Sensitivity of 5-6 day old chick embryos to some members of the chlamydia family

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Abstract. The goal of the work was to improve the diagnosis of chlamydia in animals and birds. The article presents data on experimental infection of 5-6-day and 7-9-day chicken embryos with certain types of chlamydia to study their sensitivity. Having analyzed the results obtained, it was shown that 5-6-day-old chicken embryos are the most sensitive and chlamydia are cultured in them from the first passage in higher titers compared to cultivation in 7-9-day-old chicken embryos.

1 Introduction

Chlamydia is a group of diseases caused by pathogens of the *Chlamydiaceae* family. According to the current classification, the family *Chlamydiaceae* includes one genus, *Chlamydia*, which includes seventeen species. Initially, a feature of the representatives of the *Chlamydiaceae* family was considered to be their species specificity: *C. trachomatis, C. pneumoniae* - the natural "host" is humans, *C. caviae* - guinea pigs, *C. muridarum* - mice, *C. psittaci, C. avium, C. ibidis, C. buteonis, C. gallinacean* - birds, *C. suis* - pigs, *C. felis* - cats, *C. abortus* - farm animals, C. *pecorum* - marsupials and farm animals, *C. serpentis, C. poikilothermis, C. corallus* - snakes, *C. sanzinia* – turtles [1-2].

Previously, it was believed that three types of chlamydia were dangerous to humans: *C. trachomatis*, *C. Pneumoniae*, *C. psittaci* [3]. The rest were pathogens for animals. But with the development of molecular genetic research methods, more and more information began to appear that chlamydia, which infects animals and birds, can cause a variety of clinical diseases when entering the human body [2]. Thus, *C. pecorum* and *C. abortus*, circulating among small and large livestock, affect people in direct contact with animals and cause bronchopneumonia, polyarthritis, and miscarriages in pregnant women [4].

The causative agent of a particularly dangerous disease, psittacosis, is *C. psittaci*. The disease in humans is characterized by damage to the lungs, sometimes the nervous system and parenchymal organs [5]. The main reservoir of this type of chlamydia is birds (parrots, pigeons, etc.). However, *C. psittaci* is also detected in mammals such as pigs, dogs, cats, cattle, small ruminants, etc. Cases of people becoming infected with psittacosis from various species of wild birds have been described. The main source of infection for humans are domestic birds: city pigeons (according to different sources, infection varies from 30 to

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80%), parrots, and canaries [5-6]. Parrots, especially budgerigars, are of greatest importance as a source of infection for humans and children. This circumstance is due not only to the popularity of keeping them at home, the smuggling method of their import into the country and insufficient control in pet stores, but also the highest pathogenicity of *C. psittaci* [7]. Improving the diagnosis of chlamydia in animals and birds today is an urgent task. Chicken embryos are the only universal living system for identifying all representatives of the chlamydia family. Our goal was to study the sensitivity of 5-6 day old chicken embryos to some representatives of the chlamydia family, to improve the diagnosis of the disease.

2 Materials and methods

The reference strains used in this work were *C. psittaci* “Progress”, C. abortus 252 1971-01-01, *C. trachomatis* “Burkhan”, *C. pneumonia* “A-74”, *C. recorum* 1975-01-01, as well as isolated on the basis of the Federal State Budgetary Institution "ARRIAH" two isolates from pigeons of *C. psittaci* and two isolates of *C. gallinacea* from chickens.

The studies were carried out in 5-9 day old chicken embryos (CE), free of specific pathogens (SPF). Infection was carried out into the yolk sac with 6 SPF EC for each strain and isolate. To do this, a hole was made in the shell with a punch above the center of the air chamber and a needle was inserted to a depth of 3.5–4 cm at an angle of 45° to the vertical axis in the direction opposite to the location of the embryo. Four SPF-CEs served as controls, which were injected with sterile physiological solution (0.85% NaCL, pH 7.2-7.4). The embryos were incubated at a temperature of 35-37 ºC and a relative humidity of 60 - 70% for 4-7 days. During the incubation process, embryos were ovoscoped 2 times a day. Dead embryos were stored in a refrigerator at a temperature of 4°C until dissection. At the end of the incubation period, in the absence of death, the embryos were cooled at a temperature of 4°C for 10 - 12 hours. The embryos were then dissected, the yolk sacs were removed, washed with sterile saline (pH 7.2–7.4), and impressions of the yolk sac walls were made on sterile glass slides. The prints were dried over a burner flame for 30 seconds and painted. After staining, they were examined in a light and fluorescent microscope at a magnification of 100 - 200 X.

