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UDC 576.895.132 Coccidial fauna of pheasants (*Phasianus colchicus colchicus*)

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Copyright: © The Authors (2023). Published by Herzen State Pedagogical University of Russia. Open access under CC BY-NC License 4.0. Abstract. Coprological examination of pheasants (Phasianus colchicus colchicus) in different age groups kept in cages in Absheron (Azerbaijan) found Eimeria colchicine, E. Phasiani and E. duodenalis species of genus Eimeria and Isospora spp. of genus Isospora. Two species of genus Eimeria (E. colchici and E. duodenalis) were found in faecal samples of 1-30 day old pheasants, three species of genus Eimeria (E.colchici, E.duodenalis and E.phasiani) - in 31-120 day old birds, one species of genus Isospora (Isospora sp.) and three species of genus Eimeria (E.colchici, E.duodenalis and E.phasiani) - in pheasants over 120 days of age. Generally, three species of genus Eimeria and one genus Isospora were found in pheasants in Azerbaijan. In about 60 pheasants studied, eimeria oocysts were found in 54.5% of 11 pheasants aged 1-30 days, in 66.67% of 18 pheasants aged 31-120 days, and in 82.3% of 41 pheasants over 120 days of age. Infection extensiveness of Isospora spp. among pheasants aged 31-60 days was 5.56% and Isospora sp. was found in 1.67% of the birds studied. No infection with a single species was detected; the invasion found was in the form of a mixed invasion. Among infected birds, Eimeria colchici occured most frequently (86.67%), followed by Eimeria duodenalis (77.33%) and Eimeria phasiani (33.33%). The overall infection extensiveness of pheasants with eimeria was very high - 86.67%.

Keywords: oosyst, *Eimeria*, *Eimeria* phasiani, *E. duodenalis*, *E. colchici*, *Phasianus* colchicus colchicus, Isospora sp.

Кокцидиофауна фазанов (*Phasianus colchicus colchicus*)

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Права: © Авторы (2023). Опубликовано Российским государственным педагогическим университетом им. А. И. Герцена. Открытый доступ на условиях лицензии СС ВУ-NС 4.0. Аннотация. На Абшероне (Азербайджан) в результате копрологического исследования фазанов (Phasianus colchicus colchicus) разных возрастных групп, содержащихся в клетках, были обнаружены виды рода Eimeria (Eimeria colchicine, E. phasiani, E. duodenalis) и рода Isospora (Isospora sp.). Два вида рода Eimeria (E. colchici и E. duodenalis) найдены в фекалиях 1–30-дневных фазанов, 3 вида (E.colchici, E.duodenalis и E.phasiani) - у 31–120-дневных птиц, а также 1 вид рода Isospora (Isospora sp.) и 3 вида рода Eimeria (E.colchici, E.duodenalis и E.phasiani) — у фазанов старше 120-дневного возраста. В целом у фазанов в Азербайджане было выявлено 3 вида рода *Eimeria* и 1 вид рода *Isospora*. Обследовано около 60 фазанов, ооцисты эймерий обнаружены у 11 фазанов в возрасте 1–30 дней (54,5%), у 18 фазанов в возрасте 31–120 дней (66,67%) и у 41 фазанов старше 120 дней (82,3%). Экстенсивность заражения Isospora spp. среди фазанов в возрасте 31-60 дней составила 5,56%, Isospora spp. обнаружен у 1,67% обследованных птиц. Заражение каким-либо одним видом не выявлено, обнаружена инвазия в форме смешанной инвазии. По частоте встречаемости среди зараженных птиц на первом месте оказались Eimeria colchici (86,67%), на втором — Eimeria duodenalis (77,33%), на третьем — Eimeria phasiani (33,33%). Общая экстенсивность заражения фазанов эймериями была очень высокой — 86,67%.

