Q Fever Unit, Sophia Antipolis, France

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ABSTRACT

Q fever, a commonly reported zoonosis worldwide, is caused by infection with *Coxiella burnetii*, an obligate intracellular bacterium. The infection is often asymptomatic in ruminants, but it can lead to reproductive disorders with bacterial shedding into the environment. Between 2011 and 2013, a study was undertaken in small ruminant flocks in different regions of Algeria. A total of 35 flocks were visited and 227 sera and 267 genital swabs were collected from females after abortions or the lambing period to investigate Q fever infection. Indirect ELISA was used to detect specific antibodies against *C. burnetii* and real-time PCR for detecting bacterial DNA. Our survey indicated that 58% (95% CI = 40–76%) of flocks had at least one positive animal (17 seropositive flocks) and individual seroprevalence was estimated at 14.1% (95% CI = 11.8–16.4%) (32 seropositive animals). Bacterial excretion was observed in 21 flocks (60%), and 57 females showed evidence of *C. burnetii* shedding (21.3%). These results suggest that *C. burnetii* distribution is high at the flock level and that seropositive and infected (shedder) animals can be found all over the country. Further studies are needed in other regions and on different animal species to better understand the distribution and incidence of Q fever, as well as human exposure, and to develop an adequate prophylaxis program.

1. Introduction

Coxiella burnetii is an obligate intracellular bacterium that causes the zoonotic disease Q fever. The bacterium has been found worldwide in a wide range of animal hosts, including mammals, birds and ticks [1]. Moreover, a spore-like form of *C. burnetii* can survive extracellularly, contributing to its persistence and widespread dissemination in the environment [2]. Ruminants represent the primary reservoir of this organism [3]. In these animals, Q fever is mainly asymptomatic, but can be responsible for reproductive disorders, including abortions that generally occur at the end of gestation, as well as stillbirths and delivery of weak and unviable newborns [4]. These reproductive failures are accompanied by high levels of bacterial shedding through vaginal secretions, birth products, faeces, urine, and milk [5–7]. Nevertheless, active or passive surveillance for Q fever in ruminants is rarely performed; thus, the prevalence and the incidence of Q fever cannot be accurately estimated anywhere in the world [3]. Therefore, animal infection is often revealed after the notification of human clinical cases. Outbreaks are generally associated with proximity to sheep and goats, particularly during parturition or abortion, during dry and windy weather [3,8].

In Algeria, Q fever was described for the first time in 1948 in French soldiers and then in 1956. Cases appear to be linked to contact with small ruminants [9]. In a seroprevalence study, a rate of 5.4% was reported in children under 16 years in the south of the country [10]. Another study mentioned a rate of 15.5% in inhabitants of an agro-pastoral region in the east [11]. Nevertheless, studies on Q fever in animals are still rare in Algeria [12].

The purpose of this study conducted in Algeria between 2011 and 2013 was to estimate Q fever seropositivity and shedding in small ruminant flocks. These data on the Q fever epidemiological situation in animals aims to improve the visibility of this neglected or unknown disease; enhance knowledge and facilitate future comparative studies; and participate in the development of a surveillance plan and/or appropriate monitoring for the country.

* Corresponding author.
E-mail address: khaledhamz@yahoo.fr (H. Khaled).

