Borrelia miyamotoi - an emerging risk for human health

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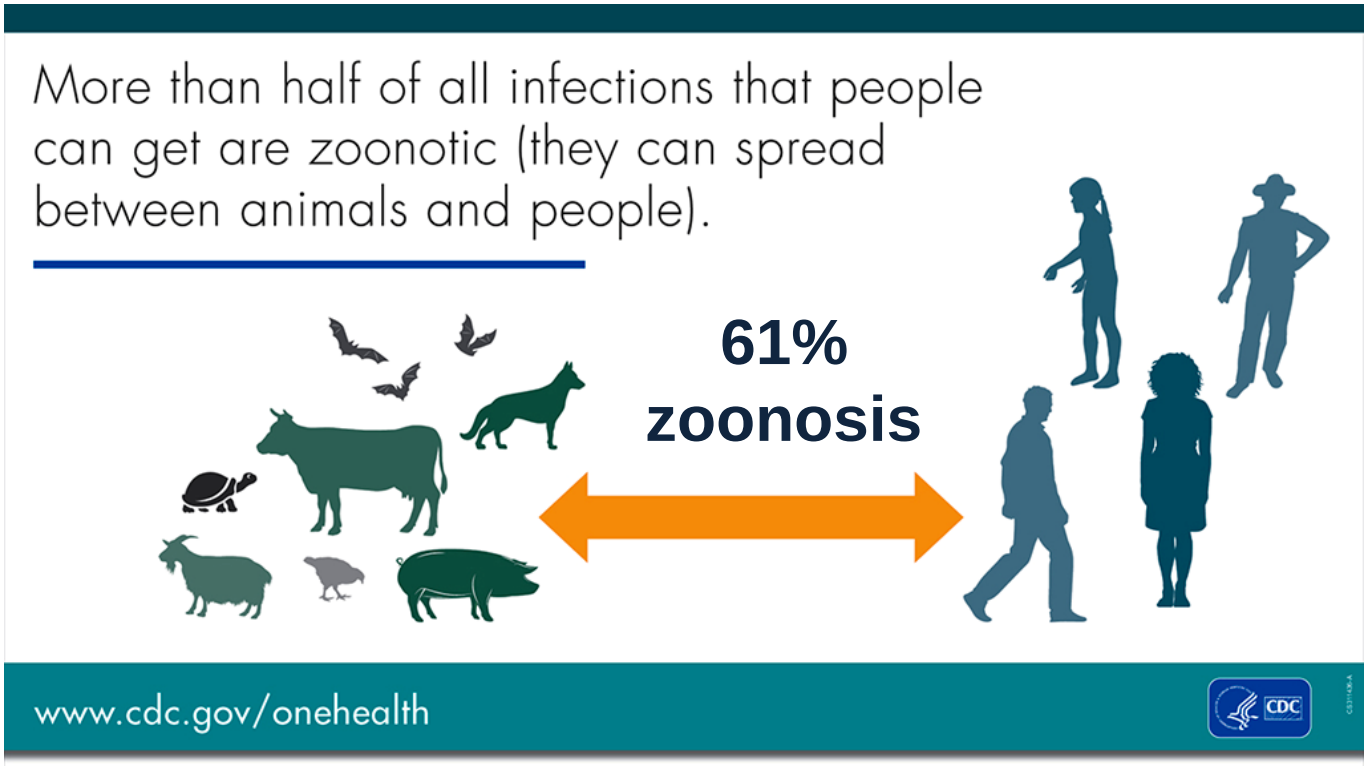
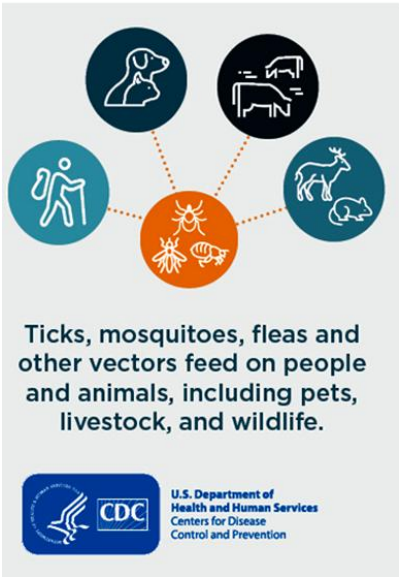
New infections diseases – a challenge for medicine

✓**29 out of the 96 major cases of human morbidity and mortality**



22% zoonosis are VECTOR-BORNE



Taylor LH, et al. 2001
WHO, 2000

Ticks as a vector of pathogens

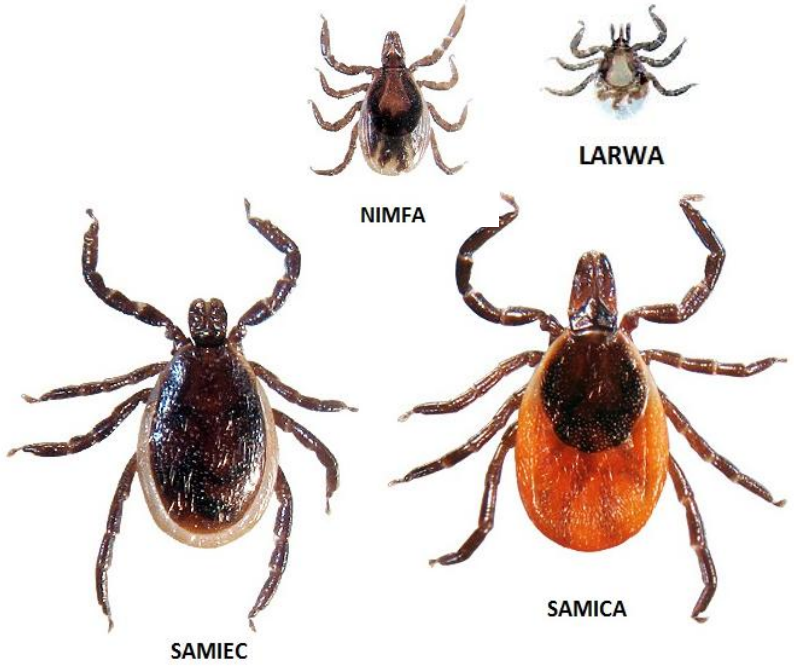
✓**after mosquitoes, ticks are the second most frequent vector of pathogens**

✓***Ixodes ricinus* the most prevalent and widely distributed of the tick species in Europe**

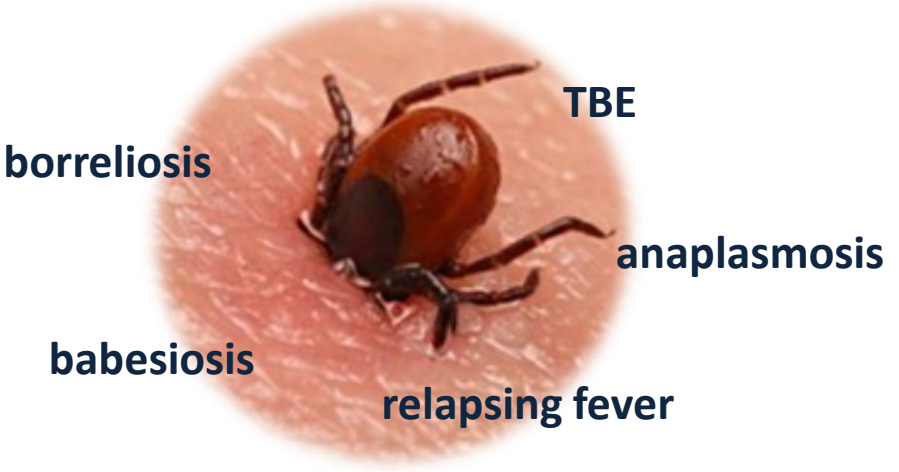
✓**in the last decades, Europe has witnessed a steady increase in cases of TBE and Lyme disease**

✓**during the past two decades, thanks to advances in the molecular biology several new rickettsial and ehrlichial diseases, and new *Borrelia* and *Babesia* genotypes have been recognized**

✓**the majority of TBDs are classified as <u>emerging infectious diseases</u> because of the growing awareness of their importance and the rising statistics for incidence in the human population**

