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DEVELOPMENT OF PCR TEST SYSTEMS FOR SPECIES DIFFERENTIATION OF CHLAMYDIAL AGENTS IN BIRDS

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Summary. According to the modern classification adopted by the 2nd European Meeting on Animal Chlamydioses and Zoonotic Implications (EMAC-2), the genus *Chlamydia* contains three species of bird chlamydial agents — *C. avium*, *C. gallinacea* and *C. psittaci*. Three PCR test systems have been developed for indication and species differentiation of these bacteria. The bases for the developed diagnostics are three pairs of oligonucleotide primers designed and synthesized to flank the DNA fragments specific for each of the three bacteria species of the *Chlamydia* genus, which are pathogenic for birds. Analytical specificity of the developed PCR test systems was confirmed by the results of amplification of 18 biological materials samples, among them 13 being *Chlamydia*-containing, one of them contained *C. avium*, one — *C. gallinacea*, and 11 — *C. psittaci*. In addition *Leptospira* and *Babesia* DNA samples were tested. The developed PCR test systems for indication and species differentiation of bird Chlamydiosis agents permit to reliably study certain aspects of chlamydial infection.

Keywords: Chlamydioses, birds, MOMP, PCR test system, species differentiation, *Chlamydia avium*, *Chlamydia gallinacea*, *Chlamydia psittaci*

Introduction. *Chlamydia* is a group of infectious diseases caused by Gram-negative intracellular bacteria of the Chlamydiales order. According to the current classification adopted at the 2nd European Meeting on Animal Chlamydioses and Zoonotic Implications (EMAC-2), 8 families (3 of them having a candidate status) are represented by 13 genera (5 with a candidate status) and 25 species (among them 7 microorganisms having a candidate status).

The mammals' and birds' pathogens are the bacteria from *Chlamydia* genus, namely: *C. abortus*, *C. avium*, *C. caviae*, *C. felis*, *C. gallinacea*, *C. muridarum*, *C. pecorum*, *C. pneumoniae*, *C. psittaci*, *C. suis* and *C. trachomatis* (Sachse, 2013; Ksyonz and Liubetskyi, 2014).

Bird Chlamydioses are caused by three bacteria species: *C. avium*, *C. gallinacea* and *C. psittaci*. In particular, *C. avium* causes diseases in pigeons and Psittaciformes (Sachse et al., 2014). *C. gallinacea* is the causative agent for of Galliformes (chickens, turkeys) diseases (Guo et al., 2016). *C. psittaci* causes Chlamydiosis in over 170 species of domestic, decorative, synanthropic and wild birds. This agent is also capable of causing disease and asymptomatic infection in various mammals, including humans (Lagae et al., 2013; Vanrompay, Ducatelle and Haesebrouck, 1995).

Currently, Ukrainian scientists and veterinary medicine practitioners do not have available diagnostic tools capable of differentiating agents of birds' chlamydial infections by species. However, such a tool is necessary both to study various aspects of the identified infections, and to develop the effective strategies for the treatment measures. The purpose of our work was to develop three PCR test systems for indicating and species differentiation of *C. avium*, *C. gallinacea* and *C. psittaci*, i.e. etiological factors of birds' Chlamydiosis.

Materials and methods. The study was carried out in the Laboratory of Animal Health and Genetics of the Institute of Pig Breeding and Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine.

184 primary sequences of the gene encoding the main outer membrane protein (MOMP), belonging to three bacterial species of the *Chlamydia* genus pathogenic for birds, namely *C. avium*, *C. gallinacea* and *C. psittaci* were obtained from the nucleotide sequences databases GenBank (USA) and analyzed to construct the primers for the species-specific PCR test systems.

The nucleotide sequences of the gene encoding MOMP of the three designated species of *Chlamydia* genus were aligned using the MEGA v. 4 and MEGA v. 7 software (Tamura et al., 2007). To develop the design of oligonucleotide primers, DNA fragments specific for each species of the *Chlamydia* genus bacteria have been selected.

Sequences of oligonucleotide primers with their annealing temperature parameters were obtained using the FastPCR software (Kalendar, Lee and Schulman, 2014). Among the calculated primers, a single pair (the direct primer and the reverse one) was selected for each species of the *Chlamydia* genus bacteria pathogenic to birds.

