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SURVEY FOR *C. ABORTUS* AND *C. PECORUM* IN RUMINANTS IN UKRAINE

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Chlamydiosis is highly prevalent in ruminants worldwide leading to serious economic losses. Laboratory diagnosis of chlamydiosis is based on direct detection of the pathogen, its antigen or DNA, or by detection of specific antibodies using serological tests. The objective of this study was to get a preliminary insight into the occurrence of chlamydial infections in domestic ruminants in different regions of Ukraine.

Indirect serological and direct molecular investigations on Chlamydiosis in different herds of Ukrainian ruminants were carried out. All samples originated from 31 farms out of seven regions of Ukraine collected during 2015 and 2016. Abortions, births of non-viable calves, and arthritis during the first year of life were observed on these farms. In total, ten and three samples (2.6 % and 0.8 %) were positive by serological investigation and real-time PCR, respectively. All bovine semen samples were negative for chlamydiae. Additionally, conjunctival swabs from calves and vaginal swab from ewe contained DNA of C. pecorum and C. abortus respectively that was confirmed by microarray assay.

More investigations including a high number of samples and other geographical locations of the country are needed to provide strategies for controlling of chlamydial infection and disease outbreaks in Ukrainian herds.

Keywords: *C. abortus, C. pecorum, microarray assay, PCR, serology*

Introduction. Chlamydiosis is highly prevalent in ruminants worldwide leading to serious economic losses. The disease is caused by *Chlamydia* which is an obligate intracellular, Gram-negative bacterium that might pose a zoonotic risk [1]. Its unique life cycle includes two stages named the elementary and the reticulate body. A third stage, the aberrant form, is supposed to remain hidden from the host immune response and to cause persistent infections [2, 3]. Several members of the genus *Chlamydia* (*C. psittaci, C. pecorum, C. abortus* and *C. pneumoniae*) can infect multiple host species [4]. *C. abortus* leads to enzootic abortion in sheep and goats causing considerable economic losses in sheep husbandry worldwide [5]. *C. pecorum* may cause subclinical infections in livestock or is the etiologic agent of encephalomyelitis in cattle and polyarthritis in sheep [6]. *C. pecorum* was also reported to infect wild ruminants and birds [7, 8, 9, 10].

Laboratory diagnosis of chlamydiosis is based on direct detection of the pathogen, its antigen or DNA, or by detection of specific antibodies using serological tests. Clinical samples for diagnosis are selected depending on the type of the disease and affected organs. In case of chlamydial abortion in ruminants, placental membranes or vaginal swabs are the samples of choice to examine by antigen detection methods or polymerase chain reaction (PCR).

To date, monitoring on chlamydiosis in ruminants in Ukraine has not been carried out and the epidemiological status of the disease remains unknown.

Objectives: The objective of this study was to get a preliminary insight into the occurrence of chlamydial infections in domestic ruminants in different regions of Ukraine.

Materials and Methods: Serological tests for the detection of chlamydial antibodies in serum samples (n=384) were conducted using the ID Screen® *Chlamydophila abortus* Indirect Multi-species ELISA (IDvet, France), the CHEKIT®-IDEXX Chlamydiosis Total Ab Test (IDEXX Laboratories) and the complement fixation test (CFT).

Vaginal and conjunctival swabs (COPAN, Italy) (n=220), placental samples and intestinal organs from aborted fetuses (cattle, sheep and goats) (n=14) as well as bovine semen samples (n=132) were also collected. All samples originated from 31 farms out of seven regions of Ukraine collected during 2015 and 2016. The DNA of each sample was extracted and investigated by quantitative real-time PCR assay (qPCR) specific for the family *Chlamydiaceae* targeting the 23S rRNA gene as described previously [11]. The chlamydial species was thereafter determined using the 23S ArrayTube microarray assay [12].

Results and discussion: Indirect serological and direct molecular investigations on Chlamydiosis in different herds of Ukrainian ruminants were carried out. Abortions, births of non-viable calves, and arthritis during the first year of life were observed on these farms. In total, ten and three samples (2.6 % and 0.8 %) were positive by serological investigation and real-time PCR, respectively. All bovine semen samples were negative for chlamydiae.

In Kherson region, an abortion in an ewe from a private backyard was observed during the study. Chlamydial DNA was detected in the vaginal swab from the aborting ewe by real-time PCR. Further analysis by ArrayTube microarray assay identified as *C. abortus* in this case (fig. 1).