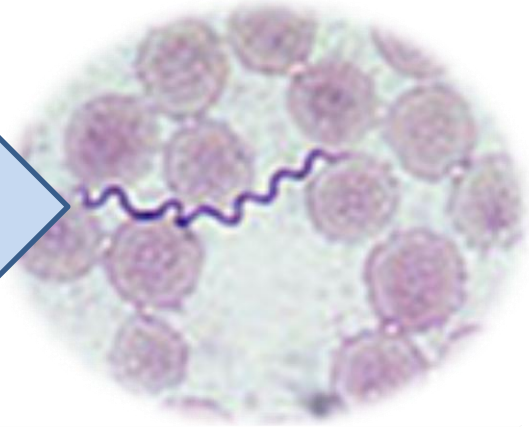


***Ixodes ricinus* – life stages**

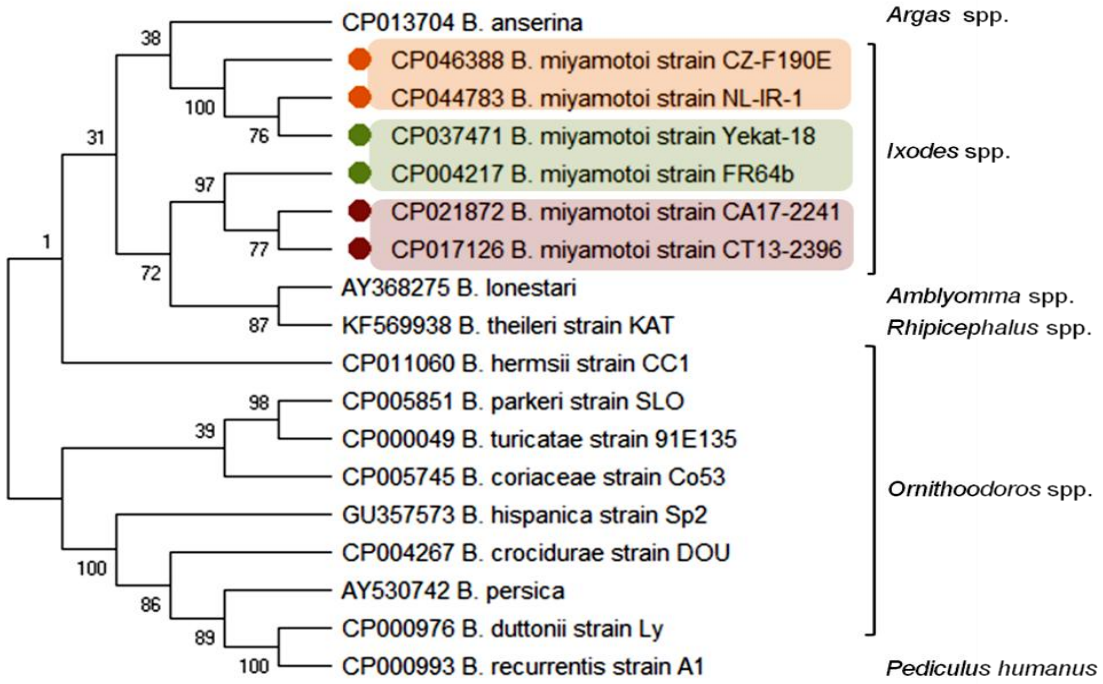


Borrelia miyamotoi

- **Gram-stain-negative bacteria**
- **cells are 0.2–0.5 mm in diameter by 3–30 mm in length**
- **with 15–20 periplasmic flagella located in the periplasmic space between the outer membrane and the protoplasmic cylinder**
- **cells are able to actively move with frequent reversal of the direction**

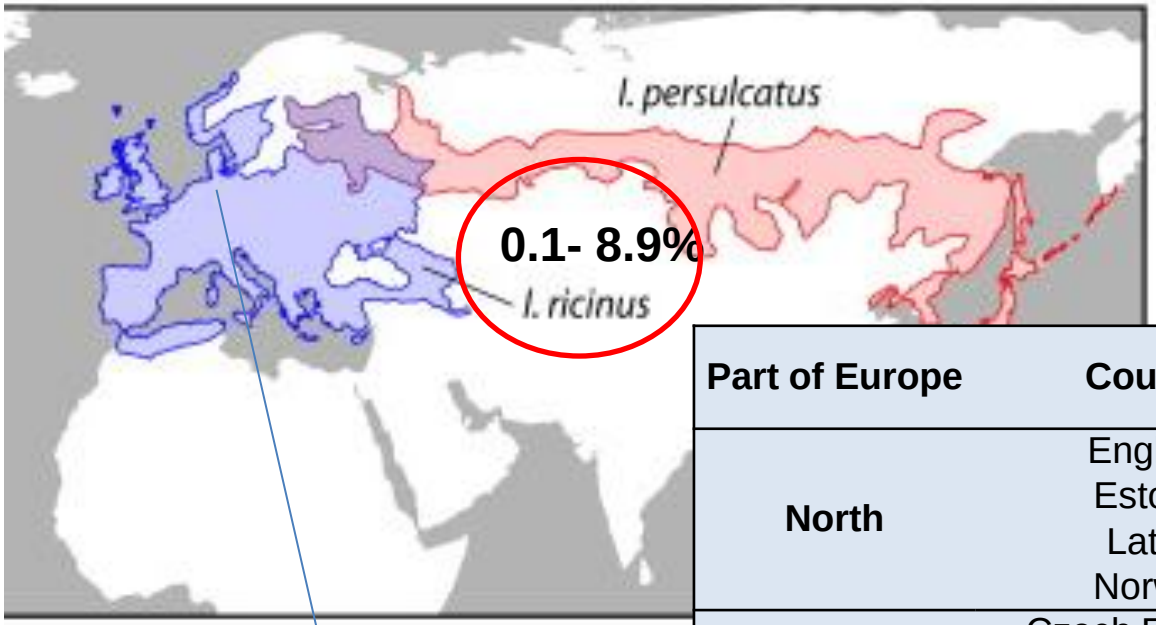


- ✓**first was detected in *I. persulcatus* ticks in 1994 in Japan**
- ✓***Borrelia miyamotoi* is included to relapsing fever spirochetes**
- ✓***B. miyamotoi* is genetically and ecologically distinct from *B. burgdorferi* sensu lato**
- ✓**both microorganisms are transmitted by the same *Ixodes* tick species**



Molecular relationships between *B. miyamotoi* and other RF *Borrelia* species based on the sequences of the *glpQ* gene selected from GenBank [Kubiak et al. 2021]

Borrelia miyamotoi in host-seeking and feeding *I. ricinus* ticks in Europe



Warmia and Mazury
0.5%

Part of Europe	Country	*B. miyamotoi* prevalence (%)
North	England	0.4 (N)[1]– 0.73
	Estonia	0.4 (*I.r.*)[2]; 2.7 (*I.p.*)[3]
	Latvia	1.1 (*I.r.*)[2]; 1.27 (*I.p.*)[3]
	Norway	0.9 – 1.3
Central	Czech Republic	3.2
	Hungary	4.8
	Poland	0.5 – 3.9
	Slovakia	0.75 – 1.0
	Switzerland	2.5
West	Belgium	1.1 – 2.4
	France	1.2 – 2.2
	Germany	0.8 – 8.9
South	Italy	0.74 (N)[1]
	Portugal	0.16
	Serbia	1.4 (N)[1]
	Spain	0.6 – 1.0

[1] (N) – nymphs; [2] (*I.r.*) – *Ixodes ricinus*;
[3] (*I.p.*) – *Ixodes persulcatus*;

[Krause et al. 2015. Kubiak et al. 2021]

Borrelia miyamotoi disease (BMD) - positive cases in Europe.