Based on the designs developed, synthesis of the oligonucleotide primers was ordered at 'Thermo Electron Corporation' (Germany). The resulting synthesized primers were diluted with sterile deionized double-distilled water to the stock concentration of 100 pM/μl and then to the use concentration of 20 pM/μl.

In addition to the primers, the test systems used reagents for the PCR manufactured by 'Fermentas UAB' (Lithuania), namely: deionized water, PCR buffer, $MgCl_2$, deoxyribonucleoside triphosphate solution (dNTP), and Taq-polymerase.

Polymerase chain reaction using the developed PCR test systems for indication and species differentiation of *C. avium*, *C. gallinacea* and *C. psittaci* was carried out in 0.6 cm^3 polypropylene microcentrifuge tubes using the 'Biometra TRIO-Thermoblosk' thermocycler (Germany) in the reaction volume of 25 μl.

The ratio of the reaction mixture and the amplification program were adjusted experimentally and practically to obtain the most distinct bands on electrophoregrammes.

Fractionation of the amplification products was performed by the method of horizontal electrophoresis in the 2.0% agarose gel in the 'Cleaver Scientific Ltd.' (UK) electrophoretic chamber with a visual assessment by means of the UV transilluminator manufactured by NVO 'Progress' (Ukraine), after staining with bromine ethidium.

Restricted vector DNA *pUC19/MspI* ('Fermentas UAB', Lithuania) was used as a DNA marker.

DNA isolation from the biological samples under study was carried out using the commercially available reagents set 'PROBA-RAPID' manufactured by LLC 'NPO DNA Technology' (Russian Federation).

Materials for testing the experimental PCR test system parameters were samples of *C. avium*, *C. gallinacea* and *C. psittaci* control DNA received from the Chlamydiosis Laboratory of Friedrich Loffler Institute (Jena, Germany); DNA samples isolated from the field chlamydia isolates from domestic, decorative, synanthropic and wild birds and DNA samples isolated from the *Babesia* stored in the Laboratory of Animal Health at the Institute of Pig Breeding and Agroindustrial Production of NAAS; DNA samples isolated from *Leptospira* received from the Museum of Microorganisms of the Leptospirosis Laboratory at the Institute of Veterinary Medicine of NAAS.

Results. Bioinformational studies of 831 primary sequences of various genes belonging to the *Chlamydia* genus bacteria (16S rRNA, RNase P RNA, MOMP) have determined the highest level of nucleotide sequence variability (97.1%) in the gene encoding the main outer membrane protein (MOMP). When aligning the fragments of the *C. avium*, *C. gallinacea*, and *C. psittaci* MOMP gene, the level of nucleotide sequences' variability was 76.2%. That is why the primary sequences of this gene received from the international bases have been used in designing oligonucleotide primers for PCR test systems for indicating and differentiating *C. avium*, *C. gallinacea*, and *C. psittaci*.

By means of the 'FastPCR' computer software, oligonucleotide primers were designed. Among a large number of primer pairs, one was chosen for each test system, considering the size of the amplified fragment flanked by them (the most convenient for electrophoretic detection) and the optimal primers annealing temperature.

Thus, in the PCR test system for indication and species differentiation of *Chlamydia avium*, the following pair of primers was used:

ChAvMOMPL: 5'-TTCTGGTGATCCTTGCGACC-3'

CHAvMOMPR: 5'-GCTCCTAAAGTTGCACAACC-3'.

In the PCR test system for indication and species differentiation of *Chlamydia gallinacea*, the following pair of primers was used:

ChGalMOMPL: 5'-CAATACCATGAATGGCAAGC-3'

ChGalMOMPR: 5'-GAAAGTTGGGTTCCAAGCTG-3'.

The following pair of primers was used in the PCR test system for indication and species differentiation of *Chlamydia psittaci*:

ChPsMOMPL: 5'-GCACTATGTGGGAAGGTGCT-3'

ChPsMOMPR: 5'-CCATTTGCTTCTGGCTGATT-3'.

