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CHLAMYDIAL INFECTION MONITORING WITHIN WILD MAMMALS IN UKRAINE

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The study was carried out on of rectal epithelial scrap samples from 117 wild mammals of 16 species, namely 39 wild swine (Sus scrofa), 4 roe deer (Capreolus capreolus), 17 red foxes (Vulpes vulpes), 5 wolves (Canis lupus), 2 raccoon dogs (Nyctereutes procyonoides), 1 badger (Meles meles), 1 polecat (Mustela putorius), 2 beavers (Castor fiber), 3 martens (Martes), 2 weasels (Mustela erminea), 2 river otters (Lutra lutra), 3 muskrats (Ondatra zibethicus), 18 hares (Lepus europaeus), 4 bobak marmots (Marmota bobak), 5 squirrels (Sciurus) and 9 mole rats (Talpa europaea) caught or shot during hunting in hunting areas of 14 regions of Ukraine.

Key words: monitoring, chlamydial infection, wild mammals, PCR, Ukraine.

The work is a fragment of the research project "Development of a system for indication and species differentiation of the Chlamydiales order bacteria", state registration No. 0119U000445.

In recent years, the discovery of new pathogens and a number of infectious diseases has steadily increased, and this mainly due to zoonoses brought from wildlife [2]. To a greater extent, information on chlamydial infections in wild animals is limited to disease cases reports and monitoring studies, most wild mammals are susceptible to chlamydial infection. Chlamydia agents are also isolated from representatives of other classes in wild fauna, namely, amphibians, reptiles, arthropods, fish, birds and mollusks [1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14, 15].

In the study of 44 wild pigs in the national park of northern Italy, bacteria of the Chlamydiales order were detected in half of them (22 pigs). In differentiating the isolates obtained, 12 of them were identified as Chlamydia suis, 5 as Chlamydia pecorum, 4 as Parachlamydia acanthamoebae, and 1 sample failed to be identified [8].

It is known that Australian koalas suffer from chlamydia, mainly Chlamydia pecorum and Chlamydia pneumoniae. Infected koalas lose reproductive capacity, often suffering from pneumonia and trachoma. Frequently, the disease has lethal consequences, and therefore causes a rapid decline in the koalas population [4].

In 2011-2014, chlamydiosis was detected in wild sea birds [3]. It is proved that 465 species of 30 bird orders around the world are susceptible to chlamydiosis [14], And Reed K. et al. described the epizooty of Chlamydiosis in the colony of African ungula frogs (Xenopus tropicalis), which was caused by Clamydophila pneumoniae [11].

Ksyonz I.M. et al., (2010) investigated epithelial scrapes samples from the rectum of 30 wild mammalians belonging to 5 species, namely samples taken from 11 wild pigs (Sus scrofa), 2 roes (Capreolus capreolus), 6 foxes (Vulpes vulpes), 8 hares (Lepus europaeus) and 3 muskrats (Ondatra zibethicus), shot at hunting on the territories of Poltava, Kharkov and Cherkasy regions in Ukraine. The diagnosis of chlamydial infection was confirmed in 11 cases (about 37 %) [1].

The purpose of the study was to clarify the epizootic status among wild mammals in different regions of Ukraine regarding chlamydial infections.

Materials and methods. To achieve this purpose, we studied 117 samples of biological material from wild mammals of 16 species with the identification of the Chlamydia genus bacteria species. The study was carried out from 2003 to 2017. Biological samples for the chlamydia test were epithelial rectum scrapings from wild mammals caught or shot during hunting in the hunting areas (in accordance with Article 6 Methods of killing, Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 "On the Protection of Animals Used for Scientific Purposes") in 14 regions of Ukraine, namely, Vinnytsia, Dnipropetrovsk, Zhytomyr, Zaporizhzhya, Ivano-Frankivsk, Kirovograd, Kyiv, Luhansk, Poltava, Sumy, Ternopil, Kharkiv, Cherkasy and Chernihiv regions. (fig. 1).

Epithelial scrapes were taken from caught animals and shot animals' carcasses by means of single-use urogenital multiprobes. The study was performed applying the polymerase chain reaction (PCR) method, which was carried out using the self-developed PCR test systems for indicating DNA of the gene encoding 16S rRNA and MOMP of the Chlamydiaceae family that cause diseases in animals and birds. In

order to determine the pathogens species in the DNA samples positive for chlamydial infection, a self-developed test system was used for the specific identification of the Chlamydiaceae family bacteria in the multiplex PCR.