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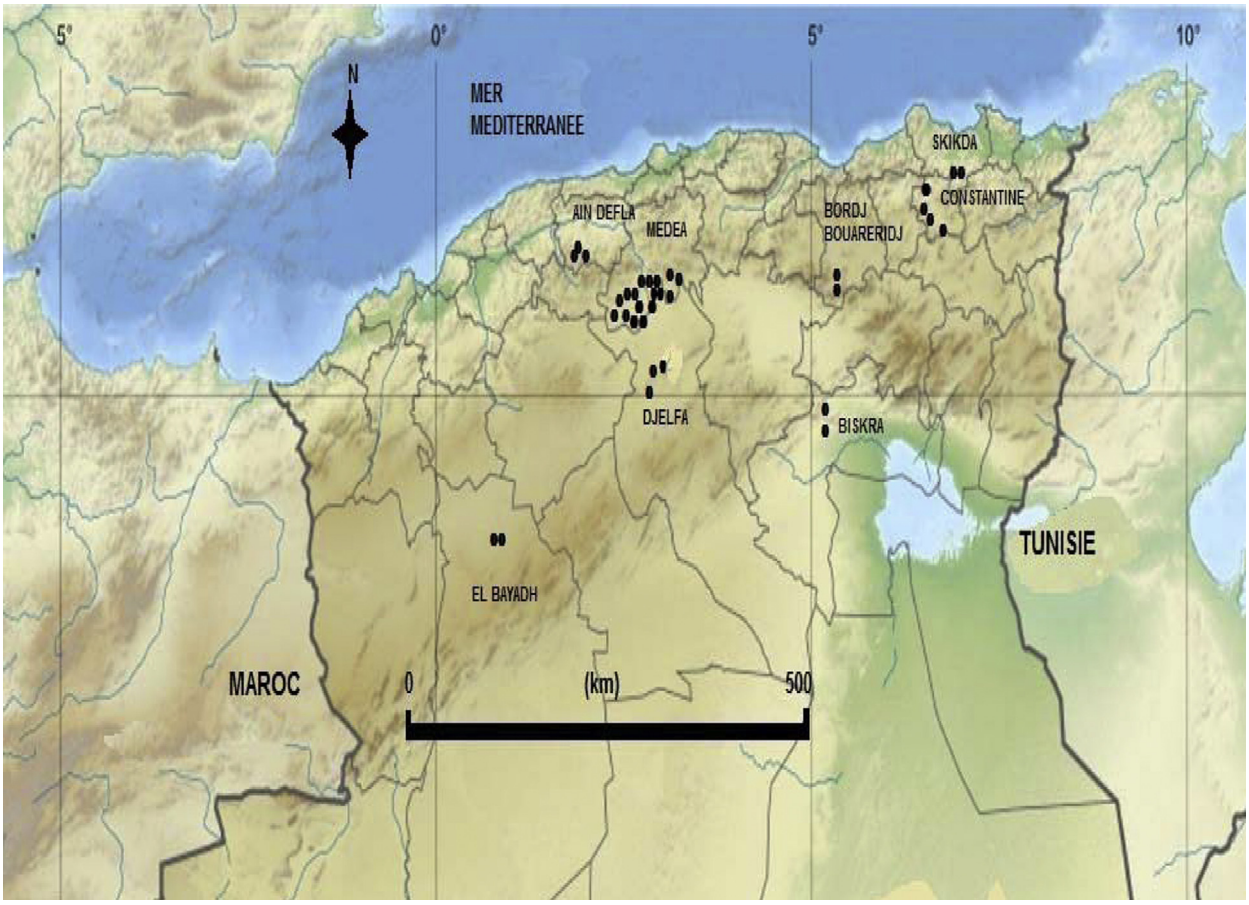

Fig. 1. Location of Algerian departments from which samples were collected (each black dot represents a tested flock).

2. Materials and methods

2.1. Study site

The study took place in eight departments of Algeria, covering the geographical and climatic diversity of the country. The chosen regions were as follows: Constantine, Skikda, and Ain Defla with a Mediterranean climate; Bordj Bouareridj, Medea, Djelfa and El Bayadh with a continental climate and Biskra with a Saharian climate (Fig. 1). According to headcount statistics for 2012 from the Algerian Ministry of Agriculture, sheep predominate the Algerian ruminant population and account for 80% of the total estimated livestock population with more than 25 million heads, including 12 million ewes. Goats are second-most common species (13%) and 58% are females. Pastoral livestock production is concentrated in the steppe (in the north-central part of the country), harbouring the largest small ruminant population in Algeria. During the summer seasons, transhumance and nomadism to the north-east and north-west become a necessity, especially from May to September when the pastures can no longer feed the flocks.

2.2. Sampling

For every farm visited, a survey was completed to provide information regarding abortion antecedents, size, composition and production system at the flock level. For each sampled animal, the species, age, symptoms (i.e. abortion or normal delivery) were also recorded. From the same animal, a blood sample and a genital swab were taken. Sampling was performed approximately one week after lambing or abortion, according to farmers' observations. In regard to local customs, the flock composition changes over time and animals are not marked. Moreover, no vaccination against Q fever has ever been administered in Algeria.

The blood sample (5 mL) was collected from the jugular vein of each animal using a vacutainer tube. Sampling was performed by qualified veterinarians as part of (routine?) sample collection for surveillance with the full consent of the farmers. Sera were separated from clotted blood by centrifugation at 1500*g* for 15 min, aliquoted into clean 1.5 mL tubes and stored at −20 °C until analysis.

The swab was rubbed on the inner vaginal wall to insure the collection of cells and intracellular bacteria. Each specimen was marked with a code including an individual sampling number and accompanied by an information sheet with the flock characteristics and tested animals. The specimen samples were analysed with the help of the OIE and the French Reference Laboratory for animal Q fever (ANSES Sophia Antipolis, France). Samples were sent to this laboratory after sending the samples under cold storage, and the transport period did not exceed 24 h.

2.3. Laboratory testing

2.3.1. ELISA

The specific anti-*Coxiella burnetii* antibodies in the serum samples were measured using a commercially available indirect enzyme-linked immunosorbent assay (ELISA) kit (LSIVET Ruminant Serum/Milk Kit, batch #: ElisaCoxLS-001, France) according to the manufacturer's instructions. The technique uses microtiter plates pre-coated with a purified *C. burnetii* antigen of ovine origin.