PCR-products were the fragments of the MOMP gene of the *Chlamydia* genus bacteria, having the sizes specific to each of the three *Chlamydia* species pathogenic to birds, namely: *Chlamydia avium* — 507 bp, *Chlamydia gallinacea* — 171 bp, *Chlamydia psittaci* — 208 bp.

Optimization of PCR conditions required selection of the reaction mixture composition and the temperature mode of amplification.

As a result of the PCR-protocol optimization, the following parameters of the reaction mixture were found to be optimal: 2.5 μl of 10-fold buffer (670 mM Tris-HCl, pH 8.8 at 25 °C, 20 mM BSA, 166 mM ammonium sulphate $(NH_4)_2SO_4$, 100 mM 2-β-mercaptoethanol) ('Fermentas UAB', Lithuania), 2.5 μl 2.5 mM dNTP ('Fermentas UAB', Lithuania), 2 μl 50 mM $MgCl_2$ ('Fermentas UAB', Lithuania), 2–3 units of Taq-polymerase (Thermus aquaticus) ('Fermentas UAB', Lithuania), 0.5 μl (0.1 opt. unit) of each primer and a sample of the DNA under study until the final concentration in the mixture; 1 μg/cm^3 deionized water to the volume of 25 μl. The amplification mixture was layered with 25 μl of mineral oil.

Optimal amplification parameters were:

94 °C	120 sec	
93 °C	30 sec	} 35 cycles
55 °C	30 sec	
72 °C	45 sec	
72 °C	300 sec	

To elaborate PCR parameters for the developed test systems and to test their analytical specificity, the following 18 biological materials were used: a sample of *C. avium* control DNA (1); a sample of *C. gallinacea* (2) control DNA; a sample of *C. psittaci* control DNA (3); a sample of DNA isolated from the *Chlamydia* field isolate of white Jacobin dove (*Columba livia*) from a private dovecote in Poltava (4); a DNA sample isolated from the field *Chlamydia* isolate of fantail-trumpeter (Torkun) dove (*Columba livia*) from a private dovecote in Poltava (5); a DNA sample isolated from the field *Chlamydia* isolate of shell parakeet (*Melopsittacus undulatus*) from a private parrot breeding farm in Karlovka (6); a DNA sample isolated from the *Chlamydia* field isolate of cockatiel (*Nymphicus hollandicus*) belonging to a resident of Poltava (7); a DNA sample isolated from the field *Chlamydia* isolate of yellow-collared lovebird (*Agapornis personatus*) from the private parrot breeding farm in Horyshniy Plavni, Kremenchuk district of the Poltava region (8); a DNA sample isolated from the field *Chlamydia* isolate of sparrow (*Passer* sp.) shot in the pig farm territory of LLC 'Svitanok', the Velyka Bahachka district of the Poltava region (9); a DNA sample isolated from the field *Chlamydia* isolate of greylag goose (*Anser anser*) shot in the lands of the Kobelyaky district of the Poltava region (10); a DNA sample isolated from the field *Chlamydia* isolate of greater flamingo (*Phoenicopterus roseus*) kept in the Kharkiv Zoo (11); a DNA sample isolated from the field isolate of silver pheasant (*Lophura nyctemera*) kept in the Kharkiv Zoo (12); a DNA sample isolated from the field isolate of vulture (*Gyps* sp.) kept in the Kharkiv Zoo (13); a DNA sample isolated from the field *Babesia canis* isolate of a dog belonging to a resident of Poltava (14); a DNA sample isolated from the field *Babesia bovis* isolate of a heifer at a private farm in Verkhy, Kamin-Kashyrskyi district of Volyn region (15); a DNA sample isolated from the *Leptospira* strain (LSU strain, *Louisiana* serovar, *Louisiana* serogroup) (16); a DNA sample isolated from the *Leptospira* strain (493 strain, *Poland*, *Polonica* serovar, *Sejroe* serogroup) (17); a DNA sample isolated from the *Leptospira* strain (*Hond Utrecht IV* strain, *Canicola* serovar, *Canicola* serogroup) (18).

At the first round of PCR the products electrophoregram of the 18 biological material samples was studied using the test system for indicating and differentiating *C. avium*. The specific size band of 507 bp was detected in a single track — No. 1 corresponding to the sample of the control *C. avium* DNA. In other 18 tracks, including negative control, there are no bands (Fig. 1).

