



Review on the Parasitic Infections in Malayan Sun Bears (*Helarctos malayanus*)

Elden bin Zoumin, Siti Sarayati Hj Abdul Mawah*, Lo Chor Wai and Farnidah Jasnje

Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Cawangan Sabah, Kota Kinabalu Campus, Locked
Bag 71, 89888 Kota Kinabalu, Sabah, Malaysia

Corresponding author: sarayati@uitm.edu.my

Accepted: 26 May 2022; Published: 24 June 2022

ABSTRACT

The Malayan sun bear is the smallest bear species on earth and remains the most diminutive studied bear species. Due to its status as a vulnerable species listed by the IUCN Red list, several conservation efforts have been made, including establishing a conservation centre where confiscated or saved bears were rehabilitated before being released into the wilderness. Thus, it is crucial to know the type of parasite that could negatively impact the bears and thus maintain their welfare in captivity. Parasitic infection is the condition of a parasite infection that has adverse effects on an organism. Intestinal parasitic infection occurs when a parasite infects the gastrointestinal tract of humans or animals. This review paper discusses the recorded parasitic gastrointestinal infections in Malayan sun bears and the methods used to identify those parasites. The review method used was descriptive review. The parasites commonly infecting the Malayan sun bears are *Ancylostoma* sp. and *Trichuris* sp. Reports in 1916 and 1929 state that *Ancylostoma* sp. and *Baylisascaris* sp. can infect the Malayan sun bear. In 2016, there was a report of *Enterocytozoan bienneusi* found in Malayan sun bear. Methods used to identify gastrointestinal parasites from faecal samples are the formalin-ether sedimentation technique, centrifugal floatation method, conventional or passive floatation method, and faecal floatation method using the McMaster counting chamber. In summary, information on the parasitic species that infect the gastrointestinal tract of Malayan sun bears is scarce. The most recent study of the gastrointestinal parasite in Malayan sun bear was in 2019, and the identification was only up to the genus level. Detailed identification of gastrointestinal parasites from the Malayan sun bears faecal samples via a molecular technique such as polymerase chain reaction is much needed. Lastly, this review was made to help veterinarians and conservationists maintain Malayan sun bears' welfare in captivity.

Keywords: *Gastrointestinal parasite, Malayan sun bear, Fecal sample*

INTRODUCTION

The Malayan sun bear (*Helarctos malayanus*) is the smallest bear species in the world. The adults can have a body length from 120 to 150 cm and weigh about 27 to 80 kg [1]. The Malayan male sun bear has a 10% to 20% larger body size than the female. They have long and protractible tongues of 20–25 cm to feed on honey and insects. Though they are the smallest bear species, they have large canines and high bite force in relation to their body sizes. Their jet-black fur is short and sleek with some underwool. A crescent-shaped pale patch on the breast varies individually in colour ranging from buff, cream, or dirty white to ochreous [1].

The Malayan sun bear is the least studied bear species in the world, and little is known about what kind of parasite could infect them. As their population in the wild has been declining at the rate of 30% for the past 30 years, several conservation efforts have been made, including establishing a rehabilitation centre. In the rehabilitation centre, Malayan sun bears saved from poachers or traps were being held captive to be treated and rehabilitated before being released back into the wild [2]. Knowing what kind of gastrointestinal parasite could infect them would help the conservationist maintain the welfare of those captive sun bears.

Intestinal parasite infection is when parasites infect the gastrointestinal tract of living organisms like humans and animals. Although able to live anywhere inside the body of its host, the parasites prefer to live in the intestinal wall. Such infection can occur due to ingesting undercooked meals or meat, drinking water contaminated with the parasite's eggs and larvae, skin penetration, and faecal-oral transmission. Parasitic diseases are among the most prevalent and important infectious diseases in wildlife. Regarding free-ranging wild animals, parasitism is the norm rather than the exception [3].