Fig. 1. Origin of the samples under study.

DNA isolation from the biological samples under study was carried out by means of the sorbent method using the "DNA-sorb-B-50" kit, which is part of the "HLA-KOM" test system for diagnosing chlamydia in animals and birds by the polymerase chain reaction method, produced by the FBUN CNIIE (Federal Budget Institution of Science "Central Research Institute of Epidemiology of the Federal Service on Customers' Rights Protection and Human well- being Surveillance"), (Rospotrebnadzor, Russia) and "Proba-Rapid" kit of DNA isolation reagents manufactured by OOO "NPO DNK-Technologiya" (Russia).

The PCR test system for indicating DNA fragments encoding 16S rRNA gene contains a pair of oligonucleotide primers: C.SP.F: 5`-GGTAATAγCGGAGGGTGCTAGC -3` and C.SP.R: 5`-CTGACACGCCATTACTAGCAA -3`, restricting the DNA fragment sizing 832 bp, which is conservative for all species of the Chlamydia genus bacteria. The primers designed by us were synthesized by "GINOSYS" (USA) and "Thermo Electron Corporation" (Germany).

The PCR test system for indicating DNA fragments encoding the major outer membrane protein (MOMP) of chlamydia contains a pair of oligonucleotide primers:

MOMPSPF: 5`-AGGTGAGTATGAAAAAACTCTT -3` and

MOMPSPR: 5`-TCGAAAACATAATCTCCGTA -3` which also restrict a conservative DNA fragment for all species of the Chlamydia genus bacteria. The indicated pair of oligonucleotide primers flanking the MOMP fragment sizing 221 bp was synthesized according to our design by "Thermo Electron Corporation" (Germany).

The multiplex PCR test system for differentiating chlamydia by species contains three upstream and five downstream oligonucleotide primers of the following design:

HAFF: 5`-GATCCTTGTGCTACTTGGTGTGA-3`,
CHPPPF: 5`-GATCCTTGCGCTACTTGGTG-3`,
CHSF: 5`-GATCCCTGCACTACTTGGTGTG-3`,
CHFER: 5`-ATTCGAGCTAGCTCCTTTATAGCC-3`,
CHPSR: 5`-ATTCGAGCTAGCTCCTTTATAGCC-3`,
CHABR: 5`-GACTACATTCAACATTTCAATTTTAGGAT-3`,
CHPNR: 5`-TTACTTAAAGAAACGTTTGGTAGTTCATT-3`,
CHSUR: 5`-TTTTGTTCCAAATAACCCAACTAAGT-3`.

The difference in the amplified fragments length of the variable MOMP gene region is due to insertions and / or deletions of the gene sites in different bacteria of the Chlamydia genus. The products of the multiplex PCR are the MOMP genes fragments with the size characteristic of the six Chlamydia genus bacteria pathogenic for animals: Chlamydia felis - 796 bp, Chlamydia psittaci - 627 bp, Chlamydia abortus - 466 bp, Chlamydia pneumoniae - 416 bp, Chlamydia suis - 358 bp, Chlamydia pecorum - 306 bp.

The primer set for the multiplex PCR test system was also synthesized by "Thermo Electron Corporation" (Germany). In addition to the oligonucleotide primers, the reaction mixture of each PCR test systems included reagents manufactured by "Fermentas UAB" (Lithuania), namely deionized water, PCR buffer, $MgCl_2$, solution of deoxyribonucleoside triphosphates (dNTP) and Taq polymerase. As DNA markers, FX 174 DNA / Hinf I, and O'Range Ruler ™ 100bp Ladder ("Fermentas UAB", Lithuania) were used.

Results of the study and their discussion. As a result of pathological studies, epithelial scrapings of 39 shot wild pigs (Sus scrofa) from 11 areas of 6 regions of Ukraine, DNA of the Chlamydiceae family

bacteria was identified in 20 samples, 10 of which were identified as Chlamydia pecorum, 8 - as Chlamydia suis and 2 - as Chlamydia abortus (tables 1).

