

## IDENTIFICATION OF CHLAMYDIAL SPECIES IN SMALL RUMINANTS WITH OCULAR INFECTIONS

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### Summary

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Detection of the many chlamydial species infecting same wild and domesticated hosts raises questions about the potential role of cross-host transmission in these infections. In this study, *Chlamydia* was detected in sheep and goat. Altogether 53 ocular swabs samples were collected from animals. The samples were examined using a molecular method for species identification. The following species were identified: *Chlamydia pecorum* (1), *Chlamydia felis* (1) in sheep and *Parachlamydia acantomoeba* (2), *Protochlamydia naegleriofila* (1) in goats with keratoconjunctivitis. Keratoconjunctivitis (IKC, „pink eye“) is commonly reported in small and large ruminants that are raised for production of meat and other goods, that is frequently caused by *Chlamydia* species. Species from family *Chlamydiaceae* can cause not only conjunctivitis, respiratory tract infections or abortions in animals, but also in humans, which indicates strong zoonotic character of the pathogen.

**Key words:** *Chlamydia* spp., goat, PCR, sheep, zoonoses

### INTRODUCTION

The family *Chlamydiaceae* belongs to the group of obligate intracellular Gram-negative bacterial pathogens that are prevalent worldwide and cause a wide range of the infections and diseases in animals and humans (Everett, 2000; Longbottom & Coulter, 2003). Most common chlamydial infections reported in ruminants were caused by species *Chlamydia abortus* and *Chlamydia pecorum*, which beside abortions in cattle, sheep and goats can also induce urogenital tract infections,

pneumonia and conjunctivitis (Longbottom, 2004). Infectious keratoconjunctivitis (IKC, „pink eye“) is a highly-infective inflammatory condition of the eye, which is commonly reported in small and large ruminants and is frequently caused by *Chlamydia* species *C. pecorum*, *C. abortus*, *C. psittaci* (Cooper, 1974; Jones, 1991; Everett, 2000; Gupta *et al.*, 2015), or mixed co-infections of mentioned species (Holzwardt *et al.*, 2011). Keratoconjunctivitis is the clinical sign

that can point out primary chlamydial infection that may not be accompanied by other symptoms and can lead to abortions of gravid animals. *C. abortus* and *C. pecorum* are known to be an etiological agents that cause late abortions in sheep known as ovine enzootic abortion (enzootic abortion of ewes, EAE) which is responsible for large economic losses in the livestock industry. Other species of *Chlamydia* that can cause abortions in domestic ruminants is *Parachlamydia acanthamoeba* (Borel *et al.*, 2007; Ruhl *et al.*, 2009) that belongs to abortigenic agents.

## MATERIAL AND METHODS

### *Study population and samples*

The sampling was done on the farm located in East Slovakia in the fall of 2016 where we collected 53 ocular swabs samples from sheep (n=33) and goats (n=20) (sheep and goats were farmed together). Animals were slightly cachectic, without specific clinical signs and had a mild eye discharge.

### *Molecular analysis*

**DNA isolation.** To identify the *Chlamydia* spp., we first isolated the DNA with DNA-sorb-AM nucleic acid extraction kit (AmpliSens, Federal State Institution of Science, Moscow, Russia) according to the manufacturer's instructions. The purified DNA was stored at -20 °C until required for PCR.

**PCR.** PCR amplification was performed using the primers U23Fa and 23Sigr that amplify the SSU (23S rRNA gene) region of DNA of *Chlamydia* spp. (Everett *et al.*, 1999). Primer U23Fa matches the sequence just after the start of the 23S rRNA gene, and 23Sigr complements the sequence approximately 600 bases downstream. PCR products were

analysed by electrophoresis of 10 µL of each 20 µL reaction mixture on a 1.5% agarose gel stained by using GoldView stain (SBS Genetech, China) and visualised fragments were compared with the positive control and the 100 bp DNA ladder. The PCR products were directly sequenced in both directions. PCR products were directly sequenced in both directions. Sequences were aligned and completed using ChromasPro, Bioedit and Clustal X, and compared with known sequences in the National Centre for Biotechnology Information GenBank database.

