Part 1. Veterinary medicine

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A STUDY OF CASEOUS LYMPHADENITIS IN GOATS FROM A SMALLHOLDER FARM IN UKRAINE

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Summary. Caseous lymphadenitis (CLA) is a chronic infectious disease of a wide range of animal species around the world, which leads to abscesses in lymph nodes and internal organs. The aim of these studies was to characterize the *Corynebacterium pseudotuberculosis* isolate obtained from infected goat from a smallholder farm in Ukraine. Clinical observation revealed two goats with concurrent external abscesses, fever, anorexia, and weight loss. Blood samples from the suspect animals were tested by ELISA. According to the obtained results, four (18.2%) from 22 sera samples were positive and one sample was questionable. *C. pseudotuberculosis* was isolated from the lymph nodes, liver, and lungs whereas the spleen, kidney, uterus, and udder did not show any growth. Finally, the obtained isolate was characterized both by biochemical tests and using multiplex PCR based on the detection of 16s rRNA, *rpo*B, and *pld* genes of *C. pseudotuberculosis*. Disease control should include elimination of infected and suspected animals from the herd, carrying out a comprehensive disinfection, and providing constant monitoring of the entire herd. Due to the absence of available data concerning the prevalence of CLA in Ukraine, further surveillance is required and an effective disease control strategy and eradication measures need to be developed

Keywords: *Corynebacterium pseudotuberculosis*, ELISA, PCR

Introduction. *Corynebacterium pseudotuberculosis* is the causative agent of caseous lymphadenitis (CLA) — a chronic infectious disease mainly in small ruminants and rarely in other mammalian species worldwide causing significant economic losses. The pathogen is characterized as a facultative anaerobic and facultative intracellular, Gram-positive coccobacillus bacterium, non-sporulating, non-encapsulated and non-motile that is capable of producing exotoxin phospholipase D (PLD), catalase, and has a mycolic acid-rich cell wall (Dorella et al., 2006). There are two biovars of the pathogen — Ovis (Nitrate non-reducing strains) and Equi (Nitrate reducing strains) (Almeida et al., 2017).

C. pseudotuberculosis can survive in the environment for several weeks which contributes to its further spread from herd to herd. The disease manifestation in goats and sheep includes mainly necrosis of the lymph glands (external form) or abscesses in internal organs (visceral form). Due to its zoonotic potential, the pathogen can cause granulomatous lymphadenitis or pneumonia in humans (Bastos et al., 2012; Peel et al., 1997).

Diagnosis of CLA includes observation of clinical signs, postmortem examination, pathogen isolation and identification, serology, and PCR (Binns, Green and Bailey, 2007; Pacheco et al. 2007).

Infected animals need to be isolated from the herd and thorough disinfection must be carried out. Chemotherapy predominantly is not practical except if the pathogen is detected early and long-term treatment is provided (Pratt et al., 2005). There are several vaccines against *C. pseudotuberculosis* based on bacterin or inactivated toxoid which reduce infection rates, but do not fully prevent the disease (De Pinho et al., 2021).

The information regarding CLA cases in Ukraine is very limited and additional investigations are required.

The aim of these studies was to characterize the *C. pseudotuberculosis* isolate obtained from infected goat from a smallholder farm in Ukraine.

Material and methods. Sera samples were obtained from 22 goats from smallholder private farm located in Kyiv Region, Ukraine. Antibodies to *C. pseudotuberculosis* were estimated using commercial ELISA ID Screen® *Corynebacterium pseudotuberculosis* Indirect (IDvet) for CLA. The cut-off values (S/P% = OD sample – OD negative control / OD positive control — OD negative control) were set at ≤ 40% negative, 40–50% questionable, and ≥ 50% positive.

Internal organs (lungs, liver, spleen, kidney, lymph nodes, uterus and udder) were selected from one dead goat for bacteriological studies. For *C. pseudotuberculosis* isolation sheep blood agar plates and CoryneBacAgar (Farmactive) with adding 10% of sterile bovine serum and 2% of potassium tellurite (K_2TeO_3) were used following incubation at 37 °C for 48 h in aerobic

conditions. Identification of the bacteria included biochemical test (urease, catalase, indole, H_2S production, and nitrate reduction, fermentation of glucose, lactose, maltose, mannose, and fructose) and multiplex PCR. The IndiSpin Pathogen Kit (Indical) was used for DNA extraction from *C. pseudotuberculosis* isolate according to the manufacturer's instruction. The multiplex PCR was provided using specific primers (Table 1) and DreamTaq Green PCR Master Mix (Thermo Scientific, USA) to detect 16s rRNA, *rpo*B, and *pld* genes of *C. pseudotuberculosis* according to the protocol as previously described Pacheco et al. (2007). The obtained products were discriminated by electrophoresis on a 1.5% agarose gel.