Table 1

Results of the study by separate Chlamydia species

Animal species	№ of animals	Chlamydia-positive	Species unidentified	C. felis	C. psittaci	C. abortus	C.pneumoniae	C. suis	C. pecorum	Detected Chlamydia genus DNA %
Wild boar (Sus scrofa)	39	20	-	-	-	2	-	8	10	51.2%
Roe deer (Capreolus capreolus)	4	2	-	-	-	2	-	-	-	50.0%
Red fox (Vulpes vulpes)	17	7	-	-	1	-	2	-	4	41.2%
Wolf (Canis lupus)	5	2	-	-	-	-	-	-	2	40.0%
Raccoon dog (Nyctereutes procyonoides)	2	0	-	-	-	-	-	-	-	-
Badger (Meles meles)	1	0	-	-	-	-	-	-	-	-
Polecat (Mustela putorius)	1	0	-	-	-	-	-	-	-	-
Beaver (Castor fiber)	2	0	-	-	-	-	-	-	-	-
Marten (Martes)	3	2	-	-	-	-	-	-	2	66.7%
Ermine (Mustela ermine)	2	1	-	-	-	-	-	-	1	50.0%
River otter Lutra lutra)	2	1	-	-	-	-	-	-	1	50.0%
Muskrat (Ondatra zibethicus)	3	1	1	-	-	-	-	-	-	33.3%
Hare (Lepus europaeus)	18	5	-	-	1	-	2	-	2	27.8%
Steppe marmot (Marmota bobak)	4	1	-	-	-	-	-	-	1	25.0%
Squirrel (Sciurus)	5	1	-	-	1	-	-	-	-	20,0%
Mole Talpa europaea)	9	1	-	-	1	-	-	-	-	11.1%
Total, pcs	117	44	1	-	4	4	4	8	23	37.6%
Total ratio by species, %	100%	37.6%	0.85%	-	3.42%	3.42%	3.42%	6.8%	19.66%	37.6%
Total ratio by species, among infected animals, %		100%	2.27%	-	9.09%	9.09%	9.09%	18.18%	52.3 %	

The total of four samples were selected from the roe deer (Capreolus capreolus), in two of them the Chlamydia abortus DNA was detected.

In the study of samples selected from 17 red foxes (Vulpes vulpes) shot in 8 areas of 5 regions, the chlamydial DNA was detected in 7 of them. Four isolates were identified as Chlamydia pecorum, 2 - as Chlamydia pneumoniae and 1 - as Chlamydia psittaci.

Among the five samples of rectal scrapes from wolves (Canis lupus) shot in hunting areas of 4 districts in three regions of Ukraine, in two samples, the Chlamydia pecorum DNA was detected.

Samples from two raccoon dogs (Nyctereutes procyonoides) hunted in the Chortkiv district of the Ternopil region were found free from chlamydial infections. Also, no Chlamydia DNA was found in specimens from a badger (Meles meles) shot in the hunting lands of the Mashivka district in the Poltava region, a polecat (Mustela putorius), caught in the lands of the Horodnya district in the Chernihiv region, and two beavers hunted in the hunting lands of the Shyshaky district in the Poltava region.

Out of the three martens (Martes) caught in the hunting lands of the Chernobai district in the Cherkasy region (2 individuals) and in the Borzna district of the Chernihiv region the Chlamydia pecorum DNA was found in one sample.

The Chlamydia pecorum DNA was detected in one of the epithelial samples taken from two ermines (Mustela erminea) caught in the Zinkiv district of the Poltava region and in the Krynychky district of the Dnipropetrovsk region.

The same result was obtained in the study of biological samples taken from two river otters (Lutra lutra) caught in the lands of the Lubny district in the Poltava region, in one of them the Chlamydia pecorum DNA being identified.

Regarding the muskrats (Ondatra zibethicus), hunted in the Sula river floodplain of the Globyno district in the Poltava region, one of the three revealed a chlamydial pathogen that could not be identified by species.

Out of 18 hares (Lepus europaeus) hunted in 10 districts of 7 regions, specimens of five individuals were found to contain chlamydial DNA. Two isolates were identified as Chlamydia pecorum, 2 - as Chlamydia pneumoniae and 1 – as Chlamydia psittaci.

Out of the four steppe marmots (Marmota bobak) caught in the territory of the National Nature Park "Dvorichansky" in the Kharkiv region, one sample of the epithelial scrap was revealed to contain the Chlamydia pecorum DNA.

In the study of five squirrels (Sciurus) caught in the Poltava district of the Poltava region (2 individuals) and in the Voznesensk district of Mykolayiv region (3 individuals), the Chlamydia psittaci DNA was detected in one sample.

Samples of epithelial scrapings from 9 moles (Talpa europaea) caught in the Poltava (4 individuals) and the Mirgorod (2 individuals) districts of the Poltava region and in the Berdyansk district of the Zaporizhzhya region (3 individuals) were subject to the study. In this case, one of the samples contained the Chlamydia psittaci DNA.