Ключевые слова: ооциста, Eimeria, Eimeria phasiani, E.duodenalis, E.colchici, Phasianus colchicus colchicus, İsospora

Introduction

According to the modern classification, coccidia belong to apicomplexan class Conoidasida (Adl et al. 2012; Perkins et al. 2000). The main characteristic feature of the Conoidasida class is presence of an ultrastructural apical complex at the anterior pole used for easy cell penetration. According to this accepted classification, coccidia (class Conoidasida, subclass Coccidiasina Leuckart, 1879) are divided into four groups: Agamococcidiorida Levine, 1979, Ixorheorida Levine, 1984, Protococcidiorida Kheisin, 1956 and Eucoccidiorida Léger & Dubos, 1910.

Among these groups, the Eucoccidiorida group is the most widely studied on a global scale and represented by more than 2,000 species (Duzsynski et al. 2015). Most of the species included in the Eucoccidiorida group are parasitic organisms and causative agents of dangerous diseases such as eimeriosis, toxoplasmosis, cryptosporidiosis and sarcocystis. Representatives of genera Eimeria and Isospora of coccidia are widespread all over the world, including Azerbaijan (Gaibova, Iskenderova 2018). Parasites belonging to coccidia are eukaryotic protists of Apicomplexa Levine, 1970 (= Sporozoa Leuckart, 1879) phylum, infecting large groups of vertebrate and invertebrate animals as well as humans. One of the most common pathologies common in livestock and poultry farms is coccidiosis. The causative agents of this disease are very widespread, they have no intermediate hosts in their life cycle, their lives are very short and they have high reproduction rates. As a result of coccidiosis, a disease of the digestive system of birds, the metabolism of proteins, fats and carbohydrates in the host's body is disrupted, as well as the activity of enzymes that catalyze these processes. Therefore, coccidia can cause frequent fatalities among juvenile animals, growth and stagnation among them and decline in the quality of products obtained from them and, consequently, lead to significant economic losses (Dallouil, Lillehoj 2006; Györke et al. 2016). The treatment and prevention costs of eimeriosis in the world amount to more than two billion euros annually (Dallouil, Lillehoj 2006).

Eimeriosis in pheasants is caused by Eimeria dispersa, E.phasiani, E.langeroni, E.pacifica, E.megalostomata, E.gennaeuscus, E.duodenalis, E.colchici, E.picta and E.tetartooimia (Lilić et al. 2013).

The aim of the research is to determine the coccidial fauna of pheasants (*Phasianus colchicus colchicus*) artificially propagated and kept in cages in Absheron and to study the extensiveness and intensity of the invasion.

Materials and methods

The research material comprised faecal samples taken from 60 pheasants (*Phasianus colchicus colchicus*) of different ages raised in backyard farms in Absheron (Azerbaijan Republic) in 2019. Only fresh faecal samples of pheasants (Phasianus colchicus colchicus) contained in cells were used in the research.

Analysis of faecal samples taken from pheasants was carried out in the Protozoology Laboratory of Zoology Institute. Faecal samples were grouped according to the age of the pheasants. Faecal samples were taken from 11 pheasants aged 1–30 days, 18 pheasants aged 31–120 and 41 pheasants over 120 days old. Faecal samples collected from pheasants were brought to the laboratory after conservation in 2.5% $K_2Cr_2O_7$ solution and analyzed.

The flotation method in saturated salt solution was used to separate oocysts from fecal samples.

To determine the invasion extensiveness and identify oocysts, the solution was centrifuged for five minutes at 3000 rpm to clean the canned faecal samples from potassium dichromate solution. After that 10 ml of distilled water was added to the precipitate and centrifuged again, and the supernatant liquid was discarded.

To separate the oocysts, 2 g were taken from precipitation, mixed with 10 ml of saturated saline solution (NaCl) and then centrifuged at 1500 rpm for 3–5 minutes. Then the oocysts collected on the solution were placed on glassware and examined under a microscope (Axio Sckope A1) magnifier (objective ×15, ocular ×40). $IE = \frac{n \cdot 100}{N}$ formula was

used in the calculation of invasion extensiveness (IE). Here: IE is invasion extensiveness; n is the number of infected birds; and N is the total number of checked individuals.