Country	Patient	Number of cases, Percentage of cases among persons studied	Case	Assay	Year of publication
The Netherlands	70-year-old man	1 single case	Meningoencephalitis	qPCR	2013
	42-year-old man	1/626, 0.16%	EM [1], asymptomatic	qPCR	2016
Germany	74-year-old woman	1 single case	Neuroborreliosis, Immunocompromised	qPCR	2016
Sweden	53-year-old woman 66-year-old woman	2 single cases	Meningitis, Immunocompetent Meningitis, Immunocompromised	PCR	2018
Poland	47-year-old man	1/133, 0.75%	Neuroborreliosis, patient with alcohol abuse	Nested PCR	2019
Austria	51-year-old woman	1 single case	Symptomatic	qPCR	2020

[Kubiak et al. 2021]

Studies from the Netherlands suggest that some 36,000 humans are bitten each year by *B. miyamotoi* (European strain) infected ticks [Wegemakers el al. 2015]

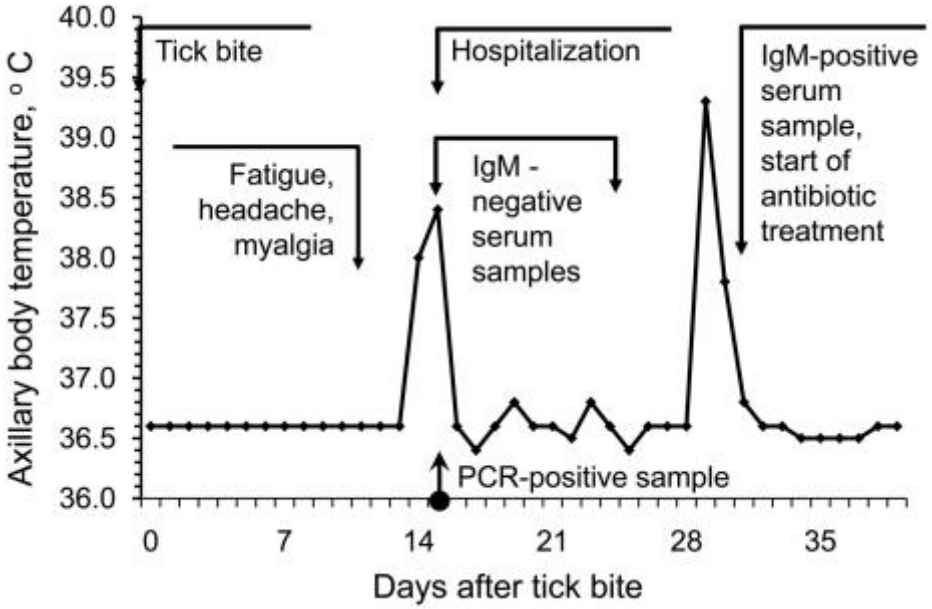
Asia – 124 cases (Russia, China, Japan) **USA – 97 cases**

Borrelia miyamotoi disease (BMD) - symptoms

typical:

✓**flu-like symptoms: fatigue, headache, chills, myalgia, arthralgia, nausea**

✓**relapsing fever episodes (~40°C)**



Relapsing fever episodes in patients with *B. miyamoti* infection [Platonov et al. 2011]

rar:

✓**thrombocytopenia**

✓**monocytosis**

✓**infections of the central nervous system (meningoencephalitis)**

no typical symptoms for borreliosis and relapsing fever:

✓**erytrama migrans**
✓**epistaxis**
✓**abortion**
✓**jaundice**
✓**severe organ failure**

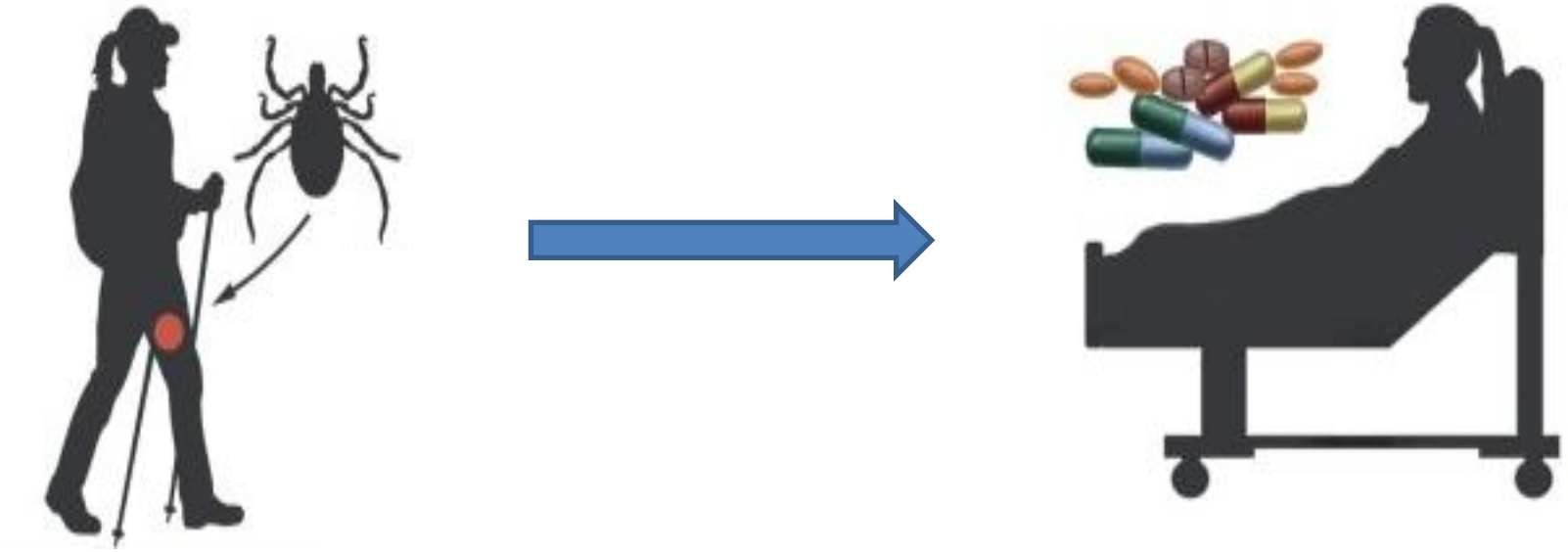
Borrelia miyamotoi disease (BMD) – treatment

therapy for *B. miyamotoi* infection has typically followed guidelines used for treatment of Lyme borreliosis:

• in cases reported in Europe, doses of 200 mg doxycycline have been used successfully once or twice daily for two weeks

•intravenous ceftriaxone 2000 mg once a day provided for two weeks has been effectively used in the case of meningoencephalitis

• probably resistance to amoxicillin



Unanswered questions regarding the pathobiology of *Borrelia miyamotoi*

What is the global epidemiological picture of *B. miyamotoi* infection?
Are the different spirochaetal variants restricted among certain tick species?
What is the ecology of this spirochaete?
What is the contribution of high incidence vertebrate species such as wild turkeys towards maintaining the ecological niche for this spirochaete?
What are the consequences of other pathogens present within ticks (including other *Borrelia*) upon the survival, persistence and transmissibility of *B. miyamotoi*?
Do different strains show differential virulence within susceptible species?
What are the full range of clinical consequences within humans?
What are human risk factors for development of clinical disease above and beyond being immunocompromised?
Does blood transfusion present a substantive risk for infection?
What is the best diagnostic approach to take, using which sample types and at what time point during infection?
What is the best regime for therapeutic management of cases?

Cutler S. et al. 2019

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Case report of swimmer's itch caused by *Trichobilharzia* spp. in north-east Poland

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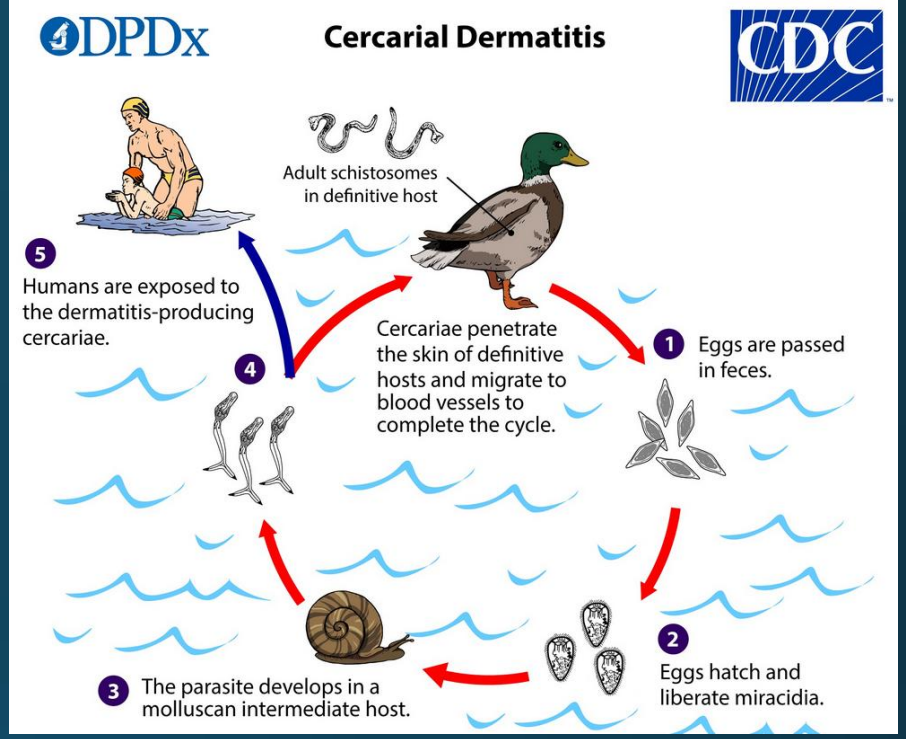
School of Public Health
Department of Medical Biology

