



Influence of temperature on sporulation of *Eimeria arloingi* and *Eimeria perforans* oocysts

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Eimeriosis of farm animals is one of the most widespread parasitic diseases in the world. In the conditions of the steppe zone of Ukraine *Eimeria perforans* is more common in rabbits and *E. arloingi* in sheep and goats. Study of factors which influence the development of these protists on the soil surface is one of the major challenges for veterinarians working for large livestock companies and fighting against eimeriosis. Environmental temperature is able to change the speed of sporulation of oocysts *Eimeria*. Five values of temperature (15, 20, 25, 30, and 35 °C) were used in a laboratory experiment *in vitro*. At 15 °C, the process of sporulation of *E. arloingi* and *E. perforans* in 100% of cases ended on the eighth day. With increase in temperature to 35 °C, the duration of sporulation decreased to three days. When the temperature was 30 °C the completion of sporulation was observed in more than 50% of the oocysts of *E. arloingi* on the second day, and for *E. perforans* on the third day. High-speed sporulation at elevated temperatures under conditions of global warming can result in the increasing spread of eimeriosis among wild and domestic animals.

Keywords: sporulation; oocysts; eimeriosis; oocysts; sporozoites; sporocysts; sheep and goats; rabbits.

Introduction

The genus *Eimeria* belongs to the protists of the Apicomplexa type and includes more than 1,700 species, they store their genetic information in the chromosomes contained in the nucleus. They feed mainly by pinocytosis (absorption of nutrients from the extracellular space by invagination of the membrane) or phagocytosis (surrounding solid particles by the cytoplasmic membrane and introducing them into the cell), and metabolic products are excreted by diffusion through the cell membrane.

Most often, these protists parasitize in the intestinal epithelium. Their life cycle is closely related to the physiological characteristics of vertebrates – their hosts. The life cycle of *Eimeria* includes three types of reproduction: sporogony, meronia and gametogony. It is the elucidation of the peculiarities of these methods of reproduction that helps to develop various methods of controlling the eimeria of wild and domestic vertebrates (Taylor et al., 2007).

Eimeriosis is usually an acute invasion of domestic and wild mammals, birds, which develops against the background of reduced immune protection of animals, is characterized by increased susceptibility to infectious and invasive diseases, and is complicated by pathogenic microorganisms. Clinical signs include diarrhea (sometimes bloody), fever, loss of appetite, weight loss, exhaustion, and, in extreme cases, death. However, often this parasitosis has a subclinical course. Eimeriosis often causes enzootic diseases in rabbits, chickens, ducks, geese, turkeys, calves, lambs, goats, and pigs, causing significant economic damage to livestock, especially through deaths. And some species of *Eimeria* can cause the death of about 80–98% of young animals, other species – lag in growth and development, deteriorating quality of livestock products, reduced productivity, susceptibility of animals to secondary bacterial and viral infections. Additional economic losses also arise from the effort taken to care for and treat infected animals (Müller & Hemphill, 2013; Fatoba & Adeleke, 2018).

In general, for most farm animal species, the level of infection is high and the incidence of clinical manifestations of the disease is low; many high-risk animals may show clinical signs of eimeriosis. Most animals become infected with eimeriosis of varying severity at the age of 1–12 months. Animals over one year of age are usually resistant to these parasites, clinical manifestations are usually absent. Clinically healthy mature animals can be a source of infection for young, animals susceptible to *Eimeria*. Unsanitary conditions contribute to the growth of infection of farm animals. The disease occurs as a result of swallowing a large number of sporulated oocysts. The stress of weaning and transporting animals can provoke disease (Almeida et al., 2011; Andrushko & Egorov, 2015; Tadesse & Feyissa, 2016; Ekawasti et al., 2019).

