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Serological investigation on *Chlamydophila abortus* infection in cattle from Albania

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Abstract

Chlamydophila abortus is a zoonotic pathogen, which can cause abortion in cattle worldwide. However, the knowledge of the epidemiology and prevalence of zoonotic diseases, like *Chlamydophila abortus* in Albania is limited. Therefore, a total of 185 blood samples were collected from cattle from different regions of Albania between January of 2010 and November of 2011. A commercial enzyme-linked immunosorbent assay was used for the detection of antibodies against *Cp. abortus*. The results showed 45.3% (43/95), 36% (27/75) and 33.3% (5/15) seropositivity among apparently healthy animals from big dairy herds, from backyard flocks and from aborted individuals, respectively. The average seropositivity (40.5%) of *Cp. abortus* in Albania was relatively high, compared to other European countries, which is probably related with the bad hygienic conditions in Albanian farms. The increased frequency of contact among the animals is assumed to be the cause of the elevated number of infected animals in big dairy herds compared to small farms. This knowledge of the prevalence of *Cp. abortus* infection is crucial for policy makers in the process of evidence-based decision making.

Keywords: Albania, Cattle, *Chlamydophila abortus*, enzyme-linked immunosorbent assay, sera

1. Introduction

The taxonomy of chlamidiaceae has been reviwe last decade. Previosly their clasification was based on fenotipical characterisation and was one genera and four species [9]. At moment, based on nucleic acid secuencing of 16 S and 23 S rRNA genes there are two distinct lineages [9]. Based on genetic relatedness this family is divaded on two genera and 9 species (Fig 1). Recentely it is proposed to include all 9 species on a single genus, *Chlamydia* species. From the family Chlamydiaceae, *Chlamydophila* (*Cp.*) *abortus* (formerly *Chlamydia* (*C.*) *psittaci* serotype 1) and *Cp. pecorum* (formerly *C. pecorum*) are known to infect cattle [10]. *Cp. psittaci* and other, Chlamydia-like organisms had been detected from bovine abortions as well [1]. These bacteria are Gram-negative, intracellular and obligate pathogens [3], known as "energy parasites" because their difficulty to generate ATP [9] . The gastrointestinal tract of animals is natyral site of *Chlamydophila* infection of animals, which shed the pathogen intermitently and for long time [9]. The elementary bodies survive for several days in envoirnmental conditions. Chlamydiae infect over 450 species of birds, several mamals, humans, and it is isolated from invertebrate species [9]. The life cycle of chlamydiae consists of two forms; the infective elementary body, which is relatively stable in the environment, and the vegetative reticular body, which evolves from the previous stadium in the host cell's cytoplasm[5]. Close contact (i.e. ingestion, inhalation) with infectious body fluids (i.e. uterine discharges, feces, urine, milk, nasal discharges) transmits the elementary bodies, which can lead to abortion in cattle in the case of *Cp. abortus* or a wide range of diseases (including pneumonia, inapparent enteric infections, encephalomyelitis, conjunctivitis, polyarthritis, mastitis, salpingitis and endometritis) in the case of *Cp. pecorum* infection [4, 11]. Rearing cattle in large, crowded herds induces the spreading and intensity of the infection of chlamydiae by increased probability of shedding animals and more frequent contact among the individuals [2]. The loss

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Table 1 Chlamydial infections of veterinary and medical importance (9).

Pathogen	Hosts	Clinical condition
Chlamydophila psittaci	Birds	Pneumonia and airsacculitis Intestinal infection and diarrhoea Conjunctivitis Pericarditis Encephalitis
	Humans (secondary hosts)	Psittacosis/ornithosis Abortion Conjunctivitis
Chlamydophila abortus	Sheep Goats Cattle Pigs	Enzootic abortion of ewes (EAE) Chlamydial abortion Chlamydial abortion Chlamydial abortion
C. felis	Cats	Conjunctivitis (feline pneumonitis)
C. caviae	Guinea-pigs	Guinea-pig inclusion conjunctivitis
C. pecorum	Sheep	Intestinal infection Conjunctivitis Polyarthritis
	Cattle	Sporadic bovine encephalomyelitis Polyanhritis Metritis
	Koalas	Conjunctivitis Urogenital infection
C.pneumoniae	Humans Horses Koalas	Respiratory infection Respiratory infection Conjunctivitis
Chlamydia trachomatis	Humans	Trachoma, inclusion conjunctivitis of infants Non-specific urethritis Respiratory disease of infants Proctitis Lymphogranulorna venereurn Arthritis
C. suis	Pigs	Intestinal infection
C. muridarum	Mice	Respiratory infection

of body condition, decreased milk production, reduced fertility rates and abortions due to chlamydial infections have serious economical significance [5]. *Cp. abortus* has also zoonotic potential, especially in pregnant women, causing spontaneous abortion, stillbirth or preterm labour, and potentially life- threatening [4, 9].

There are comercially availabel two type of vaccines, killed and temperature sensitive modified live vaccine for small rumminants for using in pregnant and prior breding respectively, no comercial vaccine is available yet for cattle [9, 10].

Serological methods such as complement fixation test and several different enzyme-linked immunosorbent assays (ELISA) are mainly used in the diagnosis and screening of animals for chlamydial infections, although there is a need to develop a more specific and sensitive test for field use [7, 12] Still, the definitive diagnosis depends on the detection of the agent by cell cultivation and molecular diagnosis [5]

A summary of medical and veterinary important clamydia is shown in Table 1 [9].