The results of studying the rectum epithelial scrapings samples of 117 wild mammals belonging to 16 species that were caught in the 14 regions territories of Ukraine indicate a sufficiently wide spread of chlamydial infection among mammals. The number of infected individuals is 44, which amounts 37.6%. This is the real situation with chlamydial infection among wild mammals in Ukraine. Perhaps, for a wider screening, the percentage will somewhat vary, but this difference will not exceed 2-3%. We have come to this conclusion taking into account that as of 2010 in the study of 30 animals, their contamination amounted 37%; in 2013, 52 animals were studied, and their contamination was 38.5%. As of today, as it has already been noted, in the study of 117 individuals, the percentage of infected ones was 37.6% [1]. That means that the difference in the diseased animal's ratio does not exceed one per cent with an increase in the studied animal's number.

Despite the fact that the main purpose of our study was to find out the occurrence of different chlamydial species in wild mammals in Ukraine, based on our study, we can also say about the prevalence of Chlamydia in some species of wild mammals. Thus, according to our study, chlamydial DNA was detected in 51% (20/39) of smears taken from wild pigs. This chlamydial prevalence is consistent with data by Hotzel et al. [10] and data by Di Francesco A et al. (2013) [8]. The prevalence of chlamydial DNA in wild boar (Sus scrofa) was slightly different from the results of Di Francesco et al. 2011 obtained in Italy, where antibody titers to chlamydiae were detected in 110 out of the 173 samples tested (63.6%) [9] and from Hotzel H et al. (2004) where chlamydial DNA has been discovered in 57.1% of the animals in a German wild boar population in Thuringia [10]. These differences in the Chlamydia prevalence are likely to be related to different regions and by different detection methods used.

Regarding species correlation, Chlamydia pecorum was found in 50% (10/20) of the chlamydia infected wild boars, 8 (40%) were positive for Chlamydia suis, and 2/ 20 (10%) for Chlamydia abortus. In the data provided by Di Francesco A et al. (2013) [8], correlation was 55 % for C. suis, 23% for Chlamydia pecorum, rest - for Chlamydia-like organisms [10]. Despite differences in the species composition of Chlamydia in the population of wild pigs, most likely due to a different region, C. pecorum and C. suis predominate in both studies among Chlamydia-positive specimens.

In our studies 7 out of the 17 (42.2%) red foxes (Vulpes vulpes) and 5 out of the 18 (27.8%) hares (Lepus europaeus) were Chlamydia-positive. Despite the fact that the evidence of the chlamydial agents presence in wild foxes and hares was in line with the data by Spalatin, J et al. (1966) [15] we cannot compare the prevalence and species composition due to the absence of information, regarding the prevalence and Chlamydiae species composition in samples from the wild foxes and hares, in the available literature sources.

Two out of the 4 samples selected from the roe deer (Capreolus capreolus) were positive for C. abortus. Candela et al.(2014), Regenscheit et al. (2012), Salinas et al. (2009) reported the circulation of Chlamydiaceae spp. and/or C. abortus in roe deer. Some of the above listed studies suggest a high prevalence of Chlamydia in samples taken from roe deer [7, 12, 13], others suggest very low occurrence of Chlamydiaceae [12]. In our case, we cannot speak about the prevalence due to lack of data. For the same

reason, we cannot give an opinion on the prevalence of chlamydial infection among other species of the animals studied.

Regarding the occurrence of different chlamydial species in wild mammals in Ukraine, it has been determined that at least 5 species of the Chlamydia genus bacteria (Chlamydia pecorum, Chlamydia suis, Chlamydia abortus, Chlamydia pneumoniae, Chlamydia psittaci) circulate among wild mammals in Ukraine, this is consistent with data by Burnard D. et al (2016), who have reported the detection of Chlamydia pecorum, Chlamydia suis, Chlamydia abortus, Chlamydia pneumoniae, Chlamydia psittaci in wild mammals [6]. Despite a range of studies having reported the detection of chlamydial species in deer, wild sheep and a variety of wild caprids, wild boar and water buffalo, white yaks, african buffalo, spotted hyenas, mice, shrews, voles and squirrels there is a lack of data about Chlamydia pecorum, Chlamydia suis, Chlamydia abortus, Chlamydia pneumoniae, Chlamydia psittaci prevalence of bacteria among wild mammals in the available literature [3, 10, 13].