**PCR reaction mix.** The volume of the PCR reaction mixture was 20 µL, from which the DNA sample was 10 µL. In these reactions, we used 8 µL of Taq polymerase HOT FIREPol Blend Master Mix (Solis BioDyne, Estonia), 10 µL of template, 0.4 µL of each primer and 1.2 µL of purified water. All reactions were carried out in BIOMETRA T-Personal Thermocycler. Cycling conditions for PCR were 95 °C for 13 min for one cycle followed by 40 cycles at 95 °C for 20 s, 65.5 °C for 60 s, and 72 °C for 60 s, and a final extension step at 72 °C for 10 min for one cycle.

## RESULTS

In the samples of ocular swabs from animals that had eye effluent we determined presence of *Chlamydia* spp. (9.43%). In the group of goats we identified *Parachlamydia acanthamoeba* (2) and *Protochlamydia naegleriophila* (1) and in sheep – *Chlamydia pecorum* (1) and *Chlamydia felis* (1).

## DISCUSSION

*Parachlamydia acanthamoeba* and *Protochlamydia naegleriophila* belong to

amoeba resisting bacteria (ARB), which are categorised among zoonotic pathogens and pose a risk of causing respiratory infections in humans that are often infected from an unknown source (Lienard, 2017). These pathogens are resistant against alveolar macrophages in humans, which can cause rapid development of infection (Greub & Raoult, 2002; Greub *et al.*, 2003), most commonly after the contact with infected secretions of aborted animals. Therefore farm workers that are in close connection with those animals are the ones that are mostly at risk. Amoebas are the main reservoir of many clinically significant pathogenic microorganisms amongst which are the species from family *Chlamydiaceae*. Species diversity of amoebas highlights their wide occurrence in various biotopes, mainly in soil and water, and allows the transmission in the environment and into the humans (Brown *et al.*, 1983). Identification of *P. acanthamoeba* and *Pr. naegleriophila* in goats pointed out that environment may be a possible vector of the spread of *Chlamydia* spp., which plays an important role in infections of not only animals, but also humans. Lower hygienic standard in farms facilitates the transmission and replication of pathogens. Detection of species *C. pecorum* in the eye swabs indicates the beginning of an infection in the sheep, which can cause abortions in gravid animals (Longbottom, 2004; Gupta *et al.*, 2015) together with *P. acanthamoeba* (Borel *et al.*, 2007). Identification of the species *Chlamydia felis* in sheep, which was detected in eye swab, is uncommon. This pathogen, which can be found in cats and sometimes in dogs, causes chronic conjunctivitis and respiratory tract infections (Baker, 1942) and was probably not reported in the eye swab of sheep. Occurrence of *C. felis* on farms may not be

unique due to the presence of shepherd dogs and cats that are used to eradicate the rodents. Also, co-infection of *C. felis* and *P. acanthamoeba* was found in nasal samples of people that were not showing any clinical signs (Corsaro & Venditti, 2015). Besides the keratoconjunctivitis and infection of respiratory tract, *C. felis* can cause pneumonia (Marrie *et al.*, 2005), hepatosplenomegaly, glomerulonephritis, endocarditis and infection of reproductive tract, that can lead to abortions, neonatal mortality and infertility in humans (Griffiths *et al.*, 1978; Pointon *et al.*, 1991).

## CONCLUSION

In this study we detected species from the family *Chlamydiaceae*, which is not necessarily host specific (*C. felis*). Either alone or in co-infection (*P. acanthamoeba*, *C. felis*, *C. pecorum*) it can cause not only conjunctivitis, respiratory tract infections or abortions in animals, but also in humans, which indicates the strong zoonotic character of the pathogen.

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