INTENSITY OF INFECTION AND MEANS OF GIARDIASIS PREVENTION AT THE FARMS OF UKRAINE

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Abstract

G. duodenalis is the most widespread among the six generally recognized species of giardiasis affecting the gastrointestinal tract of mammals. Destruction of *Giardia intestinalis* oocysts in the environment mitigates the risk of infection for animals and humans.

The objective of the study. The research is aimed at establishing the effectiveness of disinfectants against *Giardia intestinalis* cysts.

Methods. The research involved twelve farms in Ukraine. Fecal samples were taken from the tested calves aged from 1 to 3 weeks. Giardiasis was diagnosed on the basis of laboratory tests of calf feces by the Fulleborn's method. The object of study was represented by cysts of *Giardia intestinalis* obtained from the feces through combined flotation methods. After exposure for a period of 30 and 60 minutes, the supernatant was drained and the precipitate applied to a glass slide to be stained with Lugol's solution. Cysts were subject to evaluation in terms of their morphological features. Their shape, size, color, nuclei location and axon were determined under a microscope at a magnification of about 10x400. Yodosol, Kontavir, Biocontact and Bioluft were used as a disinfectant.

Results. The study carried out at dairy farms of various forms of ownership revealed that 25-50% of calves at the holdings and 50-75% of those at the farms were infected with *Giardia intestinalis*. Experiments established that Kontavir at a concentration of 1%, as a disinfectant, produced a cytotoxic effect (compressed the cyst membrane) on *Giardia intestinalis* for 60 minutes. If the drug concentration increases to 2-3%, Kontravir destroys the cyst shell releasing its contents within 30-60 minutes.

Conclusions. The prevalence of *Giardia intestinalis* has been established through studying dairy farms in four regions of Ukraine. As a disinfectant, Kontavir at a concentration of 1% produces a cytotoxic effect (compresses the cyst membrane), at a concentration of 2-3%, it completely destroys the cyst membrane.

Key words: calf giardiasis, disinfection, Giardia intestinalis biological cycle, laboratory diagnosis of giardiasis, VetExpert Giardia Ag test, microscopy.

1. Introduction

Giardia intestinalis is an important zoonotic parasite infecting the intestines of humans and various animals. However, the data on the prevalence and genetic diversity of *G. intestinalis* in different countries are limited [1]. The Giardia genus covers the most widespread intestinal flagella of vertebrates. These parasites can have a wide range of hosts, such as mammals, birds and amphibians. *G. intestinalis* is the most common among the six currently known species of Giardia. It is also important for gastrointestinal diseases in animals. *G. intestinalis* is frequently found in many mammals (dogs, fur animals, dairy cattle). The reported prevalence rates among milk animals differ significantly both in terms of geography and different diagnostic methods applied [2, 3].

Giardiasis infection [4] has been reported at the level of 34.3% for buffalo calves in the Southwest region of São Paulo, Brazil [5]. Austria and Japan showed data on mixed *Giardia intestinalis* and *Cryptosporidium parvum* infection in calves with diarrhea [6, 7]. Animals get infected from sick calves and mature animals, even if all the hygienic and disinfection standards are followed. Studies carried out at dairy farms in Henan, China, also confirm potential transmission of *Giardia intestinalis* and *Cryptosporidium parvum* by flies [8].

Giardiasis cysts (invasive stage) penetrate into the animal's body with food (orally). In the duodenum, Two trophozoites capable of asexual reproduction are formed on the basis of each cyst in the duodenum. If no treatment is applied, the giardiasis lifespan ranges from 3 to 40 days, 4 weeks on average. Giardiasis cysts and trophozoites are excreted from the body of the host with feces. Fecal excretion occurs every 8-10 days. Only cysts survive beyond the animal's body. In water they remain viable at a temperature of 4-20°C for 3 months. Mature animals rarely suffer from giardiasis. Clinical signs of giardiasis in dairy calves include diarrhea, dull coat, depression, indigestion and intoxication. Feces are liquid with a sour smell and mucus admixtures [9, 10].

Giardiasis is diagnosed using a rapid VetExpert Giardia Ag test and coprological examination. In case of giardiasis infection of low intensity, the test does not always respond sometimes showing a negative result. In this case, a microscopic test of feces in the animals is performed to confirm the diagnosis.

Therefore, the main aim of the study was to determine the incidence of giardiasis at dairy farms in Ukraine as well as to establish effectiveness of disinfectants when used against Giardia intestinalis cysts in calves. Preventive disinfection destroyed giardiasis cysts in the environment which, in its turn, mitigated the risk of giardiasis at dairy farms.