Regarding the gastrointestinal parasite infection on captive animals, some parasites are pathogenic, and some are commonly found inside the animal's gastrointestinal tract. However, gastrointestinal severe parasite infection from common parasites still brings a detrimental effect to its host. Heavy infection can cause anaemia, causing blood loss, lack of nutrients, and other effects [3]. Thus, this review explores the gastrointestinal parasite found in the Malayan sun bear (*Helarctos malayanus*) in recent years, their impact on the host, and highlights the diagnosis used to identify the parasitic gastrointestinal infection in the intestine of the Malayan sun bear.

Gastrointestinal Parasites Recorded in the Malayan Sun Bear

Ancylostoma sp. Infection

Early discovery of gastrointestinal parasites in bears dated back to 1916 by Lane [4] on the genus *Ancylostoma* in India and Ceylon. The study found the parasite *Ancylostoma malayanum* inside a Malayan sun bear held captive in India and Ceylon. Baylis and Daubney [5] discovered the same parasite in a Malayan sun bear held captive in India. Whether the parasites originated from the same bear is not known

as both studies [4, 5] did not mention the location of the Malayan sun bear being held captive in India at that time.

Ancylostoma sp. is generally known as hookworm. A review by Rogers and Rogers [6] mentioned the studies by Lane [4] and Baylis and Daubney [5], reporting the presence of the parasite *A. malayanum* in Malayan sun bear. Similarly, Mahannop et al. [7] reported the presence of *Ancylostoma sp.* eggs in the faecal sample of the Malayan sun bear in the exotic animal division at Dusit Zoo, Thailand. In Indonesia, *Ancylostoma sp.* eggs were found in the Malayan sun bear stool samples at Taman Megasatwa, Medan [8]. According to the Global Health Division of Parasitic Disease and Malaria [9], a severe infection of hookworms or *Ancylostoma sp.* can cause blood loss, which leads to anaemia and additional protein loss.

***Baylisascaris sp.* Infection**

The endoparasite *Baylisascaris transfuga* infects every bear species, including the Malayan sun bear, except the Andean spectacled bear. The parasite was discovered by Canavan [10] in the Malayan sun bear in 1926 to study the nematode parasite of vertebrates in the Philadelphia Zoological Garden and its vicinity. This record was then mentioned in a review by Sapp et al. [11].

The effect of parasite infection from the genus *Baylisascaris* in Malayan sun bear has not been reported recently. However, several reports mention its effects on other species of bears and animals. *Baylisascaris transfuga* causes neurological diseases such as epilepsy and posterior paralysis in a colony of Japanese macaques (*Macaca fuscata fuscata*) [12]. The macaques share similar habitats with the American black bear known to harbour the parasite in a safari-style zoo in Japan.

Meanwhile, a heavy infestation of *B. transfuga* in brown bears (*Ursus arctos*) is fatal since the parasite can cause granulomatous peritonitis disease, an inflammation of the abdominal tissue and the liver capsule [13, 14]. In China, the conservation effort to save the giant panda was challenged by the parasite *Baylisascaris schroederi*. This parasite is known to be fatal to the giant pandas as it causes serious health complications on them, such as obstruction of the intestinal wall [15].

***Trichuris sp.* Infection**

The parasite *Trichuris sp.*, known as whipworm, can also be found in Malayan sun bear. The eggs of *Trichuris sp.* were found inside stool samples of the Malayan sun bear in Taman Megasatwa, Medan, Indonesia [8]. Although no record of the effects of the genus *Trichuris* on Malayan sun bear is available, several studies reported that this parasite affects the health of other bear species. This parasite has been documented in the stool samples or gastrointestinal tracts of other bears, e.g., brown bears and Asiatic black bears [16-18]. Heavy infestation of *Trichuris sp.* can cause anaemia and rectal prolapse [8, 19].