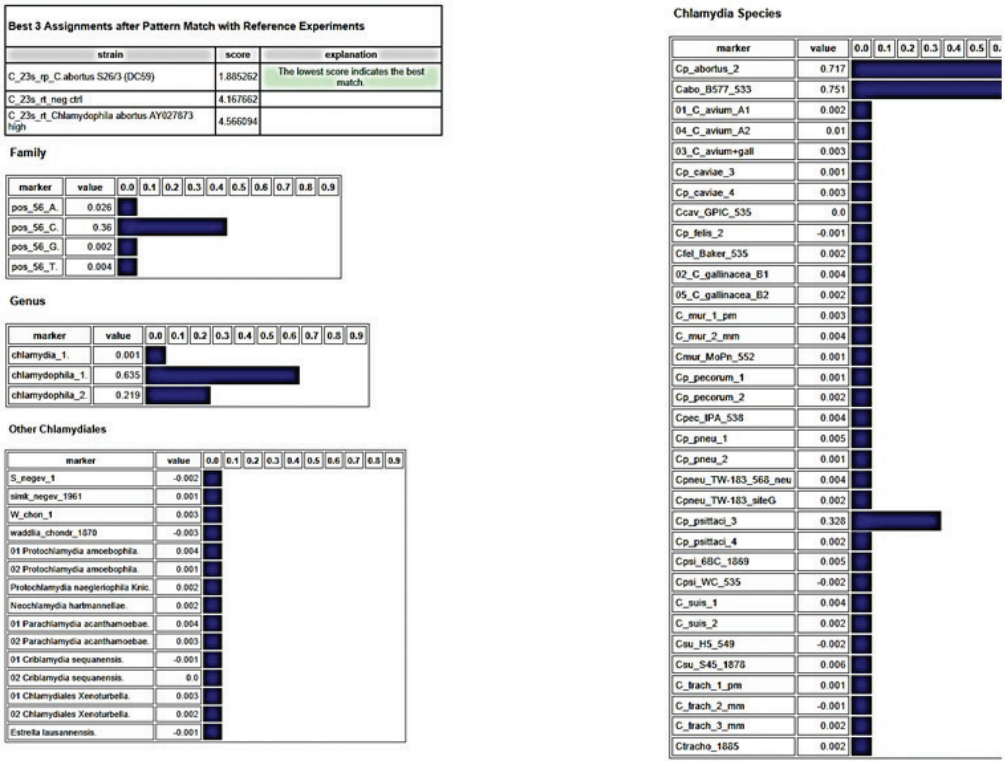

Figure 1. Report of a 23S ArrayTube microarray assay analysis of the vaginal swab obtained from the aborting ewe. *C. abortus* was identified

In a cattle farm located in Kirovograd region, conjunctivitis, rhinitis and polyarthritis were observed in calves. The farm contained 2600 dairy cows and 800 calves in total. Serum samples (n=149) from this herd were studied using CFT to detect anti-chlamydial antibodies. Of these, 26 (17.4 %) were positive (table 1). The CFT detects antibodies directed against the chlamydial lipopolysaccharide (LPS) and is genus-specific whereas the commercially available ELISA (IDvet) identifies only species-specific antibodies directed against *C. abortus*.

In our case, all CFT-positive serum samples were negative by the IDvet ELISA. Using the CHECKIT ELISA kit, which is known to show cross-reaction with anti-*C. pecorum* antibodies [13], only 15 samples (58 %) were negative. At the same time, all vaginal swabs from seropositive cows were negative for chlamydial DNA by real-time PCR. Additionally, conjunctival swabs from calves showing conjunctivitis were investigated by real-time PCR and *C. pecorum* was recognized in one of those samples by microarray assay (fig. 2).

Table 1 – Results of the serological study investigating serum samples from one cattle herd in Kirovograd region

№ of samples	cow ID	CFT results			ID-Vet ELISA	CHECKIT ELISA
		1/5	1/10	Interpretation		
1	371	**+++**	**++**	**Pos**	Neg	**Pos**
2	3221	+++	-	Quest	Neg	**Pos**
3	2565	**#**	**+++**	**Pos**	Neg	**Pos**
4	636	++	-	Quest	Neg	Neg
5	845	++	-	Quest	Neg	**Pos**
6	1591	+++	-	Quest	Neg	Neg
7	2558	+++	-	Quest	Neg	Neg
8	1419	+++	-	Quest	Neg	Neg
9	1560	+++	-	Quest	Neg	**Pos**
10	1370	**#**	**#**	**Pos**	Neg	**Pos**
11	2914	++	+	Quest	Neg	Neg
12	456	++	-	Quest	Neg	Neg
13	2328	++	-	Quest	Neg	Neg

14	359	+++	+	Quest	Neg	Neg
15	1203	#	#	**Pos**	Neg	**Pos**
16	446	#	#	**Pos**	Neg	**Pos**
17	1480	+	-	Neg	Neg	Neg
18	2442	+	-	Neg	Neg	Neg
19	277	+++	+	Quest	Neg	**Pos**
20	222	++	-	Quest	Neg	Neg
21	2840	+++	-	Quest	Neg	Neg
22	625	+++	-	Quest	Neg	**Pos**
23	2552	++	+	Quest	Neg	Neg
24	1188	++	-	Quest	Neg	**Pos**
25	1527	++	+	Quest	Neg	Neg
26	225	+	-	Neg	Neg	Neg

Although we could not detect *C. abortus* in cows during this preliminary study, continued monitoring of this pathogen in Ukrainian herds is needed due to the potential economic losses as the consequence of subclinical infertility [14]. According to other reports, variable prevalence rates ranging from <5 % to 100 % have been reported in individual animals [15]. In Germany, 13.5 % of examined cows were positive by real-time PCR for *Chlamydia sp.* in vaginal swabs [16]. In Jordan, the prevalence of antibodies against *C. abortus* in 62 cattle herds was 66.3 % [17].