2. Materials and methods of the research

The research was conducted at dairy farms in Sumy region, Ukraine, further studies were carried out at the vivarium laboratory of Sumy National Agrarian University. Fecal samples were taken from the tested calves aged 3-4 months.

Giardiasis was diagnosed through a rapid VetExpert Giardia Ag test and microscopic examination of calf feces [11]. The object of study was represented by *Giardia intestinalis* cysts obtained from the feces with combined flotation methods. Studies for giardiasis were conducted at 12 farms in four regions of Ukraine (Kharkiv, Dnipro, Sumy and Zhytomyr)

After a period of exposure lasting 30 or 60 minutes, the supernatant was drained, the precipitate was applied to a glass slide and stained with Lugol's solution. Cysts were subject to evaluation in terms of their morphological features. Their shape, size, color, nuclei location and axon were determined under a microscope at a magnification of about 10x400. Yodosol, Kontavir, Biocontact and Bioluft were used as a disinfectant. The disinfectants were manufactured by PE “Kronos Agro”, Ukraine.

3. Results

Giardiasis incidence was studied at dairy farms represented by holdings and private farms (Table 1).

Table 1

Study of giardiasis at the milk-producing farms of Ukraine

Farms	Dairy farms	
	Holdings	Private farms
Kharkiv region	+	++
Dnipro region	++	+++
Sumy region	+	++
Zhytomyr region	++	+++

Notes: «++++» – 75 - 100% *Giardia intestinalis* incidence; «+++» – 50 - 75%; «++» – 25 -50%; «+» to 25%; «–» – no *Giardia intestinalis* cysts

Experiments have established that the incidence of *Giardia intestinalis* infection in calves reached 25-50% at the holdings, 50-75% at private farms. Such a situation was due to incompliance with sanitary standards at the farms and the fact that some calves were brought from other farms. If quarantine for new calves was not followed at some of the farms, it caused widespread infection among young animals. Another challenge for many farms is the lack of giardiasis control as well as insufficient VetExpert Giardia Ag rapid test. After numerous cases of illness and deaths, measures start to be taken to prevent giardiasis (lambliosis) infection among animals and humans.

Further studies marked the effectiveness of disinfectants against *Giardia intestinalis* cysts. The disinfectants differed in terms of their composition and the mechanism of action produced on protozoan cysts.

Yodosol disinfectant contains iodine and lactic acid. It exerts an antimicrobial effect on gram-negative and gram-positive microflora since it has a fungicidal effect on yeast, candida and aspergillus, virucidal effect. Kontavir, as a combined disinfectant, contains glutaraldehyde, benzalkonium chloride, dodecyldimethylammonium chloride. The drug is also characterized by an active antimicrobial action against gram-positive and gram-negative bacteria, fungicidal, virucidal action (against DNA and RNA-containing viruses). Biocontact consists of glutaraldehyde, glyoxaldehyde and quaternary ammonium compounds. It is used for preventative disinfection in order to wipe or irrigate in case of infectious diseases of bacterial, viral, fungal nature. Bioluft disinfectant based on hydrogen peroxide and lactic acid complex produces antimicrobial and coccidiostatic action.

The effect of the above disinfectants on *Giardia intestinalis* cysts was not studied (Table 2).

Table 2

Disinvasive action on *Giardia intestinalis* cysts

Disinfectant	Drug concentration				
	1 ml/L	2 ml/L	3 ml/L	4 ml/L	5 ml/L
Yodosol	No effect	No effect	No effect	No effect	No effect
Kontavir	Oocyte compression 60 minutes later	Oocyte destruction and content release 60 minutes later	Oocyte destruction and content release 30 minutes later	-	-
Biocontact	No effect	No effect	No effect	No effect	No effect
Bioluft	No effect	No effect	No effect	No effect	No effect

The results of the experiment indicate that Kontavir at a concentration of 1% produces a cytotoxic effect by compressing the cyst shell (Figure 1).