***Enterocytozoan bienneusi* Infection**

Enterocytozoan bienneusi is microsporidia and belongs to Kingdom Fungi. This parasite commonly infects humans. It colonizes on the epithelium of the small intestines of its host, within the apical portion of the villus. This parasite can infect the host by drinking or eating water and food contaminated with its spore. The infection of *Enterocytozoan bienneusi* can cause self-limiting diarrhoea in a healthy host. However, if infection occurs on the immunocompromised host, it will cause chronic diarrhoea, which can be life-threatening. This parasite was found to infect a captive Malayan sun bear in Chengdu Zoo, and that is the first evidence of infection of *E. bienneusi* in a Malayan sun bear [20].

Other Gastrointestinal Parasites Found in Malayan Sun Bear

Another gastrointestinal parasite in the Malayan sun bear is the cestodes, mainly known as tapeworms. A Malayan sun bear was infected with the *Pentorchis arkteios* cestode in 1927 [6, 21, 22]. The only report of tapeworm infection in Malayan sun bear was in Myanmar by Meggit [21] and later reviewed by Rogers and Rogers [5] and Schaul [22]. The summary of known gastrointestinal parasites that infect Malayan sun bear and other bear species is shown in Tables 1 and 2, respectively.

Table 1: Gastrointestinal parasites recorded in the Malayan sun bear

Parasite	Reported by	Locations	Reviewed in
<i>Ancylostoma malayanum</i>	[4]	Held captive in India and Ceylon	[6]
	[5]	Held captive in India	
<i>Ancylostoma</i> sp.	[7]	Held captive in Dusit Zoo, Thailand	
	[8]	Taman Megasatwa, Medan, Indonesia	
<i>Baylisascaris transfuga</i>	[10]	Philadelphia Zoological Garden, Pennsylvania, United States of America	[11]
<i>Enterocytozoan bienneusi</i>	[20]	Chengdu Zoo, China	
<i>Trichuris</i> sp.	[8]	Taman Megasatwa, Medan, Indonesia	
<i>Pentorchis arkteios</i>	[21]	Victoria Memorial Park, Rangoon, Myanmar	[6]

Table 2: Gastrointestinal parasites recorded in other species of bears and animals

Parasite	Host	Effects	Locations	Reported by
<i>Baylisascaris transfuga</i>	American black bear	Heavy infection can cause blockage of the gastrointestinal tract.	United States of America	[22]
	Japanese macaque	Cause neurological diseases such as epilepsy and posterior paralysis.	Japan	[12]
	Brown bears	Heavy infection can cause a fatal disease known as granumalotous peritonitis.	Republic of Slovakia	[13, 14]
<i>Baylisascaris schroederi</i>	Giant panda	Heavy infection can obstruct the intestinal wall.	Republic of China	[15]
<i>Trichuris sp.</i>	Brown bears	Not mentioned in the report.	Poland	[16]
		Not mentioned in the report.	Cantabrian Mountains, Spain	[17]
	Asiatic black bears	Not mentioned in the report	Bangladesh.	[18]

Reviewing Method and Methods Used to Identify Gastrointestinal Parasites in Sun Bears

Review Methodology

The method of review used in this review paper is descriptive review. This method aims to determine the body of knowledge in a particular research topic that reveals any interpretable trend concerning pre-existing propositions, theories, methodologies and findings. By this method, several studies related to the topic of the review paper were searched, screened, and classified by specifically targeted characteristics such as research findings, research methodologies and publication year [23].

For example, in this review, since the objective is to highlight the gastrointestinal parasite infection in Malayan sun bear and the methodologies used to identify those parasites, only studies that have information about the parasitic infection in Malayan sun bear were searched. Then, those studies will be

screened to focus on the gastrointestinal parasite only and the methodologies used to identify those parasites.