The aim of this study was to investigate the seroprevalence of *Cp. abortus* in aborted cattle and in dairy herds, and to compare the results between big dairy herds and backyard flocks in Albania.

2. Material and Methods

A total of 185 blood samples from cattle were collected from different parts of Albania between January of 2010 and November of 2011. The samples originated from 3 big dairy farms (Tirana/41°19′N 19°49′E/, Durres/41°19′N 19°27′E/ and Lushnja/40°56′N 19°42′E/; managing 285, 187 and 234 heads, respectively; n=95), 20 backyard flocks (managing 3-5 heads each; n=75) and from aborted cattle (abortion happened in the third trimester; n=15). Sera were extracted by centrifugation at 3000g for 10 minutes, and stored at -20°C until further processing. The CHEKIT Chlamydia Antibody Test Kit (IDEXX Europe B.V., Koolhovenlaan, The Netherlands) was used according to the manufacturers instruction for the detection of antibodies against *Cp. abortus*. The normalization of the results was based on the formula: [(optical density (OD) sample - OD negative control)/(OD positive control - OD negative control)] × 100, using the negative and positive control sera provided in the kit, then the values were expressed as percentage of the positive control. Sera with values ≥ 40% were considered as positive, sera with values between 30-40% were considered as doubtful, and sera with values under 30% were considered as negative.

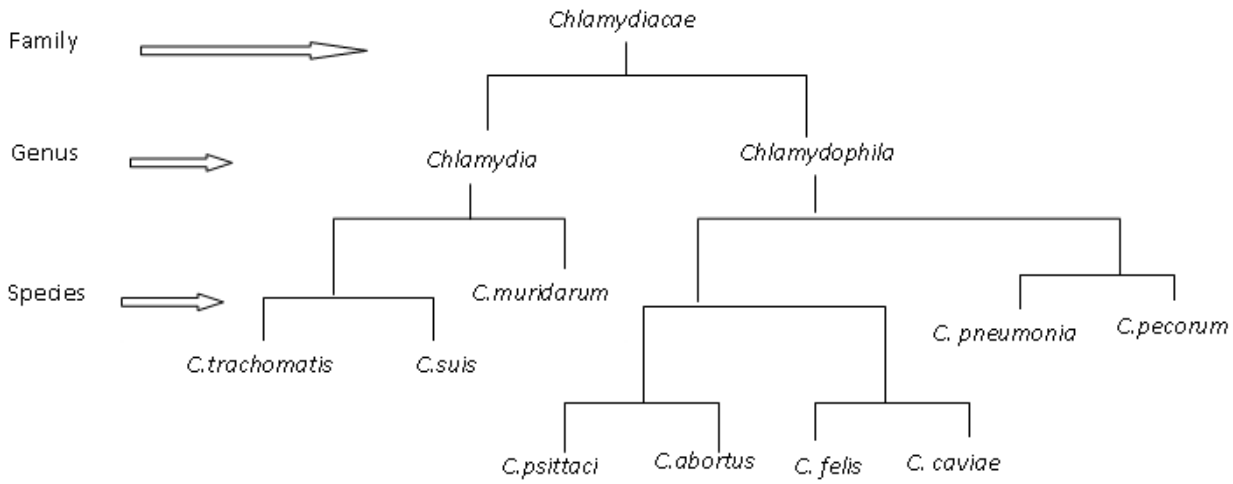

Figure 1 Chlamydiae clasification (9)

3. Results

Seventy-five (40.5%) sera were positive for antibodies against *Cp. abortus*, 102 samples were negative and 8 sera showed ambiguous values out of the 185 tested samples. Higher seroprevalence of *Cp. abortus* was detected in cattle from big dairy herds (43/95, 45.3%) than animals from small farms (27/75, 36%). Five animals were serologically positive for *Cp. abortus* from the examined 15 abortions (33.3%).

4. Discussion

The present report provides information on *Cp. abortus* seroprevalence in the cattle population of Albania, revealing that *Cp. abortus* is widespread throughout the country. Although it is notable, that cross reaction with *Cp. pecorum* could occur during the application of the used diagnostic kit, as highlighted in a previous study [12]. According to the present study the seroprevalence (40.5%) of Chlamydia in Albania is much higher, than the seroprevalences detected in other European countries; 25% in Italy [2], 19.3% in Poland [6], 3% in Germany [8], and 0.4% in Sweden [3]. This high prevalence of Chlamydia in Albania is probably related with the generally bad hygienic conditions in cattle farms. Comparing the seropositivity rate of samples from backyard flocks (36%) and from dairy herds (45.3%), the higher rate of positive cattle from the latter is probably related to the increased contact between the animals [7]. The association between miscarriages and the presence of antibodies against *Cp. abortus* was not representative, given the small number of examined animals (15 samples). However it is notable, that 5 sera were positive for Chlamydia from the 15 aborted cattle with different geographic origins.

With this study we aimed to fill the information gap and provide reliable prevalence data for *Cp. abortus* infection in Albania, as the knowledge of true prevalence is crucial for policy makers in the context of evidence-based decision making schemes. Further studies are expected to evaluate the clinical damages and economical losses caused by *Cp. abortus* and the public health relevance of this agent.

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