At the second amplification products electrophoregram of the same 18 biological material samples were studied using the test system for indicating and species differentiation of *C. gallinacean,* a 171 bp band was detected in a single track — No. 2, which corresponds to the sample of the control *C. gallinacea* DNA. In other 18 tracks, including negative control, there are no bands (Fig. 2).

At the third PCR products electrophoregram of the above 18 biological material samples studied using the test system for indicating and species differentiation of *C. psittaci*, bands of 208 bp were detected. On 11 tracks — 3–13 corresponding to the samples of *C. psittaci* control DNA and field isolates (differentiated earlier as *C. psittaci*) from doves, Psittacidae birds, sparrow, greylag goose, greater flamingo, silver pheasant, and vulture. In other 8 tracks, including negative control, there are no bands (Fig. 3).

The results of the above 18 DNA samples studies with application of the three developed PCR test systems testify that chlamydial DNA indication occurs exceptionally in the samples corresponding to each diagnosticum's specificity: at the electrophoregrams of amplification products obtained with the help of the test system designed for the species *C. avium* differentiation, the only one band sizing 507 bp has been revealed in the track corresponding to the *C. avium* control DNA sample; at the electrophoregrams of amplification products obtained with the help of the test system designed for the species *C. gallinacea* differentiation, the only one band sizing 171 bp has also been found. In the track corresponding to the the *C. gallinacea* control DNA sample; at the electrophoregrams of amplification products obtained with the help of the test system designed for the species differentiation of *C. psittaci,* 11 bands have been detected sizing 208 bp in the tracks corresponding to the *C. psittaci* control DNA sample and to the DNA isolated from the field isolates of the above species obtained from different species of birds. There have not been found any other bands at a single electrophoregram, particularly, they were absent in the tracks of the DNA samples corresponding to *Babesia* and *Leptospira* DNA samples.

Study of the 18 above mentioned DNA samples using each of the three PCR test systems was performed in 3 replicates, with similar results (Tables 1–3). However, it should be noted, that the intensity of the bands was somewhat reduced with the concentration decrease of the DNA under study in the reaction mixture.

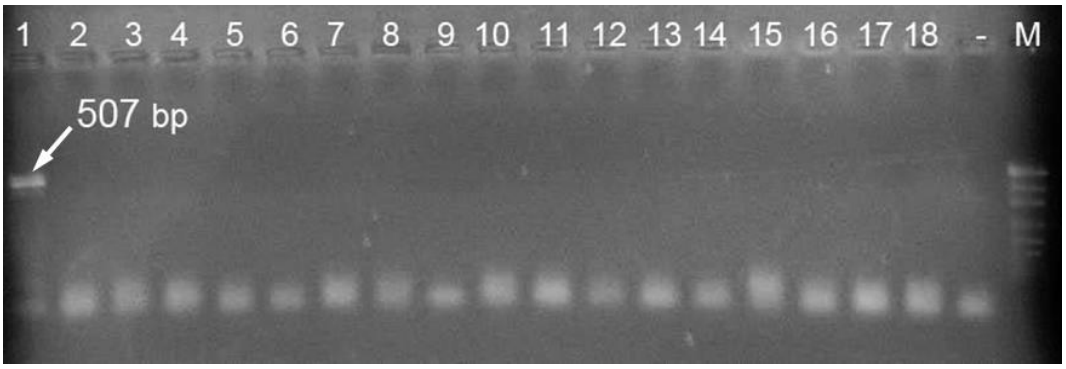

Figure 1. PCR-products electrophoregram of 18 biological material samples, studied using PCR test system for indicating and species differentiation of *Chlamydia avium* bacteria, namely: 3 control samples of *C. avium*, *C. gallinacea*, and *C. psittaci* DNA; 10 DNA samples isolated from field isolates of various bird species; 2 DNA samples isolated from the field *Babesia* protozoa isolates of a dog and cattle; 3 DNA samples isolated from *Leptospira* strains. Tracks: 1 — band of 507 bp (*C. avium*); 2–18 — negative result; «-» — negative control; M — DNA size marker *pUC19/MspI* ('Fermentas UAB', Lithuania)