The Formalin-Ether Sedimentation Technique

This method **uses** solutions of lower specific gravity than the parasite eggs or cyst, thus concentrating the latter in the sediment. Formalin fixes the eggs, larvae, oocysts, and spores, preventing them from being infectious and to preserves their morphology [24]. The formalin-ether sedimentation method was performed by suspending 0.5 g of faecal sample in a 0.9% saline solution and filtered using a layer of gauze. The filtrate was centrifuged at $700 \times g$ (2100 rpm) for 2 minutes. The sediment formed was then suspended in 10% formalin for 3 minutes. Then, 3 mL of ether was added, and the tube was shaken for 30 seconds. After that, the tube was centrifuged again, and the debris plug was recovered [25]. This method was used by Mahannop et al. [7] to identify the parasite *Ancylostoma* sp. in Malayan sun bears. Wang et al. [15] reviewed that this same method was used to identify *Baylisascaris* sp. from a faecal sample of the giant panda.

The Fecal Floatation Method

The faecal floatation method is where the faecal sample of studied animals were mixed with a special liquid with specific gravity, which causes the parasites' eggs to float on the surface of the liquid. Concentrated sugar solution (Sheater's sugar solution) or salt solutions made from zinc sulfate ($ZnSO_4$), sodium nitrate ($NaNO_3$) or sodium chloride ($NaCl$) were commonly used in this method. There are three types of floatation faecal method: the passive floatation method, centrifugal floatation method and floatation using the McMaster counting chamber slide [26].

Jenantika et al. [8] used the centrifugal floatation and faecal floatation methods using a McMaster counting chamber to identify parasite eggs from the faecal sample of Malayan sun bear. Their study identified the egg of *Ancylostoma* sp. using the centrifuge method. First, 2 g of the faecal sample was mixed with sufficient water and then stirred homogeneously. The mixture was then transferred into a centrifuge tube and centrifuged for 5 minutes at 1500 rpm. Then, the supernatant was discarded, and the centrifuge tube was filled with saturated sodium chloride ($NaCl$) solution until $3/4$ th of the centrifuge tube. The solution was centrifuged at the same speed and time, and the supernatant was discarded. The centrifuge tube was filled again with saturated $NaCl$ solution until it was complete and a convex formed at the mouth of the tube. The tube was left to stand for 10 minutes, and a cover slip was touched to the convex at the mouth of the tube and placed onto a glass slide for microscopic observation.

For the floatation method using McMaster counting chamber slide, 2 g of the sun bear's faecal sample was mixed well with 28 mL distilled water. Next, 1 mL of the mixture was mixed with 1 mL of Sheather's sugar solution and transferred into the McMaster counting chamber for observation [8]. Through this method, the author could see the egg of *Trichuris* sp. from the faecal sample of the Malayan sun bear.

In other studies of the gastrointestinal parasite of other species of bears, such as the study on *Baylisascaris* sp. in the American black bear by Schaul [22], passive floatation using sodium nitrate solution and the centrifugal floatation method using Sheather's sugar solution 454 g granulated sugar dissolved in

355 mL water) was used. The passive floatation method was done by suspending 5 g of the faecal sample in sodium nitrate solution in a centrifuge tube, and a coverslip was placed at the top of the convex formed at the mouth of the tube. The author also uses the centrifugal floatation method similar to Jenantika et al. [8], but with different solutions and centrifuge speed. Schaul [22] used Sheather's sugar solution in the centrifugal floatation method, and the faecal sample was centrifuged at 1100 rpm for 5 minutes.

Meanwhile, Štrkolcová et al. [13] used the conventional floatation method to identify *Baylisascaris sp.* eggs from faecal samples of the brown bears. The floatation method used by the author was similar to the passive floatation method by Schaul [22]. However, the authors used the Breza solution (saturated magnesium sulfate solution mixed with saturated sodium sulfate and water) and the Faust solution (371 g zinc sulfate dissolved in 1 L distilled water).