Many researchers emphasize that mammals and birds are mostly sick in warm and humid periods of the year (depending on the climatic zone, these are different periods of the year). This is due to the fact that high humidity and heat create favourable conditions for the maturation and preservation in the environment of oocysts of *Eimeria*. However, seasonal fluctuations in the infestation of mammals and birds are clearly traced in farms where animals are kept in violation of breeding technology, in unsatisfactory veterinary and sanitary and zoohygienic conditions, with poor feeding (Ruiz et al., 2006; Sazmand et al., 2012; Wondimu et al., 2019).

In Ukraine, the level of eimeriosis in animals depends on many factors, including natural and climatic zones (Table 1). Veterinary support of farms is an integral part of the technological process. World veterinary science has developed and recommends the use of chemicals throughout the breeding period to prevent eimeriosis (Fasil, 2019; Franchuk-Kryva, 2019; Kachanova & Pavlova, 2020; Yakovleva et al., 2021).

The highest intensity of invasion among the rabbit population was recorded in September (on average 800 oocysts/g of feces), among small ruminants, the peak was in February (on average, in goats, 1675, in sheep, 400 oocysts/g of feces). The lowest rates of invasion intensity among

rabbits were observed in June (on average 460 oocysts/g of feces), and among small ruminants – in April (on average in goats, 825, in sheep, 62 oocysts/g of feces).

However, the system of anti-*Eimeria* measures on a number of Ukrainian farms continues to be ineffective. At present, the peculiarities of the epizootology and species composition of the causative agents of eimeriosis are not sufficiently studied, which is a barrier to the development of an effective system for combating *Eimeria*. The distribution range of

different species of parasites of this genus remains insufficiently studied. New livestock farms of various forms of ownership have been created and continue to be created throughout Ukraine, and studies of effective measures to control eimeriosis remain relevant (Girkovyj, 2012; Bogach et al., 2015; Korjachkov, 2015; Franchuk, 2015). In this regard, it is important to study the duration of sporulation of oocysts of different species of *Eimeria* – parasites of birds and mammals to develop and improve a system of scientifically sound measures to control them.

Table 1
The spread of eimeriosis of farm animals in Ukraine

Natural climatic zone	Region	Types of farms	Species	Extensiveness of invasion, %	Intensity of invasion	Reference
Polissia (Forest zone)	Zhytomyr	rabbits (2–6 months)	<i>E. stiedae</i> , <i>E. magna</i> , <i>E. perforans</i>	57.0–59.0	157–165 parasites in 3 drops of physiological solution (<i>Eimeria</i> spp.)	Dovgij et al. (2013)
Forest-Steppe zone	Vinnytsia Kharkiv	cattle	<i>Eimeria</i> spp.	23.7	19.8 ± 1.1 oocysts/10 fields of view of the microscope	Skalchuk (2021)
		sheep	<i>E. ninaekohl-jakimovae</i> , <i>E. arloingi</i> , <i>E. faurei</i>	65.4	–	Byrka et al. (2017)
	Poltava	rabbits goats	<i>Eimeria</i> spp.	47.7 22.4	14.6 oocysts/a field of view of the microscope 73.7 ± 26.8 oocysts/g of feces	Klymenko (2015) Yevstafieva et al. (2020)
Steppe zone	Odesa	rabbits	<i>E. stiedae</i> ,	33.8	58.1 ± 6124.9 thousand of oocysts / g of feces (<i>Eimeria</i> spp.)	Franchuk (2015)
			<i>E. magna</i> ,	25.6		
			<i>E. media</i> ,	22.0		
			<i>E. perforans</i> ,	18.0		
		<i>E. intestinalis</i>	0.6			
	South part	cattle	<i>Eimeria</i> spp.	17.4	26.1 ± 1.4 oocysts/10 fields of view of the microscope	Skalchuk (2021)
	North part	cattle	<i>Eimeria</i> spp.	24.4	28.5 ± 1.2 oocysts/10 fields of view of the microscope	
Crimea	rabbits	<i>E. stiedae</i>	21.5	5–2540 oocysts / 20 fields of view of the microscope (<i>Eimeria</i> spp.)	Trofimov et al. (2013)	
		<i>E. perforans</i>	44.5			
		<i>E. magna</i>	13.6			
	<i>E. intestinalis</i>	20.4				
Dnipropetrovsk	rabbits	<i>Eimeria</i> spp.	89.2–83.9	3958–4456 oocysts / g of feces	Prus et al. (2021)	
Zaporizhzhia	rabbits	<i>Eimeria</i> spp.	77.1–71.4	751–776		