To determine the invasion intensity, the oocysts in the smears prepared separately from the faecal samples taken from each bird were counted under a microscope. The invasion intensity was determined by the number of oocysts found in one sample. Identifying features such as the morphology (the color of oocysts, the cover shape, thickness and the number of layers) of sporulated and unsporulated oocysts in the identification of eimeria, the oocysts size and form index (ratio of length to width), presence or absence of micropyle polar granules and oocyst residual body, the number and the shape of sporocysts formed as a result of sporulation were taken as a basis. The type of parasites found was determined through determinants (Pellerdy 1974; Krylov 1996).

The oocysts dimensions were measured using AxioCam Erc 5s photo camera software (AxioVision LE). After measuring at least 50 samples of each oocyst, statistical processing of the results was performed using Statistica StatSoft v.12 and Ms Excel 2016 programs.

Results

Although it is common for wild pheasants to be infected with species of *Eimeria* in na-

ture, mixed invasion is also common among pheasants kept under artificial conditions. The clinical view and lethality of the disease depend on the number of oocysts received in different ways, i.e. the dose of infection.

Eimeria colchici, Eimeria phasiani and Eimeria duodenalis of genus Eimeria were found in the pheasants studied (Fig. 1). Totally, about 60 pheasants were examined, eimeria were found in 54.5% (6/11) of 11 pheasants aged 1-30 days, 66.67% (12/18) of 18 pheasants aged 31-120 days and 82.3% (34/41) of 41 pheasants over 120 days of age. Among infected birds, Eimeria colchici occured most frequently (86.67%), followed by Eimeria duodenalis (77.33%) and Eimeria phasiani (33.33%). Except for the pheasants aged 1-30 days, E.colchici, E.duodenalis and E.phasiani were found in pheasants of all other age groups. Eimeria colchici was not found in 30-day-old pheasants. Although no infection with a single species was found, mixed invasion was observed in all cases. The overall infection extensiveness of pheasants with eimeria was very high and amounted to 86.67% (Table 1).

Infection of pheasants with *Isospora* spp. was as follows: *Isospora* oocysts were not found in the animals aged 1–30 and over 120 days. *Isospora* spp. oocysts were found in only 5.56% (1/18) of pheasants aged 31–120 days, at that time the invasion intensity was very low, only one sample had 2–3 oocysts. Deaths

Table 1

Таблица 1

umber	E. colc	hici	E. duod	enalis	E. phas	siani	Isospora	spp.	
umber				E. duodenalis		E. phasiani		Isospora spp.	
	IE	II	IE	II	IE	II	IE	II	
11	6/11 (54.55%)	5-8	6/11 (54.55%)	10-20	0/11 (0.00%)	_	0/11 (0.00%)		
18	12/18 (66.67%)	10-20	12/18 (66.67%)	12–18	5/18 (27.78%)	6–8	1/18 (5.56%)	2-3	
41	34/41 (82.93%)	3–5	23/41 (56.10%)	7–10	15/41 (36.59%)	5–6	0/41 (0.00%)		
60	52/60 (86.67%)	18–33	41/60 (77.33%)	27-48	20/60 (33.33%)	11–14	1/60 (1.67%)	2-3	
	11 18 41	$ \begin{array}{c} $	$ \begin{array}{c cccc} & & & & & \\ 11 & 6/11 \\ (54.55\%) & 5-8 \\ \hline 18 & 12/18 \\ (66.67\%) & 10-20 \\ \hline 41 & 34/41 \\ (82.93\%) & 3-5 \\ \hline 60 & 52/60 \\ \hline 18-33 \\ \end{array} $	$ \begin{array}{c ccccc} & & & & & & & \\ \hline 11 & 6/11 \\ (54.55\%) & 5-8 & 6/11 \\ (54.55\%) \\ \hline 18 & 12/18 \\ (66.67\%) & 10-20 & 12/18 \\ (66.67\%) \\ \hline 41 & 34/41 \\ (82.93\%) & 3-5 & 23/41 \\ (56.10\%) \\ \hline 60 & 52/60 & 18-33 & 41/60 \\ \end{array} $	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	