INTRODUCTION

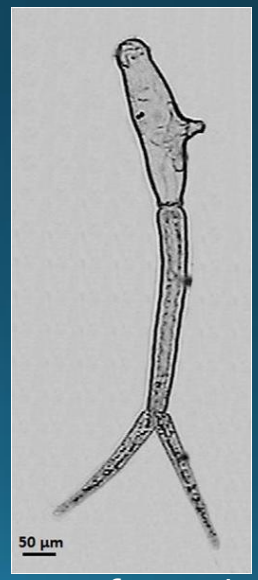
What is swimmer's itch?

- Swimmer's itch or human cercarial dermatitis (HCD) is an inflammatory skin reaction caused by cercariae of Avian schistosomes.
- These parasites are released from infected snails into fresh and salt water.They are capable of penetrating human epidermis; however, the majority of larvae die within 24 hours of establishing an infection.
- Swimmer's itch is found throughout the world and is more frequent during summer months.

The aim of this study was to determine the etiology of skin lesions in people bathing in Pluszne Lake using molecular analysis.



Life Cycle

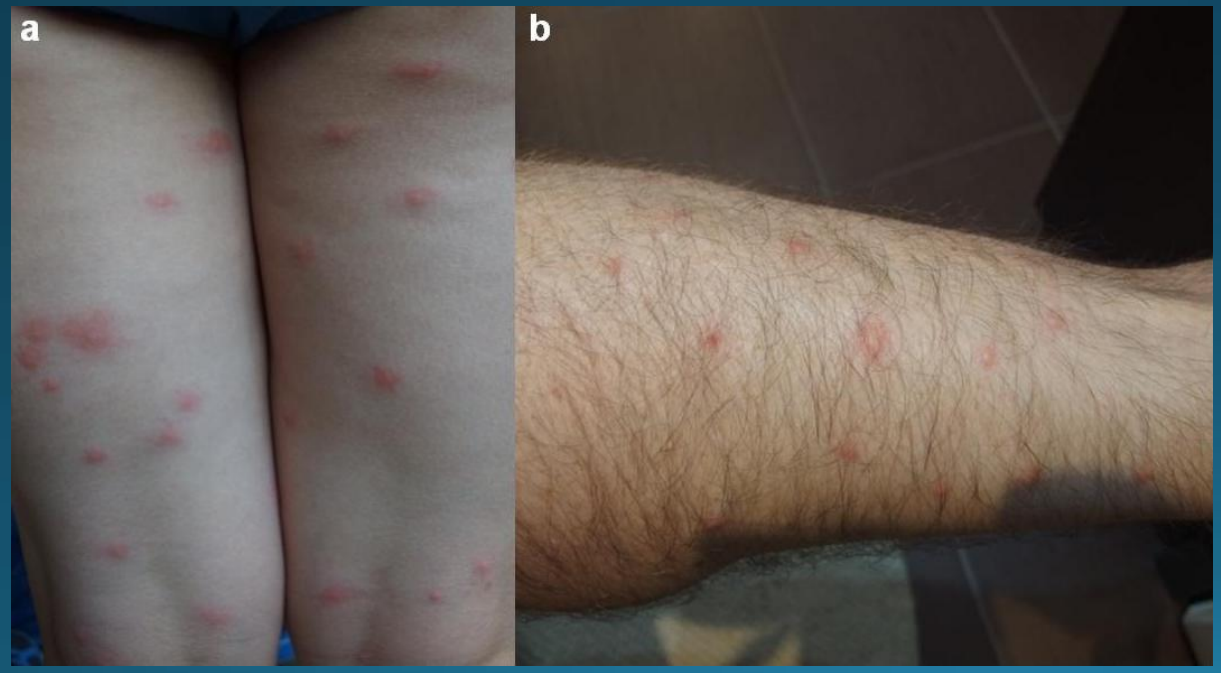


Cercaria of *T. szidati*

MATERIAL & METHODS

Medical case description

On 5th June 2018 a boy, aged 13, and his father, aged 46, presented at our Department complaining of skin lesions on torso and limbs characterized by intense pruritus. A few hours be-fore the symptoms appeared, both the boy and his father had bathed in the lake. Initially, a few hours following water exposure, the only symptom in both cases was pruritus; several hours later numerous, round skin eruptions appeared, about 1-2 centimetres in diameter, which were erythematous and edematous, bright red in colour, with some lesions containing centrally located vesicles filled with serous content. The greatest number of skin lesions were observed on lower limbs and the buttocks, with smaller number of lesions found on the forearms and lower body, with only a few isolated eruptions on the remaining body parts. The treatment included oral anti-pruritic agents (antihistamines) and topical anti-inflammatory agents (mometasone) in the form of ointment. In both cases the symptoms resolved after 10 days following the first reaction.



Multiple erythematous papules 1 day after infection: on the legs of a 13-year-old boy (a) and on the leg of a 46-year-old adult man (b).

MATERIAL & METHODS

Snails collection

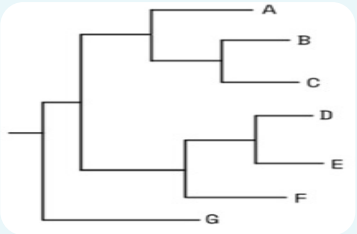
In the summer of 2018 a sample of 397 snails was obtained from 4 bathing sites (Pluszne Lake: 53°35′26″N, 20°23′55″E).

Cercariae collection

Each snail was then placed in a glass beaker with distilled water and subject to 1-hour light stimulation in order to induce cercariae expulsion. Furcocercariae with pigmented eye spots were collected into 1.5 ml tubes with 95% ethanol and frozen (-20˚C) until DNA extraction was performed.

Molecular identification of parasites

DNA extraction was done using the QIAamp DNA Mini Kit (QIAGEN). ITS (1,2 and 5.8S) region of furcocercariae was amplified using the forward primer Its5Trem and the reverse primer Its4Trem.



Phylogenetic analysis

Alignment of sequences was done in MEGA 10. For the phylogenetic tree reconstruction, neighbor-joining analysis was conducted using Kimura’s two-parameter model, followed by 1000 replicates of bootstrap resampling.

RESULTS

- Cercariae of bird schositosomes were detected in 3 out of 4 studied bathing sites in Lake Pluszne.
- All the positive samples selected by means of amplification of ITS gene region of ribosomal DNA underwent genotyping. 5 sequences were obtained (MT041668-MT041672)