Material and methods

The studies were carried out in the laboratory of the Department of Parasitology and Veterinary and Sanitary Expertise of the Dnipro State Agrarian and Economic University in 2021. Fecal samples were taken from ruminant ungulates and rabbits naturally infected with *Eimeria* kept in the Educational, Research and Production, Clinical and Diagnostic Center of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University. Examination of feces for the presence of *Eimeria* spp. carried out by the McMaster method.

In the climatic conditions of the steppe zone of Ukraine, several species of *Eimeria* were found in ruminants and rabbits. In goats, *E. arloingi* oocysts were found in 73% of cases (extensiveness of invasion), *E. ninaekohljakimovae* – 11%, *E. parva* – 12%, *E. alijevi* – 4%. In sheep, *E. ninaekohljakimovae* – 85% and *E. parva* – 15% were registered. In rabbits, two types of eimeria were found: in 78% – *E. perforans*, in 28% – *E. stiedae*.

As a result of the research, the types were determined according to the main characteristics of the *Eimeria* of Ukraine:

– *E. arloingi* – oocysts of round, ellipsoidal or oval shape, 20–31 × 16–23 μm; the oocyst wall consists of two layers; there is a micropyle, and the micropolar cap is barely noticeable;

– *E. ninaekohljakimovae* – round or ellipsoidal oocysts, 16.5–27.5 × 13.3–23.1 μm; the polar cap and micropyle are absent;

– *E. parva* – oval or ellipsoidal oocysts, 9.9–18.7 × 7.7–13.3 μm; the wall of the oocysts is smooth, pale yellow, there is no micropyle;

– *E. perforans* – oocysts of ellipsoidal or oval shape, 13–31 × 11–20 μm, transparent or with a pink tint, with a smooth shell, no micropyle;

– *E. stiedae* – oocysts are oval or ellipsoidal, 31–42 × 17–25 μm in shape, have a smooth yellow-brown shell; there is a micropyle at the narrowed pole.

Non-sporulated oocysts contain germ mass. After culture, the sporulated oocysts contain 4 sporocysts. Each sporocyst contains two sporozoites. For the experiment, we used five temperature regimes (15, 20, 25, 30 and 35 °C) of a TCO-80 MICROmed thermostat. Samples of excrement from goats and rabbits (2 g each) were put separately in beakers (n =

3) and placed in a thermostat. Oocyst sporulation was monitored every day. The percentage of sporulated and non-sporulated oocysts in each sample was calculated. *Eimeria arloingi* (Marotel, 1905) Martin, 1909 oocysts and *E. perforans* (Leuckart, 1879) Sluiter and Swellengrebel, 1912 oocysts were used in the experiment. Results are presented as arithmetic mean ± standard error (x ± SE).

Results

According to the research results, sporulation in *E. perforans* oocysts occurred more slowly than in *E. arloingi* (Table 2). The longest sporulation process was recorded at 15 °C – eight days; more than 50% of sporulated oocysts were identified on the fifth day of the experiment. At 15 °C on the seventh day of the experiment, more than 90% of sporulated oocysts were recorded.

At 20 °C, sporulation of *E. perforans* and *E. arloingi* oocysts ended on the sixth day of the experiment. However, only on the fourth day was there a noticeable increase in the number of sporulated oocysts (more than 50%). At a temperature of 25 °C, sporulation of both studied types of *Eimeria* was 100% completed on the fifth day of the experiment. More than 50% of sporulated oocysts were also recorded on the fourth day of the experiment, compared with a temperature of 20 °C.