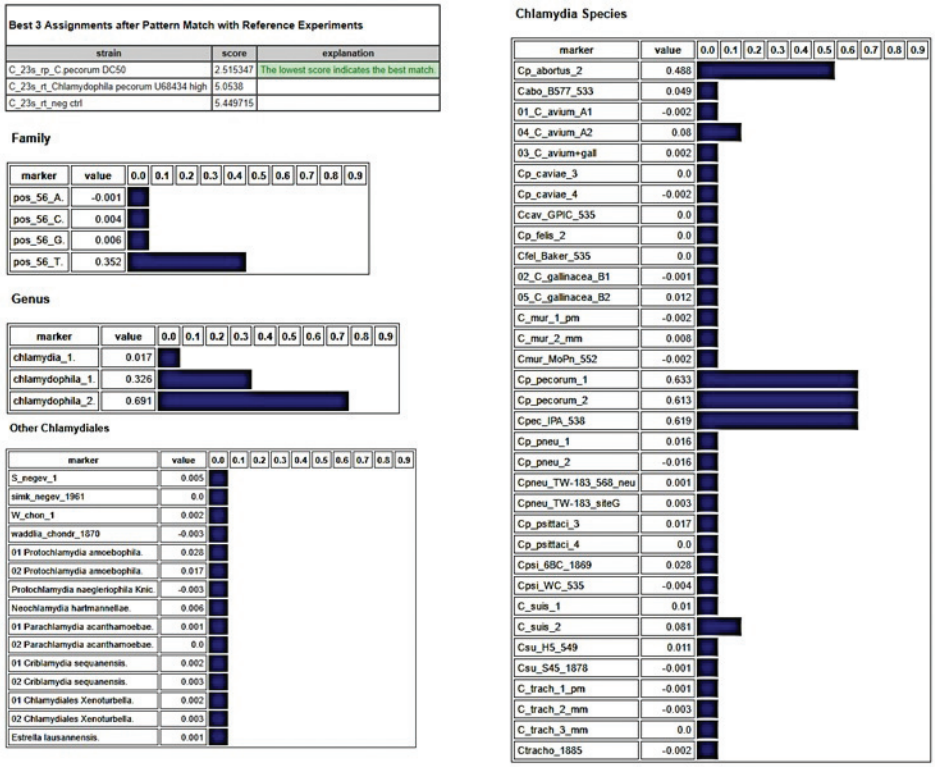

Figure 2. Report of the 23S ArrayTube microarray assay analysis of the sample obtained from a calf with conjunctivitis. *C. pecorum* was identified

In this pilot study, we focused on the necessity to implement the European standards in Ukrainian veterinary laboratories to improve the serological diagnosis of animal chlamydiosis. CFT was previously recommended by OIE but is not as sensitive and specific as ELISA tests [18]. Isolation of Chlamydia in cell culture is laborious and needs special expertise rendering this technique unsuitable for routine diagnosis in clinical samples. Nowadays, PCR is a very useful and rapid routine method for Chlamydia detection and can be used in combination with serological tests.

Conclusion. Preliminary results by serological and molecular investigations indicate the circulation of chlamydial infection in ruminants in Ukraine. More investigations including a high number of samples and other geographical locations of the country are needed to provide strategies for controlling of chlamydial infection and disease outbreaks in Ukrainian herds.

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ВИЯВЛЕННЯ *C. ABORTUS* ТА *C. PECORUM* У ЖУЙНИХ ТВАРИН В УКРАЇНІ

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Хламідіоз є досить поширеним захворюванням серед жуйних тварин у всьому світі, що призводить до серйозних економічних втрат. Лабораторна діагностика хламідіозу ґрунтується на прямому виявленні збудника, його антигенів або ДНК, а також шляхом виявлення специфічних антитіл за допомогою серологічних тестів.

Мета даного дослідження полягала в тому, щоб отримати попереднє уявлення про виникнення хламідійних інфекцій серед домашніх жуйних у різних регіонах України.

З цією метою проводилися непрямі серологічні та прямі молекулярно-генетичні дослідження щодо хламідіозу в різних господарствах. У обстежених господарствах спостерігалися аборти, народження нежиттєздатного молодняку та артрит протягом першого року життя. У цілому, десять і три зразки (2,6 % і 0,8 %) були позитивними за результатами серологічних досліджень та за ПЛР у реальному часі, відповідно. Усі зразки сперми від бугаїв-плідників не містили генетичний матеріал хламідій. Крім того, кон'юнктивальні мазки від телят та вагінальний зіскрібок від вівцематки містили ДНК C. pecorum і C. abortus відповідно, що було підтверджено за допомогою аналізу ДНК-мікрочіпів.

Для розробки ефективної стратегії боротьби проти хламідійної інфекції і запобігання спалахів захворювань в господарствах України необхідно провести масштабні дослідження з охопленням інших географічних районів.

Ключові слова: *C. abortus, C. pecorum, аналіз мікрочипами, ПЛР, серологія*