Оооцисты кокцидий, обнаруженные у фазанов

Coccidia oocysts found in pheasants

IE — invasion extensiveness; II — invasion intensity

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among birds were not observed. Generally, infection with *Isospora* spp. was detected in 1.67% (1/60) of the birds studied. The infection intensity of pheasants with eimeria was as follows: *E.colchici* — 18–33 oocysts, *E.duodenalis* — 24–48 oocysts, *E.phasiani* — 11–14 oocysts (Table 1).

Dimensions of oocysts separated from faecal samples of juvenile birds were smaller than the dimensions of oocysts separated from older birds. Thus, two species (*E.colchici* and *E.duodenalis*) of genus *Eimeria* in faecal samples of pheasants aged 1–30 days, three species (*E.colchici*, *E.duodenalis* and *E.phasiani*) of genus *Eimeria* in birds aged 31–120 days, one species (*Isospora* sp.) of genus *Isospora* and three species (*E.colchici*, *E.duodenalis* and *E.phasiani*) of genus *Eimeria* were found in pheasants over 120 days of age. Generally, three species of genus *Eimeria* and one species of *Isospora* were found in pheasants (*Phasianus colchicus colchicus*) in Azerbaijan.

Eimeria colchici Norton 1967 (Syn: *Eimeria colchica* Gottschalk 1972)

The oocysts are oblong, ellipse-shaped, more rounded than on the other side, colorless. Hard-to-notice micropile and polar granules. Sporocysts have a well-observed Stieda body. The maximum length of *E. colchici* oocysts found in pheasants (*Ph.colchicus colchicus*) is 33.50 μ m; the minimum length — 17.80 μ m; the maximum width is 22.0 μ m; the minimum width — 13.0 μ m; on average the size is 25.84 ± 5.71 × 16.68 ± 3.19 μ m. The form index varies between 1.37–1.60; on average it is 1.55 ± 0.07 (Table 2).

Eimeria duodenalis Norton, 1967

Oocysts are round or short oval-shaped. The cover wall is smooth, $1.0-1.3 \mu m$ thick, the color of the inner layer varies from reddish-brown to dark brown, the outer layer is brownish-yellow. Micropile abscent. The maximum length of oocysts found in pheasants (*Ph.colchicus colchicus*) is 22.90 μm , the minimum length — 20.00 μm , the maximum width is 19.20 μm , the minimum width — 17.40 μm ; on average the size is $20.83 \pm 1.26 \times 18.07 \pm 0.72 \ \mu\text{m}$. The form index varies between 1.10–1.20; on average it is 1.15 ± 0.03 (Table 2).

The inside of recently excreted oocysts is completely filled with large granular cytoplasm. As the oocytes begin to spore, they take the shape of a balloon; at this time, the cytoplasm separates from the oocyst wall and accumulates in the centre. The sporulated oocysts also form four oval and in some cases round sporocysts. The Stieda body is present. The dimensions of spores are $10.8-11.3 \times 5.4-$ 6.7, on average — $11.0 \times 5.9 \mu$ m. Two commashaped sporozoites develop in each sporocyst. The dimensions of spores are $4.5-5.4 \times 3.8 4.6 \mu$ m, on average — $4.8 \times 4.2 \mu$ m. Although oocysts have a residual body, sporocysts do not have a residual body.

Eimeria phasiani Tyzzer, 1929 (Syn. *Eimeria phasiana*, lapsus in Tyzzer, 1929)

Oocysts have elliptical or stretched elliptical shape. The dimensions are 23.5– $32.40 \times 16.19-18.99 \mu m$, on average — $28.41 \pm 2.60 \times 17.80 \pm 1.60 \mu m$. The oocyst wall is smooth, double-contoured, yellowgreen. The form index varies between 1.45– 1.71, on average — 1.60 ± 0.07 . No micropile and no residual body.