At a temperature of 30 °C, more than 50% of sporulated oocysts were found already on the second day of the experiment in *E. arloingi* and on the third day in *E. perforans*. 100% of sporulated *E. perforans* and *E. arloingi* oocysts were determined on the fourth day of the experiment.

At a temperature of 35 °C, 100% sporulation of the oocysts of the studied *Eimeria* species was already observed on the third day of the experiment; on the second day, more than 50% of the sporulated oocysts of the two studied species were found.

Thus, on average, sporulation of oocysts occurs starting from the second day of the experiment, and the peak of sporulation in the majority of oocysts falls on the third or fourth day. The most favourable conditions for sporulation of *Eimeria* oocysts were recorded at 35 °C (*E. perforans* – 62.4% of sporulated oocysts on the second day of the study; *E. arloingi* – 71.9%).

Table 2Sporulation rate (%) of *E. arloingi* and *E. perforans* oocysts depending on temperature ($\bar{x} \pm SE$, $n = 3$)

Species	Temperature, C	Durations, days							
		1	2	3	4	5	6	7	8
<i>E. perforans</i>	15	0	2.78 ± 0.11	14.77 ± 0.19	32.98 ± 0.82	59.41 ± 1.18	84.26 ± 0.46	94.28 ± 0.39	100.00 ± 0.00
	20	0	10.55 ± 1.18	23.40 ± 0.91	57.88 ± 1.05	87.46 ± 0.71	100.00 ± 0.00	–	–
	25	0	17.40 ± 0.52	34.78 ± 2.46	77.30 ± 0.40	100.00 ± 0.00	–	–	–
	30	0	43.84 ± 0.32	82.84 ± 0.59	100.00 ± 0.00	–	–	–	–
	35	0	62.44 ± 0.96	100.00 ± 0.00	–	–	–	–	–
<i>E. arloingi</i>	15	0	6.73 ± 0.59	17.10 ± 0.85	38.66 ± 1.20	63.28 ± 0.70	89.24 ± 1.05	97.05 ± 0.58	100.00 ± 0.00
	20	0	14.69 ± 1.09	33.55 ± 0.92	64.54 ± 1.51	93.58 ± 1.04	100.00 ± 0.00	–	–
	25	0	22.09 ± 0.59	43.60 ± 0.72	83.91 ± 1.52	100.00 ± 0.00	–	–	–
	30	0	53.84 ± 1.02	88.89 ± 0.71	100.00 ± 0.00	–	–	–	–
	35	0	71.92 ± 1.03	100.00 ± 0.00	–	–	–	–	–

Discussion

Coccidiosis (and one of its forms – eimeriosis) is widespread throughout the world. Some authors study the influence of various factors on the viability of oocysts and the duration of their sporulation. Waldenstedt et al. (2001) determined the effect of litter moisture on sporulation of *E. maxima* oocysts. The experiment was carried out using litter of different moisture content: 16%, 42%, and 62%; *E. maxima* sporulation occurs most intensively at the lowest litter moisture content (16%), and in conditions with the highest moisture content (62%), this process is inhibited.

The importance of bedding material for the survival of *Eimeria* oocysts is highlighted by Soliman et al. (2018). They added superphosphates, meta-bisulfides and charcoal additives to the bedding material. All of these additives have been shown to be effective in inhibiting sporulation and reducing the viability of *Eimeria* oocysts. The regular use of these substances in poultry farms can become one of the therapeutic and preventive measures for reducing the survival rate and inhibiting the maturation of oocysts of different species of the genus *Eimeria*.