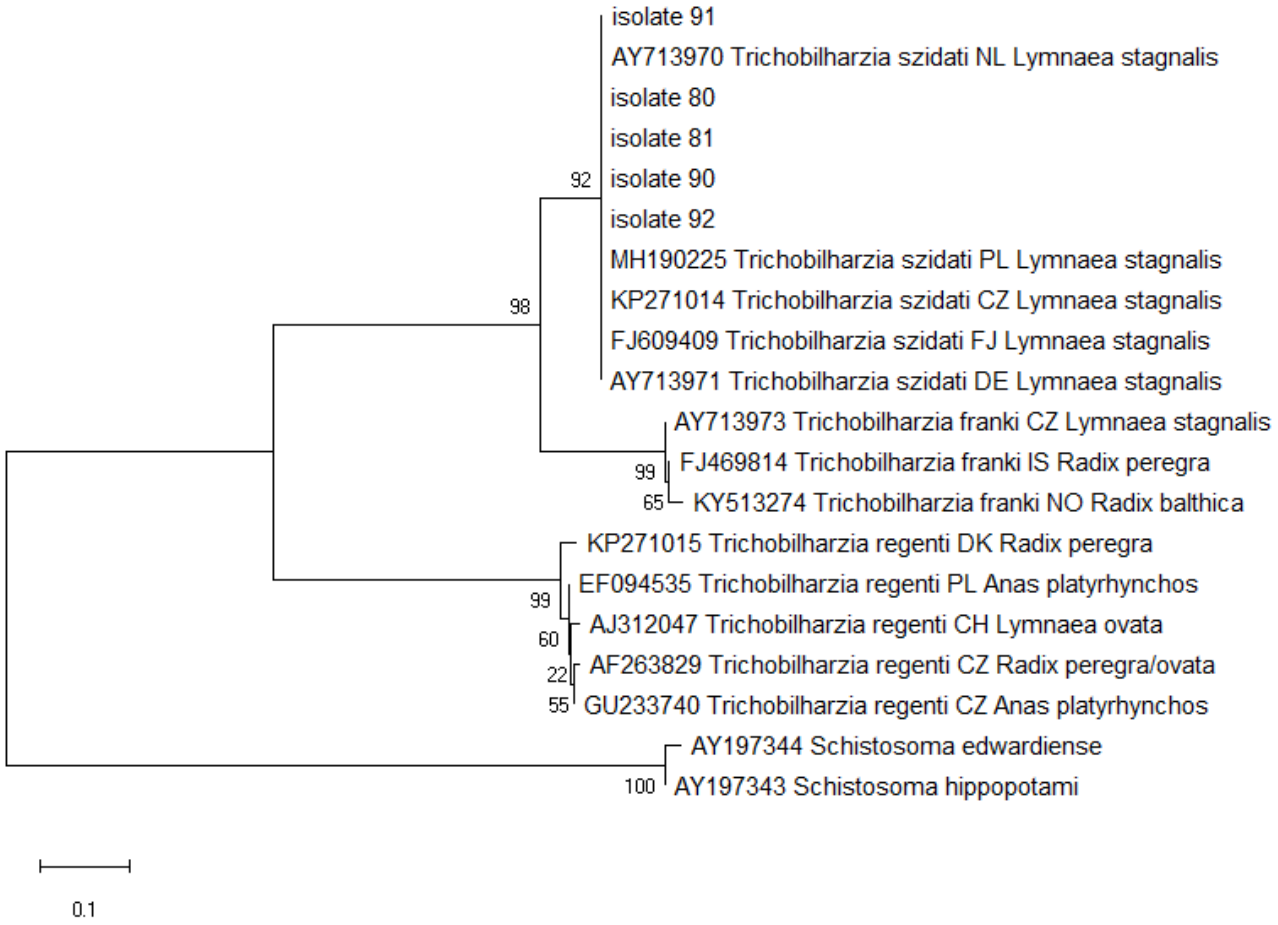
Table 1. The number of snails infected by *Trichobilharzia szidati* in Lake Pluszne

Sampling site for snails	Number of examined snails	Infected *L. s*	Non-infected *L. s*	Infected *R.* spp.	Non-infected *R.* spp.	Infected *P. c*	Non-infected *P. c*	*T. szidati* prevalence (%)
Location 1 53°36'23.3"N 20°25'05.8"E	105	1	80	–	24	–	–	1.23
Location 2 53°36'19.3"N 20°25'07.5"E	80	–	14	–	61	–	5	–
Location 3 53°36'12.3"N 20°25'06.4"E	92	1	54	–	37	–	–	1.81
Location 4 53°36'05.1"N 20°25'00.6"E	120	3	98	–	15	–	4	2.97
Total	397	5	246	–	137	–	9	1.25*

L. s – Lymnaea stagnalis, R. spp. – *Radix* spp., *P. c – Planorbarius corneus,* *total prevalence including all collected snails from Lake Pluszne

The analysis demonstrated that European DNA sequences show low variation within and between *T. szidati* populations. It is also of note that there is no variation regarding intermediate host. For *T. szidati* species it is primarily *Lymnaea stagnalis*

The phylogenetic tree of isolates of European bird schistosomes based on the partial sequences of ITS region.
The tree includes definitive and intermediate host species infected and the country of occurrence



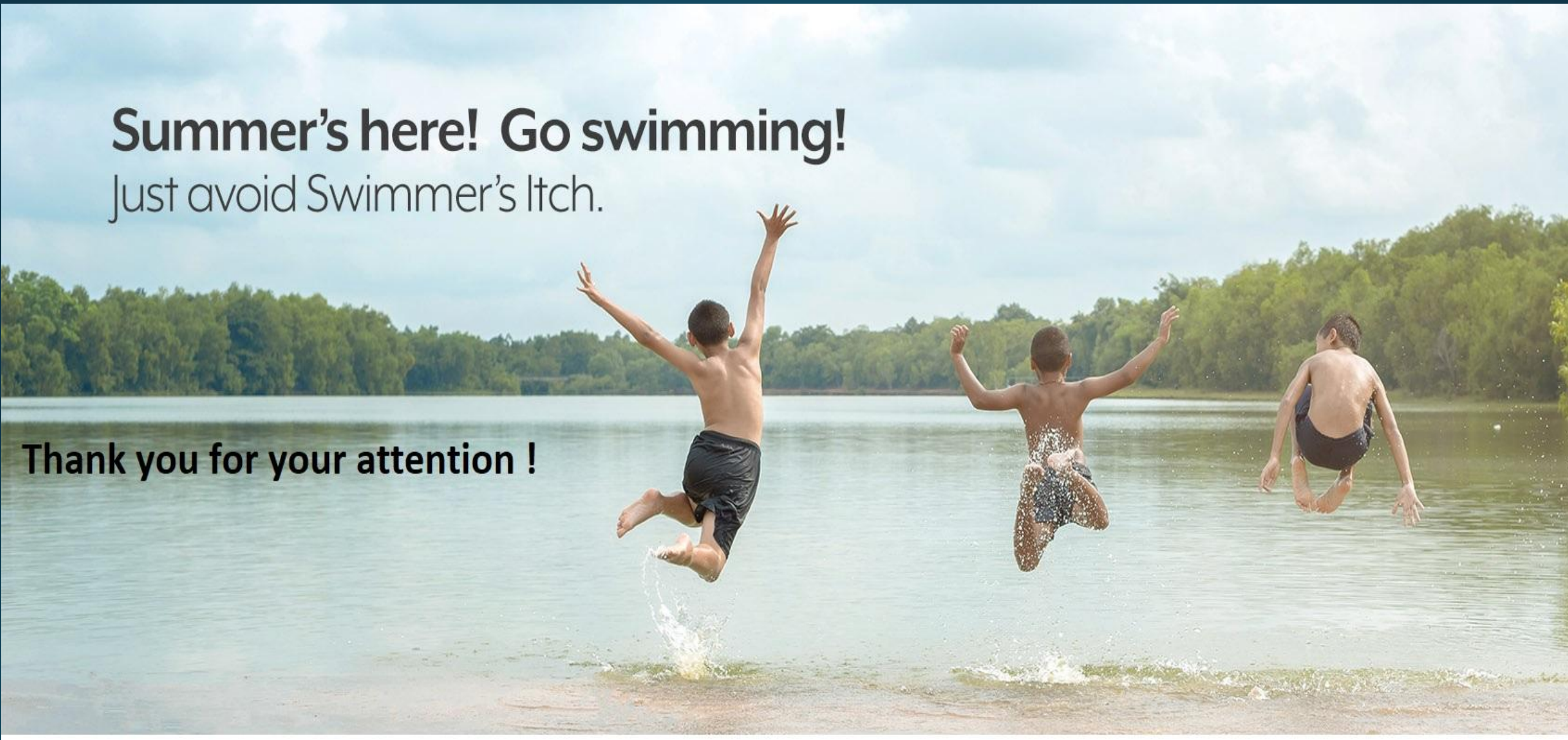
Abbreviations: NL, Netherlands; PL, Poland; CZ, Czech Republic; FJ, Finland; DE, Germany; IS, Iceland; NO, Norway; DK, Denmark; CH, Switzerland

CONCLUSION

- Both our own observations and other scientific reports suggest there is a rationale for instituting of screening studies in the regions of recreational potential, which include the area of north-east Poland.
- An important aspect is dermatologic diagnostics, which primarily relies on history-taking and examining skin lesions.
- In some cases misdiagnosis may be the case, which is associated with rather low specificity of symptoms as well as the state of medical knowledge in that respect.
- Moreover, there is a need to conduct research in the area of north-east of Poland in the future covering water bodies varying in trophicity and origin.