In recently excreted oocysts, the granular protoplasmic mass extends to both poles and takes a broad oval shape. Sporulated oocysts contain one to two polar granules. Micropile abscent. In sporulated oocysts, four stretched sporocysts with elliptical shape are formed. The maximum size of spores is $16.2 \times 8.1 \mu$ m, the minimum size is $11.1 \times 5.7 \mu$ m, and the average size is $13.1 \times 5.9 \mu$ m. There is the Stieda body at the tip of the sharp point. Two comma-shaped sporozoites are observed in each spore. The dimensions are $3.5-7.9 \times 2.7-5.4$, on average $-5.9 \times 4.0 \mu$ m. A small grain-shaped residue is noted only in spores.

Isospora sp. oocysts found in the faecal samples are ellipse- or ovoid-shaped. The wall is two-layered. Micropile abscent. The dimensions of oocysts are 29.0– $32.89 \times 20.85-27.11 \mu m$, on average —



Fig. 1. Coccidial fauna of pheasants (*Phasianus colchicus colchicus*): A — Eimeria colchici; B — Eimeria duodenalis; C — Eimeria phasiani; D — İsospora sp.

Рис. 1. Кокцидиальная фауна фазанов (*Phasianus colchicus colchicus*): *A — Eimeria colchici; B — Eimeria duodenalis; C — Eimeria phasiani; D — İsospora sp.*

 $31.03 \pm 1.90 \times 23.99 \pm 3.19$ µm, and form index is 1.29 (Table 2).

Residual bodies are found in oocysts and sporocysts. The results of the measurements show that the shape of oocysts found in the isolates is stable while their dimensions (length and width) differ slightly.

Discussion

Coccidiosis caused by Eimeria can result in a large number of deaths and significantly reduce productivity of pheasants raised in artificial conditions. Coccidiosis still remains one of the dangerous diseases of animals. In natural conditions pheasants are rarely infected with only one of the Eimeria species. Infection usually occurs as a mixed infestation. Clinical signs of the disease depend on the number of oocysts. Regular contamination of the environment by sick birds with oocysts leads to infection of healthy birds.

Currently 10 species of genus Eimeria are known to parazitize pheasants (Peilerdy 1974). Pheasants are reported to be infected with Eimeria colchici, Eimeria dispersa, Eimeria duodenalis, Eimeria gennaeuscus, Eimeria langeroni, Eimeria megalostomata, Eimeria pacifica, Eimeria picta, Eimeria phasiani and Eimeria tetartooimia of genus Eimeria (Norton 1976; 1986; McQuistion 1987; Vanparijs et al. 1990; Lilić et al. 2013). Among these species, Eimeria colchici is a pathogenic and common species in pheasant farms and distinguished by high reproduction rates (Norton 1976). Norton reports the discovery of E. duodenalis and E. colchici species in England (Norton 1967).

Golemansky reports five species E.duodenalis Norton, 1967, E.megalostomata Ormsbee, 1939, E. pacifica Ormsbee, 1939, Eimeria colchici Norton, 1967 — of genus Eimeria parasitizing pheasants in Bulgaria (Golemansky 2017). Although Musaev and Aliveva (Musaev, Alieva 1983) reported on the discovery of E. phasiani, E. pacifica, E. duodenalis and Isospora sp. in pheasants in Azerbaijan, they provided no information about their size. Musaev, Gaibova and others state that one species of Eimeria, E. phasiani, was found in pheasants in Azerbaijan (Musaev et al. 1998). It was reported that in the United States, E. colchici and E. phasiani species of Coccidia cause eimeriosis among pheasants (Gerhold et al. 2010).