There is a lot of data on the effect of plant extracts on the oocysts of these parasitic protozoa. In our earlier published work, experiments on the effect of essential oils on the sporulation of *E. magna* (Perard, 1925), rabbit parasites, are described. The essential oils of 14 plant species (*Piper cubeba*, *Cananga odorata*, *Pelargonium graveolens*, *Citrus sinensis*, *Eucalyptus globulus*, *Lavandula angustifolia*, *Picea abies*, *Citrus paradisi*, *Pterocarpus santalinus*, *Abies sibirica*, *Juniperus communis*, *Melaleuca alternifolia*, and *Cyzygium aromaticum*) were studied. The essential oil of *Cinnamomum verum* had the greatest effect on the sporulation of *E. magna* oocysts. When exposed to an aqueous emulsion of the essential oil of this plant for 72 hours, 100% death of oocysts was recorded. *Syzygium aromaticum* oil was less effective. Under its influence, about 54% the death of partially sporulated oocysts was observed. It was also found that essential oils of *P. abies*, *C. paradisi*, *P. santalinus*, *A. sibirica*, *J. communis*, and *M. alternifolia* can delay the sporulation of *E. magna* oocysts (Boyko et al., 2021).

Gadelhaq et al. (2018) investigated the ability of two herbal extracts, *Allium sativum* and *Moringa olifera*, to suppress or disrupt the sporulation of *Eimeria* oocysts, a chicken parasite. However, no positive result was observed. Arfan Zaman et al. (2015) *in vitro* evaluated the effect of *Curcuma longa* leaves, *Artemisia absinthium* seeds, and *Saussurea lappa* roots on the sporulation of *Eimeria tenella*: the extract of *C. longa* had the highest inhibitory effect.

Fatemi et al. (2015) compared the effect of different *Artemisia annua* extracts on the sporulation rate of *E. acervulina*, *E. necatrix*, and *E. tenella* oocysts. Ethanol (96%) and petroleum extracts of *A. annua* inhibit the sporulation of oocysts of the studied *Eimeria* species at concentrations of 2:1000 and 5:1000. In this case, the proportions of inhibition of oocysts compared with the control were 31% at a concentration of 5:1000 and 29% – 2:1000 for petroleum extract. Ethanol extract (96%) had a stronger effect: 34% and 46% inhibition of sporocyst development for concentrations of 5:1000 and 2:1000, respectively.

The effect of temperature on the sporulation of parasitic protozoan oocysts is described by many authors around the world. According to Pyziel & Demiaszkiewicz (2015), the efficiency of sporulation of *E. bovis* oocysts (Zublin, 1908), a parasite of the European bison *Bison bonasus* (Linnaeus, 1758), increases with increasing temperature; sporulation of

oocysts of this *Eimeria* species at a temperature of 18 °C and the addition of a solution of potassium dichromate ($K_2Cr_2O_7$) occurs within six days. An increase in temperature by 5 °C under the same conditions leads to a reduction in the maturation time of oocysts by two days. At a temperature of 28 °C and the addition of a $K_2Cr_2O_7$ solution, *E. bovis* oocysts complete the sporulation process already on the second day. A similar experiment was carried out by these authors also in sterilized water: the results were very different; sporulation was much slower. At a temperature of 23 °C without adding a $K_2Cr_2O_7$ solution, this process ended 9 days later, that is, on the 13th day. At a temperature of 3–5 °C in sterilized water, this process was completed only after 16 weeks. We have obtained similar results for *E. perforans* and *E. arloingi*. With an increase in temperature, a decrease in the duration of oocyst sporulation was recorded. However, the time spent on this process under the same temperature conditions was different for *E. bovis* and *E. perforans*, as well as for *E. arloingi*. At 15 °C, the duration of sporulation of *E. perforans* and *E. arloingi* was much shorter (100% in 8 days) than *E. bovis*, the process of sporulation of which was completed on the 13th day at a higher temperature (23 °C). Perhaps this difference is associated with the species characteristics of parasites, as well as with other microclimatic conditions, including humidity indicators.