Conclusion

In our study, most frequently, animals were infected with Chlamydia pecorum (table 1). Chlamydial pathogens species was found in 52.3% of the infected animal's number. In particular, Chlamydia pecorum was found in 50% of the chlamydia infected wild boars, 57% of infected foxes, 100% of chlamydia infected wolves, martens, ermines, otters, marmots, 40% of infected birds, 50 moles, i.e. it is the predominant pathogen in 9 species of mammals we examined. Chlamydia suis is also isolated from wild pigs, this pathogene is detected in 40% of infected animals belonging to the above species. 10% of the pathogen isolates obtained from pigs are defined as Chlamydia abortus. Chlamydia abortus was detected in samples of two chlamydia infected roe deer, which makes 100%. The fourth species of the pathogen detected in 28.6% of isolates obtained from foxes and in 40% from hares is Chlamydia pneumoniae. The fifth pathogen species is Chlamydia psittaci, it was differentiated in 14.3% of chlamydia isolates obtained from infected foxes, in 20% - from infected rabbits, in 100% - from infected moles and in 100% - from infected squirrels. Regarding the isolate from a muskrat, where chlamydia species were not identified, it might be related to Chlamydia muridarum, which was not covered by the design of the developed multiplex PCR test system for species differentiation.

Chlamydia psittaci, Chlamydia pecorum, Chlamydia suis, Chlamydia pneumoniae Chlamydia abortus are the most common species among wild mammals in Ukraine. The lack of standardized methods for studying chlamydial infections in wild animals and impossibility of longitudinal and / or whole population studies makes it difficult to accurately assess the significance of chlamydial infections in the wildlife. Further research is needed to determine the range of cross-species transmission.

Thus, wild mammals are the reservoir of chlamydia pathogens in nature. This should be taken into account by livestock breeders, ensuring the absence of contacts between domestic animals and wildlife, as well as by the zoos veterinary doctors and by those controlling wild animals entering pet shops, since chlamydiosis is an interspecific zoonotic disease.

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Реферати

МОНІТОРИНГ ХЛАМІДІЙНОЇ ІНФЕКЦІЇ СЕРЕД ДИКИХ ССАВЦІВ В УКРАЇНІ

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Проведено дослілження зразків епітеліальних зіскрібків з прямої кишки від 117 диких ссавців 16 видів, а саме 39 диких свиней (Sus scrofa), 4 козуль (Capreolus capreolus), 17 червоних лисиць (Vulpes vulpes), 5 вовків (Canis lupus),2 єнотоподібних собак (Nyctereutes procyonoides), 1 борсука (Meles meles), 1 тхора (Mustela putorius), 2 бобів (Castor fiber), 3 куниць(Martes), 2 горностаїв (Mustela ermine), 2 річкових видр (Lutra lutra), 3 ондатр (Ondatra zibethicus), 18 зайців (Lepus europaeus), 4 степових бабака (Marmota bobak), 5 білок (Sciurus) та 9 кротів (Talpa europaea) виловлених або відстріляних під час полювання у мисливських угіддях 14 регіонів України.

Ключові слова: Монітринг, хламідійна інфекція, дикі ссавці, ПЛР, Україна.

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МОНИТОРИНГ ХЛАМИДИЙНОЙ ИНФЕКЦИИ СРЕДИ ДИКИХ МЛЕКОПИТАЮЩИХ УКРАИНЫ

Ксенз І.Н, Зезекало В.К., Передера С.Б., Щербакова Н.С., Передера Ж.А., Коне М.С., Рак Т.М., Кравченко С.А., Канивец Н.С.

Исследование проводилось на образцах ректальных эпителиальных скребков 117 диких млекопитающих 16 видов, а именно 39 диких свиней (Sus scrofa), 4 косули (Capreolus capreolus), 17 лисиц (Vulpes vulpes), 5 волков (Canis lupus) , 2 енотовидных собаки (Nyctereutes procyonoides), 1 барсук (Meles meles), 1 хорек (Mustela putorius), 2 бобра (Castor fiber), 3 куницы (Martes), 2 ласки (Mustela erminea), 2 речные выдры (Lutra lutra) 3 ондатры (Ondatra zibethicus), 18 зайцев (Lepus europaeus), 4 сурка (Marmota bobak), 5 белок (Sciurus) и 9 кротов (Talpa europaea), пойманных или застреленных во время охоты в охотничьих угодьях 14 областей Украины.

Ключевые слова: мониторинг, хламидийная инфекция, дикие млекопитающие, ПЦР, Украина.

Рецензент Пилипенко С.В.