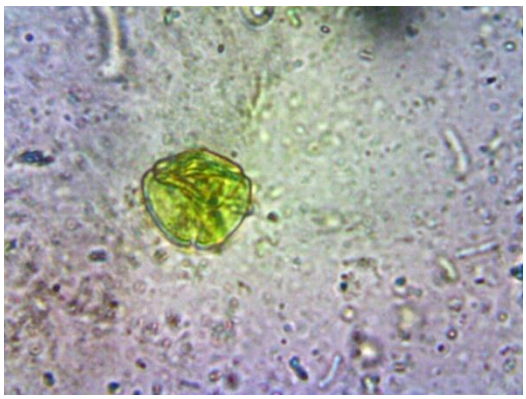

Figure 1. Compression of the *Giardia intestinalis* cyst shell due to Kontavir action

When the concentration increases to 2-3%, the cyst shell is destroyed (Figure 2). We believe that this effect is associated with the presence of dodecyldimethylammonium chloride as a disinfectant, which disrupts intermolecular interactions and causes dissociation of lipid layers in the shell. When combined with glutaraldehyde, it leads to destruction of protein structures in the *Giardia intestinalis* cyst shell.

Figure 2. Shell destruction and content release for the *Giardia intestinalis* cysts due to Kontavir action

Other disinfectants (Yodosol, Biocontact and Bioluft) produced no effect on the *Giardia intestinalis* cysts (Figure 3). Therefore, we do not consider it efficient to use them as disinfectants against giardiasis at the farms.

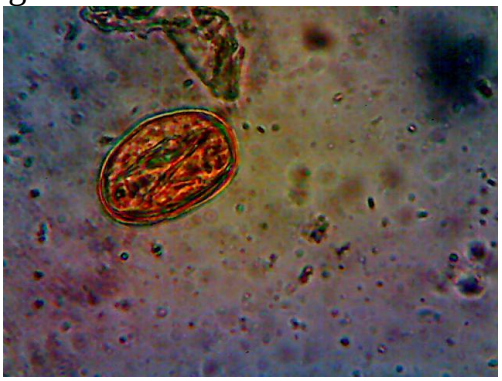

Figure 3. *Lamblia intestinalis* cysts, normal condition

4. Discussion

Monitoring of the *Giardia intestinalis* incidence in calves at twelve dairy farms in four regions of Ukraine showed that a percentage of 25-50% at holdings and 50-75% at farms [12, 13]. The experiment allowed us to determine *Giardia intestinalis* prevalence at cattle farms. However, depending on the technology of detention and sanitary and hygienic conditions, the degree of damage may differ [14, 15].

It has been experimentally established that Kontavir at a concentration of 1% produced a cytotoxic effect (compressing the cyst membrane), at a concentration of 2-3% it destroyed the cyst membrane. Therefore, Kontavir can be recommended for further research for the purpose of disinfection in the event of giardiasis at dairy farms [16].

Research limitations. The research is limited by a small number of the livestock and the genotype.

Prospects for further research. Study of Kontavir effectiveness and of the giardiasis incidence at dairy farms.

The prevalence of *Giardia intestinalis* was studied at dairy farms in four regions of Ukraine. Kontavir effectiveness against *Giardia intestinalis* cysts was proven in vitro.

Empirical studies showed that the incidence of giardiasis in calves is lower at large enterprises, holdings, where sanitary and hygienic standards are complied with and preventive disinfection of premises for animals is carried out.

The practical value of the research lies in determining the *Giardia intestinalis* prevalence at dairy farms in Ukraine. As it was established, Kontavir, as a disinfectant, destroys giardiasis cysts in the environment.

References

[1] Jian, Y., Zhang, X., Li, X., Karanis, G., Ma, L., & Karanis, P. (2018). Prevalence and molecular characterization of Giardia duodenalis in cattle and sheep from the Qinghai-Tibetan Plateau Area (QTPA), northwestern China. *Veterinary parasitology*, *250*, 40–44. https://doi.org/10.1016/j.vetpar.2017.12.001

[2] Ballweber, L. R., Xiao, L., Bowman, D. D., Kahn, G., & Cama, V. A. (2010). Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends in parasitology*, *26*(4), 180–189. https://doi.org/10.1016/j.pt.2010.02.005

[3] Gherman, C. M., Kalmár, Z., Györke, A., & Mircean, V. (2018). Occurrence of Giardia duodenalis assemblages in farmed long-tailed chinchillas Chinchilla lanigera (Rodentia) from Romania. *Parasites & vectors*, *11*(1), 86. https://doi.org/10.1186/s13071-018-2652-8

[4] Minetti, C., Taweenan, W., Hogg, R., Featherstone, C., Randle, N., Latham, S. M., & Wastling, J. M. (2014). Occurrence and diversity of Giardia duodenalis assemblages in livestock in the UK. *Transboundary and emerging diseases*,*61*(6), e60–e67. https://doi.org/10.1111/tbed.12075