Comparison of the conclusions of their research with the conclusions of similar studies conducted around the world shows that coccidia are widespread among pheasants. We found *Eimeria colchici, Eimeria phasiani* and *Eimeria duodenalis* of genus *Eimeria* in the pheasants studied in Absheron. The most frequently occurring species was *Eimeria colchici* (86.67%).

E.phasiani and *E. duodenalis* species are found in Germany. Both species were found in 41% of the pheasants studied. Reports reveal that *E.phasiani, E. duodenalis,* and *E. colchici* species were found in England (Williams 1978) and USA (Fuller et al. 2008). Fisher notes that the infection extensiveness of pheasants in Iowa farms with *E.phasiani* species is 75–94% (Fisher 1973). In Illinois, the infection extensiveness with *E.duodenalis* species is 57.5%, and the infection extensive-

Table 2

Dimensions of oocysts recorded in spontaneously infected pheasants

Таблица 2

Coccidia		Dimensions o	f oocysts, µm	Form index		
		min–max	M ± SD	min–max	M ± SD	
E. phasiani	length	23.5-32.40	28.41 ± 2.60		1.60 ± 0.07	
	width	16.19–18.99	17.80 ± 1.60	1.45–1.71	1.00 ± 0.07	
E. colchici	length	17.80-33.50	25.84 ±5.71	1.37-1.60	1.55 ± 0.07	
	width	13.00-22.00	16.68 ± 3.19			
E. duodenalis	length	20.00-22.90	20.83 ± 1.26	1.10–1.20	1 15 + 0.02	
	width	17.40-19.20	18.07 ± 0.72		1.15 ± 0.03	
<i>Isospora</i> sp.	length	29.00-32.89	31.03 ± 1.90	1 10 1 00	1.20 + 0.00	
	width	20.85-27.11	23.99 ± 3.19	1.10-1.90	1.29 ± 0.09	

Сведения о размерах ооцист у спонтанно зараженных фазанов

ness with *E.phasiani* species is 8.8% (McQuistion 1987).

A comparative study of the infection of pheasants with different species of Eimeria and Isospora depending on their age shows that E.colchici and E.duodenalis infection prevailed in 1-30-day-old chickens and amounted to 54.55% in both cases. The intensity of E. duodenalis infection in birds was two times higher than that of E. colchici. Out of 18 pheasants aged 31-120 days, oocysts of E. colchci and E. duodenialis were detected in 12 individuals (66.67% in both cases), E. phasiani — in five (27.78%), and Isospora spp. — in one (5.56%). *Isospora* oocysts were not found in any of the samples taken from 41 pheasants older than 120 days, E. colchci were found in 34 (82.93%), E. duodenialis - in 23 (56.10%) and *E. phasiani* — in 15 (36.59%).

Thus, the comparison of the conclusions of our research with the results of the similar studies shows that the infection extensiveness of pheasants with *E. colchici* and *E. duodenalis* species is very high in Azerbaijan. It is 86.67% and 77.33%, respectively, while the infection extensiveness with *İsospora* species is very low — 1.67%.

In order to prevent and treat coccidiosis, protection measures should be based on regular parasitological control of pheasants in accordance with modern zoohygiene requirements.

Conclusion

As a result of the coprological examination of pheasants (*Phasianus colchicus colchicus*) kept in cages in Absheron, three species (*Eimeria colchici, Eimeria phasiani, Eimeria duodenalis*) of genus *Eimeria* and one species (*Isospora* sp.) of genus *Isospora* were found to be parasitic.

Among infected birds, *Eimeria colchici* occured most frequently (86.67%), followed by *Eimeria duodenalis* (77.33%) and *Eimeria phasiani* (33.33%). The overall infection extensiveness of pheasants with eimeria was very high and amounted to 86.67%. *Isospora* sp. oocysts were found in 1.67% of the pheasants examined.

The results of the measurements show that the shape of oocysts found in the isolates is stable and their dimensions (length and width) differ slightly.

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