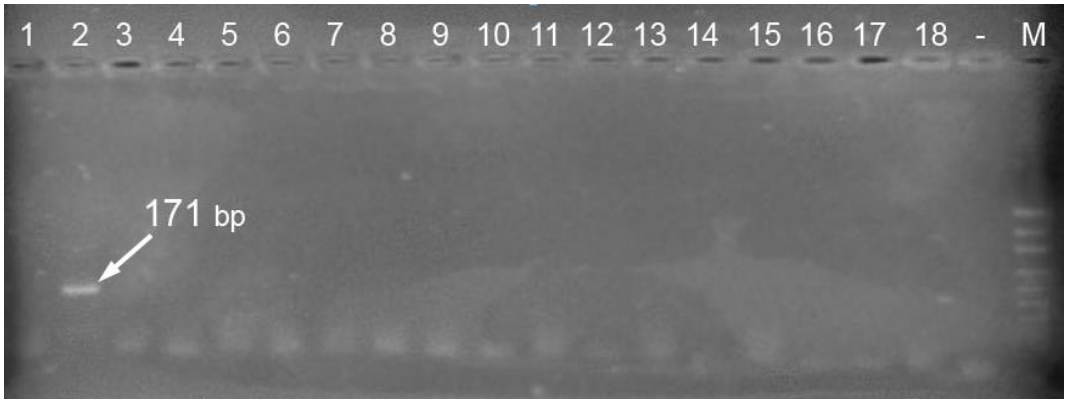

Figure 2. PCR-products electrophoregram of 18 biological material samples, tested using PCR test system for indicating and species differentiation of *Chlamydia gallinacea* bacteria, namely: 3 control samples of *C. avium*, *C. gallinacea*, and *C. psittaci* DNA; 10 DNA samples isolated from field isolates of various bird species; 2 DNA samples isolated from the field *Babesia* protozoa isolates of a dog and cattle; 3 DNA samples isolated from *Leptospira* strains. Tracks: 2 — band of 171 bp. (*C. gallinacea*); 1, 3–18 — negative result; «-» — negative control; M — DNA size marker *pUC19/MspI* ('Fermentas UAB', Lithuania)

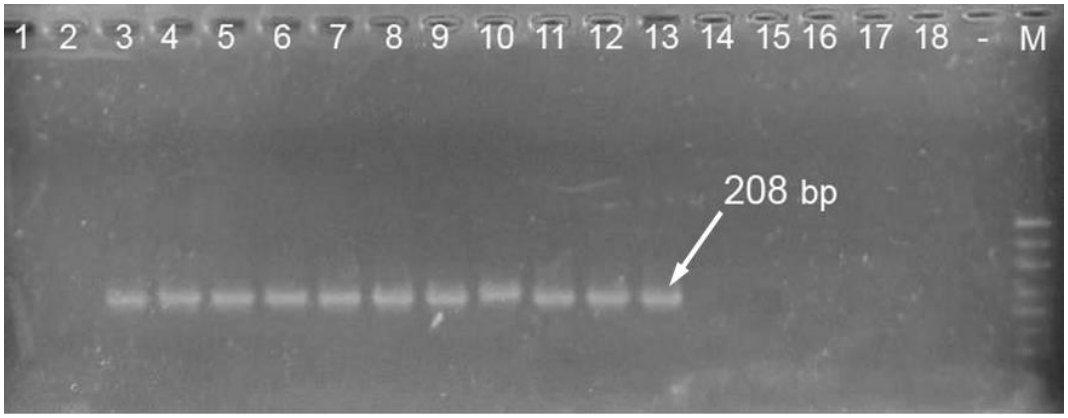

Figure 3. PCR-products electrophoregram of 18 biological material samples, studied using PCR test system for indicating and species differentiation of *Chlamydia psittaci*, bacteria, namely: 3 control samples of *C. avium*, *C. gallinacea*, and *C. psittaci* DNA; 10 DNA samples isolated from field isolates of various bird species; 2 DNA samples isolated from the field *Babesia* protozoa isolates of a dog and cattle; 3 DNA samples isolated from *Leptospira* strains. Tracks: 3–13 — band of 208 bp (*C. psittaci*); 1, 2, 14–18 — negative result; «-» — negative control; M — DNA size marker *pUC19/MspI* ('Fermentas UAB', Lithuania)