What can be done to reduce the risk of swimmer's itch?

- Do not swim near or wade in marshy areas where snails are commonly found
- Towel dry or shower immediately after leaving the water
- Do not attract birds (e.g., by feeding them) to areas where people are swimming.


Summer's here! Go swimming!
Just avoid Swimmer's Itch.
Thank you for your attention !

Influence of Proton Pump Inhibitors (PPI) and Histamine Receptor 2 Antagonists on *Blastocystis* ST3 and Selected Microorganisms of Intestinal Microbiota *in vitro*

Małgorzata Lepczyńska, Ewa Dzika, WenChieh Chen i Chien-Yu Lu

BLASTOCYSTIS ST3

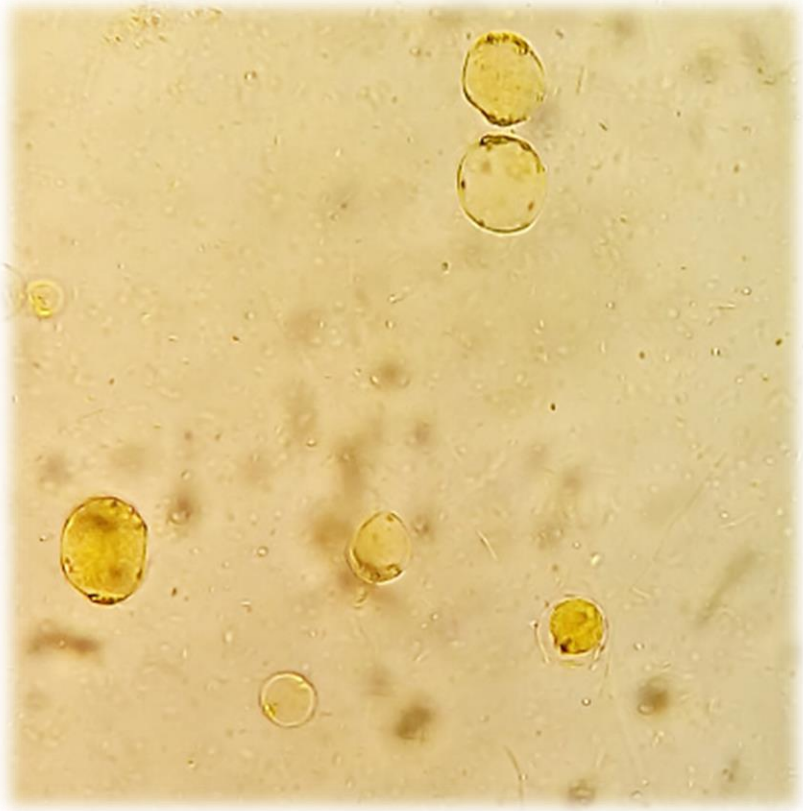
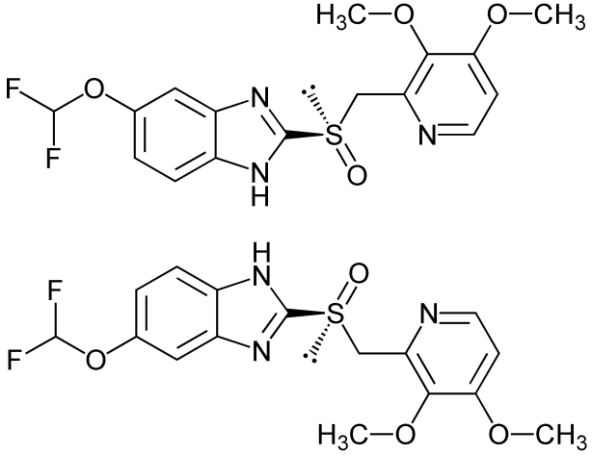


Fig. 1. *Blastocystis* ST3, laboratory culture on Jones' medium; Lugols liquid; under 400x magnification. Photo: M. Lepczyńska

- ***Blastocystis*** is the most common, unicellular intestinal protozoan worldwide
- 17 subtypes were described in the literature (ST1-17)
- The influence on the microorganism most probably have:

1. species composition of bacteria and fungi in the human intestine;
2. diet, medicines, life style

Proton Pump Inhibitors (PPI)



- **PPI** – pantoprazole and esomeprazole – are commonly prescribed medications to treat a variety of medical conditions, including gastroesophageal reflux disease, gastric and duodenal ulcers, non-steroidal-anti-inflammatory drug-induced enteropathy, Zollinger-Ellison syndrome, dyspepsia, and *Helicobacter pylori* infection

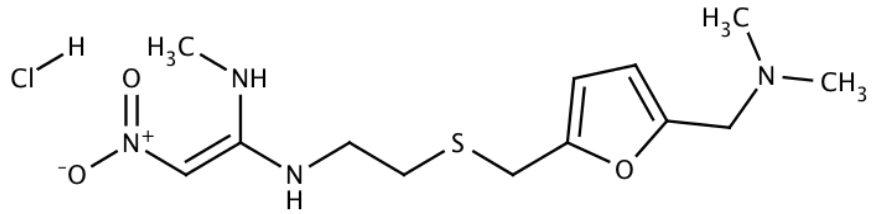
- **PPI** can irreversibly inhibit the H+/K+ ATP pumps of parietal cells in the stomach lining, thus suppress acid production and increase the gastric pH, leading to changes in the composition of gut microbiota and parasitic colonization

- According to the Pérez-Villanueva et al. (2011) research – PPIs have been demonstrated to kill certain human protozoans *in vitro*, such as *Giardia lamblia, Entamoeba histolytica* and *Trichomonas vaginalis*

Histamine type-2 receptor antagonists (H2 blockers)

- **H2 blockers** – cimetidine and ranitidine act by binding to type 2 histamine receptors on the basolateral surface of gastric parietal cells

- H2 blockers interfere with the pathways of gastric acid production and secretion



Aim of the study

- The current study was aimed to determine the *in vitro* sensitivity of selective gut microbiota (*Blastocystis* ST3, *Lactobacillus rhamnosus*, *Enterococcus faecium*, *Escherichia coli*, *Candida albicans)* to PPIs (pantoprazole – PAN and esomeprazole – ESO) and H2 blockers (ranitidine – RAN and cimetidine – CIM) in cell cultures compared to metronidazole as a antibacterial and antiparasitic drug.

Materials

- ***Blastocystis*:** Culture on Jones' medium, 37°C, anaerobic conditions. Subcultivation: every 2-3 days

- **Bacteria:** A lyophilized stock of the organisms in Micro Swab form obtained from ATCC – *L. rhamnosus*, *E. faecium*, *E. coli*. Culture on TSB at 37°C. Subcultivation: every 2 days

- **Fungi:** A lyophilized stock of the organisms in Micro Swab form purchased from ATCC – *C. albicans.* Culture on Sabouraud broth at 24.5°C. Subcultivation: every 6 days

Microorganism	Number of CFU/mL
Escherichia coli	**1.19×10^{9}**
Enterococcus faecium	**1.22×10^{9}**
Lactobacillus rhamnosus	**1.28×10^{9}**
Candida albicans	**1.79×10^{6}**
***Blastocystis* ST3**	**2.9×10^{5}**