Table 1 — List of the primers, using for detection and identification of *C. pseudotuberculosis* isolate (Pacheco et al. 2007)

Primer name	Sequence 5'-3'	Target gene	Length, bp
16S-F	ACCGCACTTTAGTGTGTGTG	16s rRNA	816
16S-R	TCTCTACGCCGATCTTGTAT		
C2700F	CGTATGAACATCGGCCAGGT	*rpo*B	446
C3130R	TCCATTTCGCCGAAGCGCTG		
PLD-F	ATAAGCGTAAGCAGGGAGCA	*pld*	203
PLD-R	ATCAGCGGTGATTGTCTTCCAGG		

Results. CLA outbreak among goats was registered in a farm where 60 sheep, 25 goats, two cows, and two horses were kept together in one building. The goat flock consisted of animals with unknown epidemiological data. These new animals were not quarantined or tested for major infections. Clinical observation revealed two goats with concurrent external abscesses, fever, anorexia, and weight loss. The enlarged lymph nodes are characterized by a spherical shape, up to 12 cm in size, painless, and not fused to the skin. Blood samples from suspect animals were tested by ELISA. According to the obtained results, four (18.2%) from 22 sera samples were positive (S/P% = 72, 82, 123, and 196) and one sample was questionable (S/P% = 41).

Bacteriological findings showed the appearance of round, brilliant, dry colonies, surrounded by a hemolysis zone. The biochemical characteristics of the culture were as follows: Catalase-positive, Urease-positive, Nitrate reductase-negative, Indole-negative, H_2S-negative, fermentation of glucose, maltose, mannose, and fructose. Gram-staining of the cells showed gram-positive cocci-bacilli that are typical for microorganisms belonging to the family Corynebacteriaceae. *C. pseudotuberculosis* was isolated from the lymph nodes, liver, and lungs whereas the spleen, kidney, uterus, and udder did not show any growth.

Pathogen identification was provided by multiplex PCR (Fig. 1). All three fragments were successfully amplified that showed the presence of 16s rRNA, *rpo*B, and *pld* genes of *C. pseudotuberculosis*.

Discussion. CLA is a chronic infectious disease of a wide range of animal species throughout the world that leads to lymph node enlargement and the occurrence of abscesses in internal organs (Dorella et al., 2006). We report here a single case of CLA among goats on a farm in Kyiv Region, Ukraine.

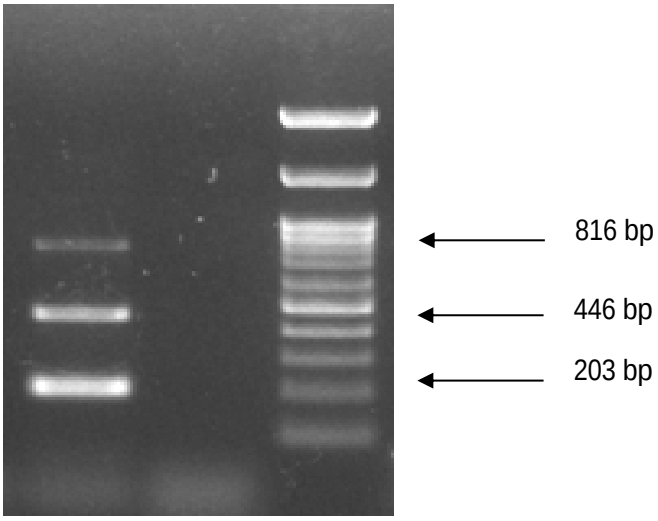

Figure 1. The multiplex PCR result of the obtained *C. pseudotuberculosis* isolate for detection of 16s rRNA, *rpo*B, and *pld* genes: S — *C. pseudotuberculosis* DNA, N — negative control (distilled water), M — DNA marker (Thermo Scientific, USA).

The animals were kept for milk production, which poses a risk to humans due to the zoonotic potential of the pathogen. In addition, infected animals were housed with other species that could also be infected with *C. pseudotuberculosis*: cattle (Mathewos and Fesseha, 2022), horses (Spier and Whitcomb, 2014; Corbeil, Morrissey and Léguillette, 2016), and sheep (Ruiz et al., 2020). However, *C. pseudotuberculosis* infection was only confirmed in goats on this farm by serology, bacteriology and molecular identification. The seroprevalence among goats on the farm was 18.2%, which was consistent with other studies (Washburn et al., 2013; Jung, et al., 2015; Costa et al., 2020). Thus, *C. pseudotuberculosis* was recovered from the lungs, liver and lymph nodes, which agreed with the results published by other authors (Baird

and Fontaine, 2007; El Damaty et al., 2023). Finally, the isolate obtained was characterized both by biochemical tests and by PCR. Disease control should include elimination of infected and suspect animals from the herd, comprehensive disinfection, and continuous monitoring of the entire herd. A control program must be established to prevent the spread of *C. pseudotuberculosis* among small ruminants in Ukraine.

Conclusions. An outbreak of CLA in the goat herd was confirmed by serological, microbiological and molecular techniques. Due to the lack of available data on the prevalence of CLA in Ukraine, further surveillance is needed and an effective disease control strategy and eradication measures need to be developed.

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