Table 1 — Testing results of the PCR test-system developed to indicate *Chlamydia avium* bacteria as to its analytical specificity and sensitivity

No.	Type of the sample studied	Study result										
		DNA amount in the reaction mixture									Band size	Agent species
		2 µl (20 µg/ml)			1 µl (20 µg/ml)			0.5 µl (20 µg/ml)				
		1 repeat	2 repeat	3 repeat	1 repeat	2 repeat	3 repeat	1 repeat	2 repeat	3 repeat		
1	Control DNA of *C. avium*	+	+	+	+	+	+	+	+	+	507	*C. avium*
2	Control DNA of *C. gallinacea*	–	–	–	–	–	–	–	–	–	–	–
3	Control DNA of *C. psittaci*	–	–	–	–	–	–	–	–	–	–	–
4	Chlamydial field isolate from white Jacobin dove (*Columba livia*)	–	–	–	–	–	–	–	–	–	–	–
5	Chlamydial field isolate from fantail-trumpeter (Torkun) dove (*Columba livia*)	–	–	–	–	–	–	–	–	–	–	–
6	Chlamydial field isolate from shell parakeet (*Melopsittacus undulatus*)	–	–	–	–	–	–	–	–	–	–	–
7	Chlamydial field isolate from cockatiel (*Nymphicus hollandicus*)	–	–	–	–	–	–	–	–	–	–	–
8	Chlamydial field isolate from yellow-collared lovebird (*Agapornis personatus*)	–	–	–	–	–	–	–	–	–	–	–
9	Chlamydial field isolate from sparrow (*Passer* sp.)	–	–	–	–	–	–	–	–	–	–	–
10	Chlamydial field isolate from greylag goose (*Anser anser*)	–	–	–	–	–	–	–	–	–	–	–
11	Chlamydial field isolate from greater flamingo (*Phoenicopterus roseus*)	–	–	–	–	–	–	–	–	–	–	–
12	Chlamydial field isolate from silver pheasant (*Lophura nycthemera*)	–	–	–	–	–	–	–	–	–	–	–
13	Chlamydial field isolate from vulture (*Gyps* sp.)	–	–	–	–	–	–	–	–	–	–	–
14	Field isolate *Babesia canis* from dog	–	–	–	–	–	–	–	–	–	–	–
15	Field isolate *Babesia bovis* from heifer	–	–	–	–	–	–	–	–	–	–	–
16	Leptospira strain LSU	–	–	–	–	–	–	–	–	–	–	–
17	Leptospira strain 493 *Poland*	–	–	–	–	–	–	–	–	–	–	–
18	Leptospira strain *Hond Utrecht IV*	–	–	–	–	–	–	–	–	–	–	–

Table 2 — Testing results of the PCR test-system developed to indicate *Chlamydia gallinacea* bacteria as to its analytical specificity and sensitivity