Table 1. Concentration of the microorganisms used in experiment

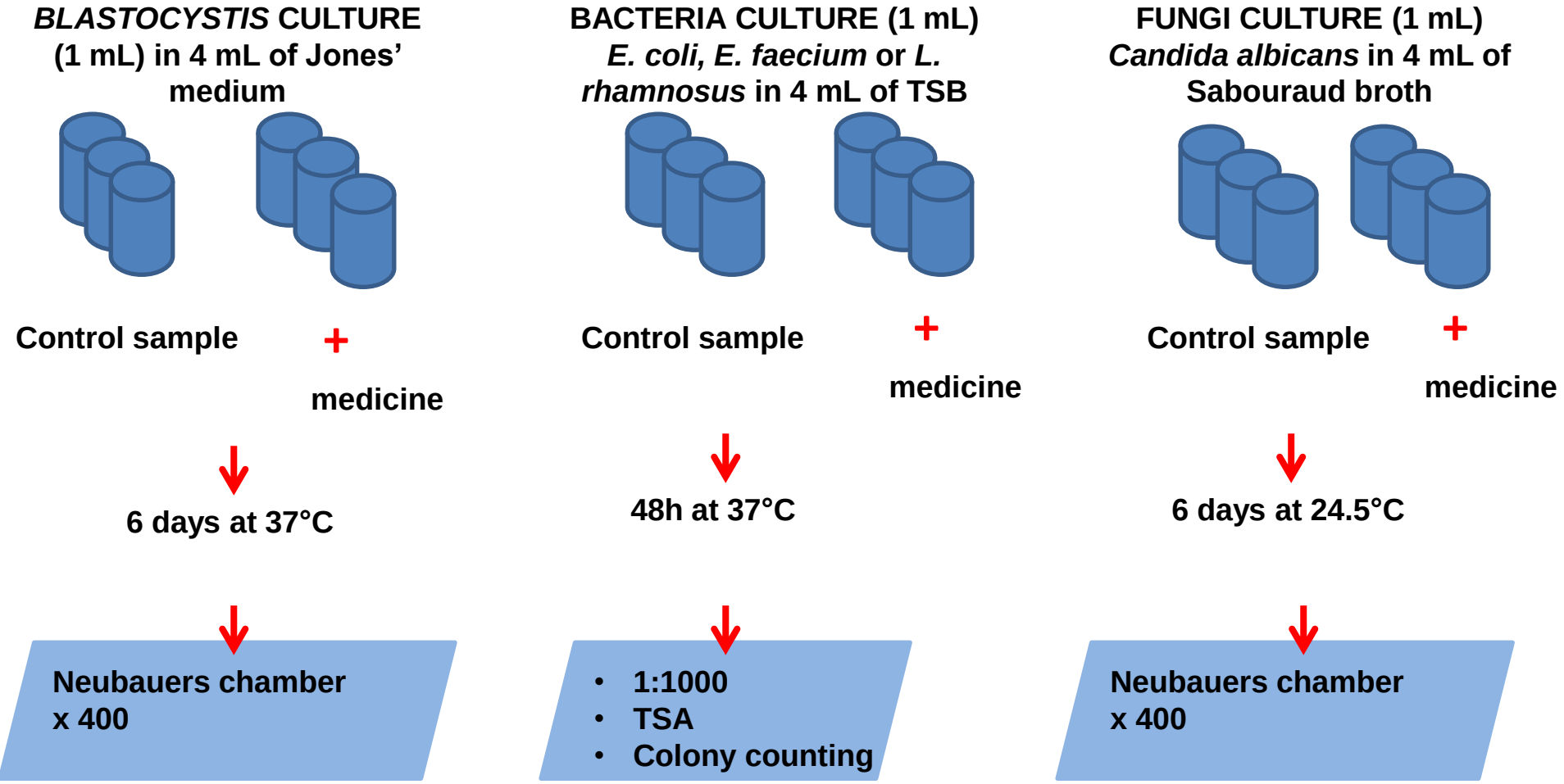
Methods

Experimental set up:

1. Stock solutions of medicines with final concentration of **2 mg/mL** were prepared**.** Three dilutions were used in the experiment: **0.1, 0.06** and **0.02 mg/mL**

2. The final pH value of the solutions was 8.5, 5.8, 5.2 and 6.2 for pantoprazole, esomeprazole, both H2 blockers cimetidine and ranitidine, and metronidazole, respectively.

Methods



All experiments were repeated three times, and the average values reported as results

Fig. 2. The schematic picture of the experimental set up *in vitro.* * Medicine = PAN, ESO, CIM, RAN, MTZ

Methods

Statistical analysis – significance in difference between the drug treatment and the controls was tested by **Student t-test** (GraphPad Prism 8).

The Pearson Chi square was used to compare the effectiveness between medications.

Two-way ANOVA test – was used to determine the influence of the pH condition.

Three-way ANOVA (Tukey's test) was used to evaluate the influence of the drug concentrations adjusted to the incubation time.

A *p* value of **<0.05** was considered statistically significant.

Results

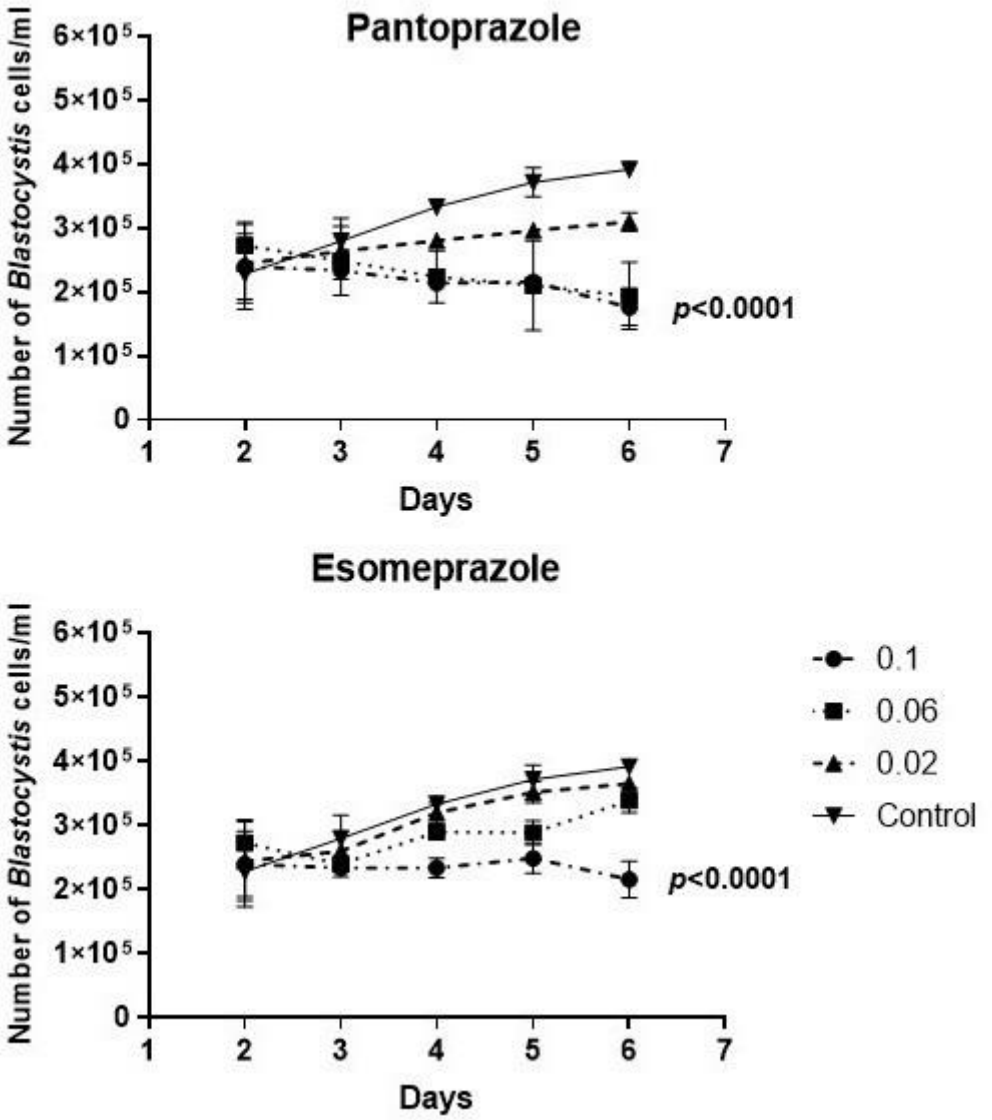


Fig. 3. The influence of different concentrations of pantoprazole and esomeprazole on *Blastocystis* ST3 development according to time of co-incubation