[5] de Aquino, M., Harvey, T. V., Inácio, S. V., Nagata, W. B., Ferrari, E. D., Oliveira, B., Albuquerque, G. R., Widmer, G., Meireles, M. V., & Bresciani, K. (2019). First description of*Giardia duodenalis* in buffalo calves (*Bubalus bubalis*) in

southwest region of São Paulo State, Brazil. *Food and waterborne parasitology*, *16*, e00062. https://doi.org/10.1016/j.fawpar.2019.e00062

[6] Lichtmannsperger, K., Hinney, B., Joachim, A., & Wittek, T. (2019). Molecular characterization of Giardia intestinalis and Cryptosporidium parvum from calves with diarrhoea in Austria and evaluation of point-of-care tests. *Comparative immunology, microbiology and infectious diseases*, *66*, 101333. https://doi.org/10.1016/j.cimid.2019.101333

[7] Matsuura, Y., Matsubayashi, M., Nukata, S., Shibahara, T., Ayukawa, O., Kondo, Y., Matsuo, T., Uni, S., Furuya, M., Tani, H., Tsuji, N., & Sasai, K. (2017). Report of fatal mixed infection with Cryptosporidium parvum and Giardia intestinalis in neonatal calves. *Acta parasitologica*, *62*(1), 214–220. https://doi.org/10.1515/ap-2017-0026

[8] Zhao, Z., Dong, H., Wang, R., Zhao, W., Chen, G., Li, S., Qi, M., Zhang, S., Jian, F., Zhao, J., Zhang, L., Wang, H., & Liu, A. (2014). Genotyping and subtyping Cryptosporidium parvum and Giardia duodenalis carried by flies on dairy farms in Henan, China. *Parasites & vectors*, *7*, 190. https://doi.org/10.1186/1756-3305-7-190

[9] Xie, S. C., Zou, Y., Chen, D., Jiang, M. M., Yuan, X. D., Li, Z., Zou, F. C., Yang, J. F., Sheng, J. L., & Zhu, X. Q. (2018). Occurrence and Multilocus Genotyping of *Giardia duodenalis* in Yunnan Black Goats in China. *BioMed research international*, *2018*, 4601737. https://doi.org/10.1155/2018/4601737

[10] Rumsey, P., & Waseem, M. (2020). Giardia Lamblia Enteritis. In *StatPearls*. StatPearls Publishing.

[11] Feng, Y., & Xiao, L. (2011). Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clinical microbiology reviews*, *24*(1), 110–140. https://doi.org/10.1128/CMR.00033-10

[12] Dixon, B., Parrington, L., Cook, A., Pintar, K., Pollari, F., Kelton, D., & Farber, J. (2011). The potential for zoonotic transmission of Giardia duodenalis and Cryptosporidium spp. from beef and dairy cattle in Ontario, Canada. *Veterinary parasitology*, *175*(1-2), 20–26. https://doi.org/10.1016/j.vetpar.2010.09.032

[13] Feng, Y., Ryan, U. M., & Xiao, L. (2018). Genetic Diversity and Population Structure of Cryptosporidium. *Trends in parasitology*, *34*(11), 997–1011. https://doi.org/10.1016/j.pt.2018.07.009

[14] Hatam-Nahavandi, K., Mohebali, M., Mahvi, A. H., Keshavarz, H., Najafian, H. R., Mirjalali, H., Rezaei, S., & Rezaeian, M. (2016). Microscopic and Molecular Detection of *Cryptosporidium andersoni* and *Cryptosporidium xiaoi* in Wastewater Samples of Tehran Province, Iran. *Iranian journal of parasitology*, *11*(4), 499–506.

[15] Cho, Y. I., Han, J. I., Wang, C., Cooper, V., Schwartz, K., Engelken, T., & Yoon, K. J. (2013). Case-control study of microbiological etiology associated with calf diarrhea. *Veterinary microbiology*, *166*(3-4), 375–385. https://doi.org/10.1016/j.vetmic.2013.07.001

[16] Hatam-Nahavandi, K., Ahmadpour, E., Carmena, D., Spotin, A., Bangoura, B., & Xiao, L. (2019). Cryptosporidium infections in terrestrial ungulates with focus on livestock: a systematic review and meta-analysis. *Parasites & vectors*, *12*(1), 453. https://doi.org/10.1186/s13071-019-3704-4

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