No.	Type of the sample studied	Study result										
		DNA amount in the reaction mixture									Band size	Agent species
		2 µl (20 µg/ml)			1 µl (20 µg/ml)			0.5 µl (20 µg/ml)				
		1 repeat	2 repeat	3 repeat	1 repeat	2 repeat	3 repeat	1 repeat	2 repeat	3 repeat		
1	Control DNA of *C. avium*	–	–	–	–	–	–	–	–	–	–	–
2	Control DNA of *C. gallinacea*	+	+	+	+	+	+	+	+	+	171	*C. gallinacea*
3	Control DNA of *C. psittaci*	–	–	–	–	–	–	–	–	–	–	–
4	Chlamydial field isolate from white Jacobin dove (*Columba livia*)	–	–	–	–	–	–	–	–	–	–	–
5	Chlamydial field isolate from fantail-trumpeter (Torkun) dove (*Columba livia*)	–	–	–	–	–	–	–	–	–	–	–
6	Chlamydial field isolate from shell parakeet (*Melopsittacus undulatus*)	–	–	–	–	–	–	–	–	–	–	–
7	Chlamydial field isolate from cockatiel (*Nymphicus hollandicus*)	–	–	–	–	–	–	–	–	–	–	–
8	Chlamydial field isolate from yellow-collared lovebird (*Agapornis personatus*)	–	–	–	–	–	–	–	–	–	–	–
9	Chlamydial field isolate from sparrow (*Passer* sp.)	–	–	–	–	–	–	–	–	–	–	–
10	Chlamydial field isolate from greylag goose (*Anser anser*)	–	–	–	–	–	–	–	–	–	–	–
11	Chlamydial field isolate from greater flamingo (*Phoenicopterus roseus*)	–	–	–	–	–	–	–	–	–	–	–
12	Chlamydial field isolate from silver pheasant (*Lophura nycthemera*)	–	–	–	–	–	–	–	–	–	–	–
13	Chlamydial field isolate from vulture (*Gyps* sp.)	–	–	–	–	–	–	–	–	–	–	–
14	Field isolate *Babesia canis* from dog	–	–	–	–	–	–	–	–	–	–	–
15	Field isolate *Babesia bovis* from heifer	–	–	–	–	–	–	–	–	–	–	–
16	Leptospira strain LSU	–	–	–	–	–	–	–	–	–	–	–
17	Leptospira strain 493 *Poland*	–	–	–	–	–	–	–	–	–	–	–
18	Leptospira strain *Hond Utrecht IV*	–	–	–	–	–	–	–	–	–	–	–

Table 3 — Testing results of the PCR test-system developed to indicate *Chlamydia psittaci* bacteria as to its analytical specificity and sensitivity

No.	Type of the sample studied	Study result										
		DNA amount in the reaction mixture									Band size	Agent species
		2 µl (20 µg/ml)			1 µl (20 µg/ml)			0.5 µl (20 µg/ml)				
		1 repeat	2 repeat	3 repeat	1 repeat	2 repeat	3 repeat	1 repeat	2 repeat	3 repeat		
1	Control DNA of *C. avium*	–	–	–	–	–	–	–	–	–	–	–
2	Control DNA of *C. gallinacea*	–	–	–	–	–	–	–	–	–	–	–
3	Control DNA of *C. psittaci*	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
4	Chlamydial field isolate from white Jacobin dove (*Columba livia*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
5	Chlamydial field isolate from fantail-trumpeter (Torkun) dove (*Columba livia*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
6	Chlamydial field isolate from shell parakeet (*Melopsittacus undulatus*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
7	Chlamydial field isolate from cockatiel (*Nymphicus hollandicus*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
8	Chlamydial field isolate from yellow-collared lovebird (*Agapornis personatus*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
9	Chlamydial field isolate from sparrow (*Passer* sp.)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
10	Chlamydial field isolate from greylag goose (*Anser anser*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
11	Chlamydial field isolate from greater flamingo (*Phoenicopterus roseus*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
12	Chlamydial field isolate from silver pheasant (*Lophura nycthemera*)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
13	Chlamydial field isolate from vulture (*Gyps* sp.)	+	+	+	+	+	+	+	+	+	208	*C. psittaci*
14	Field isolate *Babesia canis* from dog	–	–	–	–	–	–	–	–	–	–	–
15	Field isolate *Babesia bovis* from heifer	–	–	–	–	–	–	–	–	–	–	–
16	Leptospira strain LSU	–	–	–	–	–	–	–	–	–	–	–
17	Leptospira strain 493 *Poland*	–	–	–	–	–	–	–	–	–	–	–
18	Leptospira strain *Hond Utrecht IV*	–	–	–	–	–	–	–	–	–	–	–

Thus, the electrophoregram results indicate the adequacy and analytical specificity of the developed PCR test systems.

Conclusion. The developed PCR test systems, included oligonucleotide primers flanking different sized DNA fragments of the gene encoding MOMP of three *Chlamydia* species, permitted the detection and identification of *C. avium*, *C. gallinacea*, and *C. psittaci* DNA, which is pathogenic to different bird species.

Indication and differentiation of *Chlamydia* by species is provided by a visual assessment of the amplified fragments due to different band sizes at electrophoregrams in 2.0% agarose gel.

Testing of the developed PCR test systems for indicating and species differentiation of chlamydial infections agents in birds demonstrated their high sensitivity and analytical specificity.

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