For subsequent passage, the walls of the yolk sacs from each sample were separately combined into one sample. From the combined sample, a 10% suspension was made in sterile physiological solution (pH 7.2-7.4) and chicken embryos free of specific pathogens were infected. The second and third passages were carried out according to the procedure of the first passage.

After each passage, the biomaterial was checked for the presence of chlamydia using the PCR method: “Test system for detecting DNA of Chlamydia spp.” (“InterLabService”), followed by differentiation for C. psittaci, *C. Abortus, C. Trachomatis, C. Pneumonia, C. Recorum* test - with the “GenPak®DNA-Fluo PCR test” and “Amplisense® chlamydia trachomatis-fl” systems . For DNA extraction, a set of reagents for isolating total RNA/DNA from clinical material "RIBO-PREP" (InterLabService) was used.

Staining of preparations and cultivation of chlamydia on chicken embryos was carried out according to the “Guidelines for the laboratory diagnosis of chlamydial infections in animals dated June 30, 1999 No. 13-7-2/643”.

3 Results and Discussion

To improve the methodology for increasing sensitivity developed in and approved for work in veterinary laboratories of the Russian Federation (“Guidelines for the laboratory

diagnosis of chlamydial infections in animals dated June 30, 1999 No. 13-7-2/643") - isolation of chlamydia on developing chicken In embryos, an experimental infection of chicken embryos of different ages (5-6 days of age) free from specific pathogens was carried out with some types of chlamydia, including standard strains of C. psittaci "Progress", *C. abortus* 252 1971-01-01, *C. trachomatis* "Burkhan", *C. pneumonia* "A-74", *C. recorum* 1975-01-01, as well as two isolates from pigeons *C. psittaci* and two isolates of *C. gallinacea* from chickens isolated on the basis of the Federal State Budgetary Institution "ARRIAH" comparison of their sensitivity. The results of studies studying the sensitivity criterion of chicken embryos to some representatives of the chlamydia family are presented in Tables 1, 2.

Table 1. Detection of chlamydia in chicken embryos 5-6 days old, free from specific pathogens, using light microscopy in yolk sac cells.

No	Pathogen name	Detection of chlamydia by light microscopy in yolk sac cells			Identification results in RT-PCR after each passage		
		First passage	Second passage	Third passage	First passage	Second passage	Third passage
1	*C. pecorum*	+	+	+	+	+	+
2	*C. abortus*	+	+	+	+	+	+
3	*C. psittaci*	+	+	+	+	+	+
4	Isolate No. 1 (*C. Psittaci*)	+	+	+	+	+	+
5	Isolate No. 2 (*C. Psittaci*)	+	+	+	+	+	+
6	Isolate No. 3 (*C. gallinacea*)	+	+	+	+	+	+
7	Isolate No.4 (*C. gallinacea*)	+	+	+	+	+	+
8	*C. trachomatis*	+	+	+	+	+	+
9	*C. pneumoniae*	+	+	+	+	+	+

"+" - detection of chlamydia

As can be seen from Table 1, all types of chlamydia were cultured in yolk sac cells starting from the first passage. Each passage was monitored by light microscopy and confirmed by RT-PCR.

In parallel, 7–9 day old chicken embryos free from specific pathogens were infected to study and compare their sensitivity to some representatives of the genus Chlamydia. The results are presented in Table 2.

Table 2. Detection of chlamydia in chicken embryos 7 - 9 days old using light microscopy in yolk sac cells.