Results

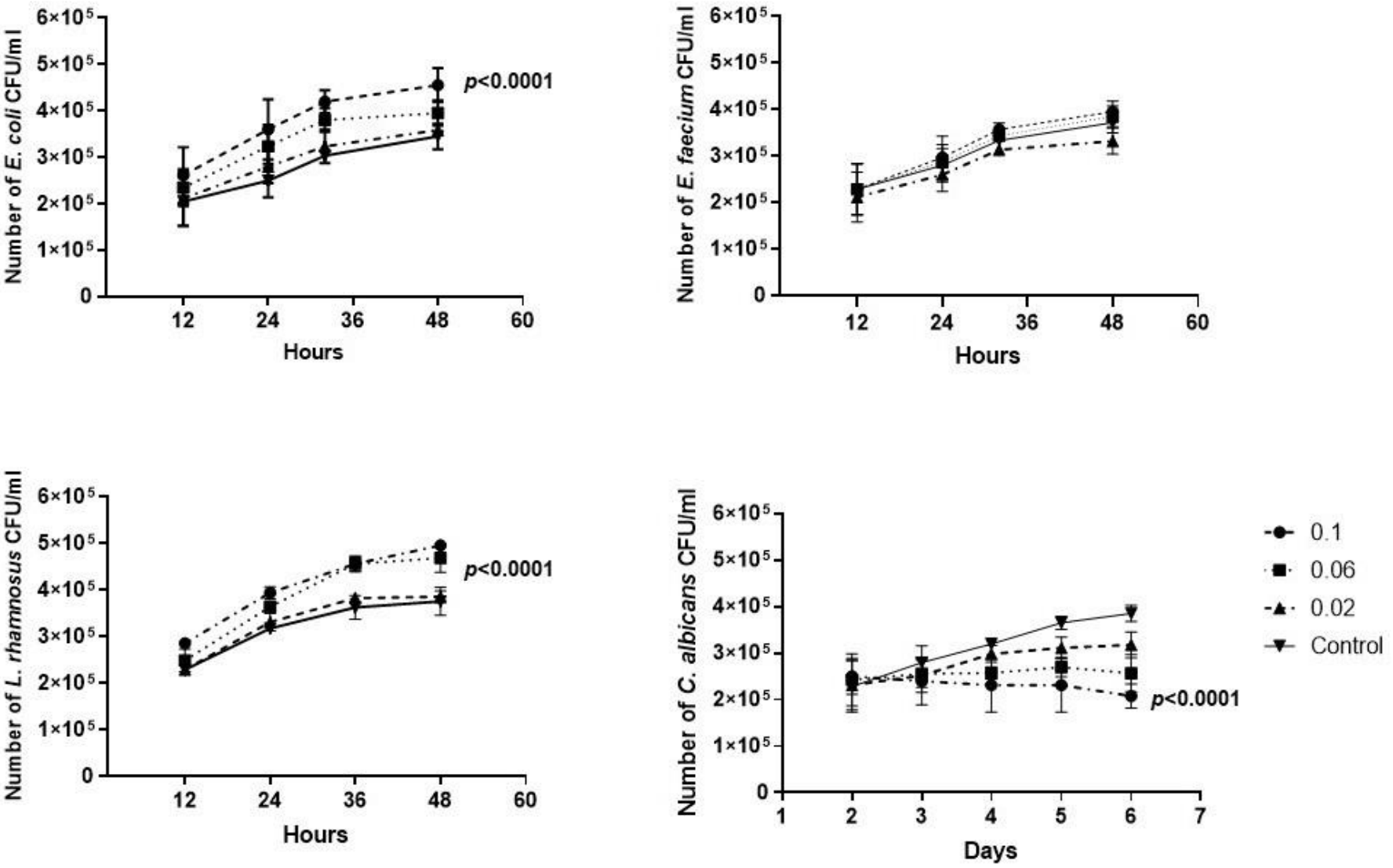


Fig. 4. The influence of different concentrations of pantoprazole on chosen microorganisms development according to time of co-incubation

Results

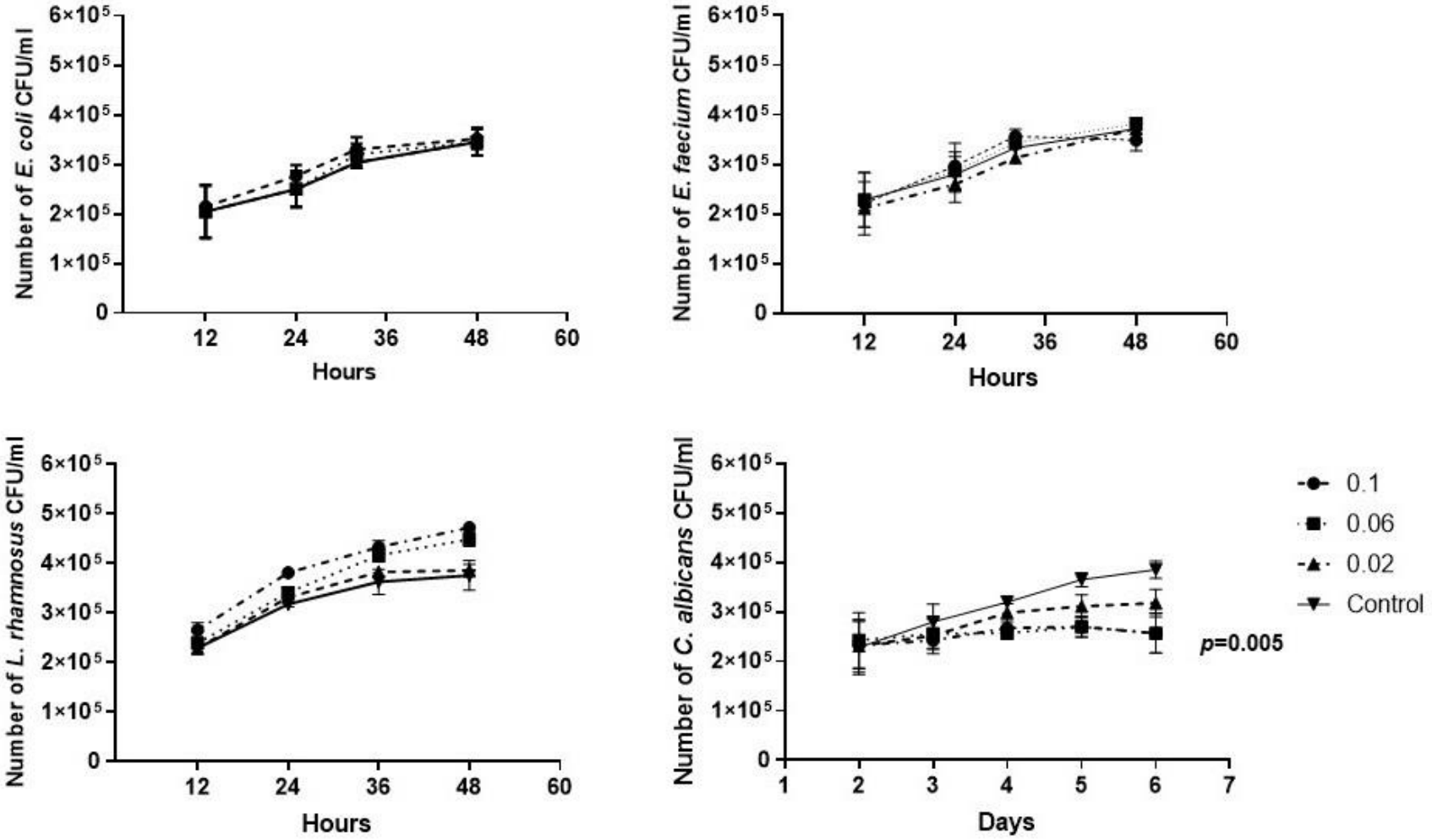


Fig. 5. The influence of different concentrations of esomeprazole on chosen microorganisms development according to time of co-incubation

Conclusions

1. **<u>The research showed that</u>:**

- ✓ PPIs are more effective at inhibition of ***Blastocystis* ST3** and ***C. albicans*** proliferation *in vitro* than antiparasitic drug – metronidazole
- ✓ PPIs causing the increase of ***Lactobacillus*** multiplying
- ✓ Esomeprazole support ***E. coli*** growth
- ✓ H2 blockers does not have any influence on the investigated microorganisms

2. **<u>The influence of PPI on the investigated microorganisms may be:</u>**

- ✓ direct – through the chemical structure of the medicines (benzimidazole derivatives) which act like antiparasitic drugs
- ✓ indirect – modulation of commensal bacteria in the intestine associated with alteration of pH values

Summary

1. Due to their high safety and tolerability, PPIs can be considered for clinical **treatment of intestinal protozoan infections**

2. Further studies are required to prove and explain the PPIs mechanism of action against *Blastocystis* ST3, *C. albicans*, *E. coli* and *L. rhamnosus* **at the cellular level** and to establish the clinically ideal doses and regimens

Thank you for your attention