Graat et al. (1994) report that the most important aspect of the development of *E. acervulina* is the timing of the onset of sporulation. Together with this, temperature is the most important regulator of this process. According to the results of the experiment, Graat et al. (1994) a temperature of 33 °C contributed to a much faster completion of the sporulation process than a temperature of 21 °C.

According to the results of other experiments (Marquardt et al., 1960), the temperature at the time of the onset of sporulation is also of great importance. In 50% of *E. zurnii* oocysts (parasites of cattle), the sporulation process began after 65 hours at 20 °C and much faster – after 36 hours at 25 °C. In this case, normal sporulation of *E. zurnii* oocysts occurs at a temperature of 8.0–32.5 °C. At temperatures below 12 °C, the sporulation time increases. But already a temperature of 35 °C leads to morphological abnormal changes. The optimum temperature for sporulation of *E. zurnii* oocysts was about 30 °C. An experiment on the effect of temperature on the process of sporulation of oocysts was carried out on the same species of *Eimeria* (Marquardt, 1960). He showed that *E. zurnii* cannot sporulate at high temperatures. The first sign of high temperature damage to the oocysts of this *Eimeria* species is a decrease in the number of normally sporulating oocysts. Sporulation of *E. zurnii* is faster at 35 °C than at lower temperatures (25 °C). At the same time, the number of normally sporulating oocysts is significantly reduced. According to Marquardt (1960), *E. zurnii* sporulation occurs in two stages. This is a preliminary segmentation (the process of which is shortened by exposure to high temperature), as well as the subsequent process of sporulation (segmentation itself), which stops in these temperature conditions.

Similar experiments were carried out by Schneiders et al. (2020). These authors point out that a subsequent increase in temperature may, on the contrary, slow down the process of oocyst development. According to their data, the viability of sporozoites declines when exposed *in vitro* for at least 60 minutes at a temperature of 55 °C, which is confirmed by their morphological changes. The development of merozoites is significantly inhibited when the temperature rises 2 °C higher than the optimal *in vitro* incubation temperature.

Temperature also affects the number of oocysts that are released into the environment. According to Ruiz et al. (2006), the highest intensity of eimeriosis invasion in goats from semi-arid zones was recorded in the hot season. Eight species of *Eimeria* (*E. ninakohlyakimovae*, *E. arloingi*, *E. alijevi*, *E. caprina*, *E. christenseni*, *E. jolchijevi*, *E. caprovina*, *E. hirci*) have been recorded in goats of the arid desert zone of Gran Canaria (Spain). Ruiz et al. (2006) found that the oocyst shedding rate of the detected *Eimeria* species was related to herd size as well as the prevailing climatic conditions in the area.

The data obtained by us can be used in the future to improve the rules for carrying out anti-eimeriosis measures for specific species of host animals in various climatic zones of the world. Global climatic changes are already increasing the intensity of the spread of many parasitic organisms (Makaida et al., 2021), and in the future the spread of eimeriosis may intensify due to the acceleration of the formation of their sporozoites in the environment. A decrease in the time from the moment of excretion of feces into the environment until the moment when they can effectively infect the next animal, by one or two days, can dramatically increase the prevalence of eimeriosis under the conditions of using various technologies for raising farm animals (Gutyj et al., 2019).

Conclusion

One of the main factors that influence the process of sporulation of eimerian oocysts is the temperature of the environment. Under *in vitro* conditions, with a decrease in temperature to 15 °C, sporulation in *E. perforans* (parasites of rabbits) and *E. arloingi* (parasites of small ruminants) slowed down to 8 days. At a temperature of 35 °C, sporulation of oocysts of the studied species of parasitic protozoa ended on the third day in an *in vitro* experiment. These data can be used in the future when planning preventive measures in the fight against eimeriosis of farm animals to reduce the use of chemically synthesized disinfectants, in order to reduce the toxic load on agroecosystems, as well as on natural ecosystems.

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