No	Pathogen name	Detection of chlamydia by light microscopy in yolk sac cells			Identification results in RT-PCR after each passage		
		First passage	Second passage	Third passage	First passage	Second passage	Third passage
1	*C. pecorum*	-	+	+	+	+	+
2	*C. abortus*	-	+	+	+	+	+
3	*C. psittaci*	±	+	+	±	+	+
4	Isolate No. 1 (*C. Psittaci*)	+	+	+	+	+	+
5	Isolate No. 2 (*C. Psittaci*)	+	+	+	+	+	+
6	Isolate No. 3 (*C. gallinacea*)	+	+	+	+	+	+
7	Isolate No. 4 (*C. gallinacea*)	±	+	+	±	+	+
8	*C. trachomatis*	-	+	+	±	+	+
9	*C. pneumoniae*	-	+	+	±	+	+

"+" - detection of chlamydia; "-" - chlamydia was not detected; "±" is a dubious result.

As can be seen from Table 2, when carrying out three consecutive passages, chicken embryos of 7-9 days of age turned out to be less sensitive at the first passage to some types of chlamydia, including isolated isolates, and in particular to *C. pecorum, C. abortus, C. psittaci*, isolate No. 4 (*C. gallinacea*), *C. trachomatis*, *C. pneumonia*. According to the results of light microscopy, all chlamydia were detected only at the second and third passages.

During microscopy of preparations prepared according to the "Guidelines for laboratory diagnosis of chlamydial infections in animals dated June 30, 1999 No. 13-7-2/643," changes were observed in the form of characteristic cytoplasmic inclusions. Figure 1 shows a specimen where multiple round-shaped intracytoplasmic inclusions were noted. Figure 2 shows a preparation of an imprint of the walls of the yolk sac of chick embryos that were injected with sterile saline solution.

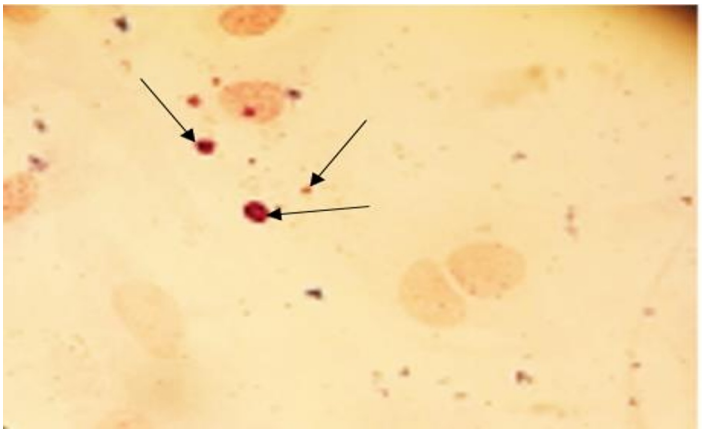

Fig. 1. Yolk sac cells infected with *C. pneumonia* (200X magnification).

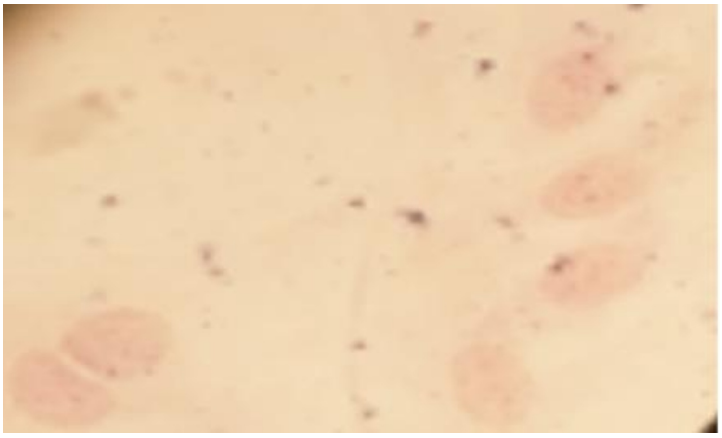

Fig. 2. Yolk sac cells (control) (200X magnification).

Next, experiments were carried out on the cultivation of chlamydia in the same chicken embryos to determine the infectious titer. The results are presented in Tables 3 and 4.

Table 3. Determination of the infectious activity of chlamydia in chicken embryos 5 - 6 days old.

No	Pathogen name	Average infectious titer of the pathogen $TCD_{50/ml}$	Detection of chlamydia by light microscopy in yolk sac cells			Identification results in RT-PCR after each passage
			First passage	Second passage	Third passage	
1	*C. pecorum*	4.35 lg $TCD_{50/ml}$	+	+	+	+
2	*C. abortus*	6.45 lg $TCD_{50/ml}$	+	+	+	+
3	*C. psittaci*	6.87 lg $TCD_{50/ml}$	+	+	+	+
4	Isolate No. 1 (*C. Psittaci*)	5.75 lg $TCD_{50/ml}$	+	+	+	+
5	Isolate No. 2 (*C. Psittaci*)	5.25 lg $TCD_{50/ml}$	+	+	+	+
6	Isolate No. 3 (*C. gallinacea*)	4.0 lg $TCD_{50/ml}$	+	+	+	+
7	Isolate No. 4 (*C. gallinacea*)	4.0 lg $TCD_{50/ml}$	+	+	+	+
8	*C. trachomatis*	5.75 lg $TCD_{50/ml}$	+	+	+	+
9	*C. pneumoniae*	6.45 lg $TCD_{50/ml}$	+	+	+	+

"+" - detection of chlamydia

As can be seen from Table 3, during three successive passages in chicken embryos 5–6 days old, various types of chlamydia were cultivated in infectious titers from 4.0 to 6.87 lg $TCD_{50/0,1ml}$. Over the course of three consecutive passages, the infectious titer did not decrease.

Table 4. Determination of the infectious activity of chlamydia in chicken embryos 7 - 9 days old.

No	Pathogen name	Average infectious titer of the pathogen $TCD_{50/ml}$	Detection of chlamydia by light microscopy in yolk sac cells			Identification results in RT-PCR after each passage
			First passage	Second passage	Third passage	
1	*C. pecorum*	3.75 lg $TCD_{50/ml}$	-	+	+	+
2	*C. abortus*	5.00 lg $TCD_{50/ml}$	-	+	+	+
3	*C. psittaci*	4.00 lg $TCD_{50/ml}$	±	+	+	+
4	Isolate No. 1 (*C. Psittaci*)	3.75 lg $TCD_{50/ml}$	+	+	+	+
5	Isolate No. 2 (*C. Psittaci*)	5.0 lg $TCD_{50/ml}$	+	+	+	+
6	Isolate No. 3 (*C. gallinacea*)	3.25 lg $TCD_{50/ml}$	+	+	+	+
7	Isolate No. 4 (*C. gallinacea*)	3.0 lg $TCD_{50/ml}$	±	+	+	+
8	*C. trachomatis*	4.00 lg $TCD_{50/ml}$	-	+	+	+
9	*C. pneumoniae*	4.00 lg $TCD_{50/ml}$	-	+	+	+

"+" - detection of chlamydia; "-" - chlamydia was not detected; "±" is a dubious result.

As can be seen from Table 3, during three consecutive passages in chicken embryos 7 - 9 days old, chlamydia were cultivated in lower infectious titers than when cultivated in chicken embryos 5 - 6 days old and ranged from 3.0 to 5. 0 lg $TCD_{50/0.1\ ml}$, which indicates the comparability of the sensitivity of the three living systems. Over the course of three consecutive passages, the infectious titer did not decrease.

4 Conclusion

- All studied types of chlamydia were cultured in the yolk sac cells of 5-6 day old chick embryos, starting from the first passage. Each passage was monitored by light microscopy and confirmed by RT-PCR.
- When carrying out three consecutive passages, chicken embryos of 7-9 days of age turned out to be less sensitive to some types of chlamydia at the first passage, including isolated isolates, including *C. pecorum*, *C. abortus*, *C. psittaci*, isolate No. 4 (*C.*

gallinacea), *C. trachomatis, C. pneumonia*. According to the results of light microscopy, all chlamydia were detected only at the second and third passages.

- The infectious titer of chlamydia obtained by cultivation in chicken embryos 5 - 6 days old was almost one logarithm higher than in chicken embryos 7 - 9 days old.
- These experiments make it possible to improve the method of isolating chlamydia of animals and birds in developing chicken embryos.

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