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique where a small fragment of deoxyribonucleic acid (DNA) is amplified into millions of copies so that it can be studied thoroughly. The process involves the use of the enzyme of Taq-DNA polymerase (a thermo-stable enzyme that controls the DNA amplification process), a set of DNA primers which target the selected genome segment to be amplified and multiple rounds of DNA synthesis to amplify the targeted segment [27, 28].

Li et al. [20] used this method to study the *Enterocytozoon bieneusi* in captive wildlife at zoological gardens in China. Through the nested PCR method, the author manages to identify the parasitic microsporidia in Malayan sun bear that were held captive in Chengdu Zoo. Briefly, the method started with extracting the genomic DNA from the faecal sample using a specific DNA Extraction kit for stool samples.

After the extraction of genomic DNA, the targeted ITS gene of the parasite was amplified through PCR. After the ITS gene was amplified, the targeted microsatellites (MS1, MS3 and MS7) and minisatellite (MS4) were amplified. The secondary product of the PCR reaction was visualized by gel electrophoresis, and the targeted DNA band was extracted from the gel. It was sent to Invitrogen (Shanghai, China) for two-directional sequencing analysis [20].

Summary of the Study of Parasite Infection in the Malayan Sun Bear

Status of the Study of Parasite Infection in Malayan Sun Bear

Identifying the gastrointestinal parasites in the Malayan sun bear and their effect on its health is crucial. Jenantika et al. [8] and Li et al. [20] are the researchers who have provided the latest data about possible parasites that can infect the gastrointestinal tract of the Malayan sun bear in Asia and Southeast Asia. Despite other reports that mentioned the parasites found in Malayan sun bears, they are more than ten years old and have been reviewed or mentioned many times in the latest article. The report by Jenantika et al. [8] is the latest parasite report in the Malayan sun bear, but they only identify the parasite up to genus level.

Most reviewed studies use microscopy techniques to identify the parasites' genus and species. The only study by Li et al. [20] used a molecular approach to identify *Enterocytozoan bienneusi*.

The study of parasite infection in Malayan sun bears and their effects are vital as this data can help save this endangered bear species. The latest data on the types of parasites that infect the Malayan sun bear can help the authorities involved in the conservation of the Malayan sun bear, provide a place with better sanitation, and control the spread of gastrointestinal parasite parasites in the conservation centre. By providing the info about the type of gastrointestinal parasite that could infect Malayan sun bear and their effect through this review paper, veterinarians and conservationists at the rehabilitation centre can determine the type of anti-parasitic treatment for the infected bear thus maintaining their welfare in the rehabilitation centre.

Recommendation for Using Molecular Approach to Identify Parasite in Malayan Sun Bear

A molecular approach to identify the parasite found in Malayan sun bear is recommended as it provides more accurate data since it detects the DNA of the parasite. Polymerase chain reaction (PCR) is a way to identify the parasite species via a molecular approach [20].

In PCR, the targeted DNA of the parasites was amplified or replicated into millions or billions of copies so that it can be used to study the DNA molecule in detail. The method started by extracting the genomic DNA from the sample using an extraction kit designed specifically for the selected sample. For example, if the DNA is meant to be extracted from a faecal sample, then the extraction kit that must be used is a kit that was designed to extract DNA from faecal samples [20, 27, 28].

After the extraction of genomic DNA from the samples, the process continues by amplifying or replicating the targeted DNA using specific primers and PCR kits. In PCR, the thermocycler setting for annealing must be set following the type of primers used. Table 3 shows the annealing temperatures that were used by Li et al. [20] in their PCR process to identify *Enterocytozoan bienneusi*.

Table 3: Primers and annealing temperatures that were used to detect *Enterocytozoan bienneusi*

Targeted gene	Primer sequences (5'–3')	Annealing temperature (°C)	Expected product size (bp)
ITS	F1: GATGGTCATAGGGATGAAGAGCTT, R1: AATACAGGATCACTTGGATCCGT	55	410
	F2: AGGGATGAAGAGCTTCGGCTCTG, R2: AATATCCCTAATACAGGATCACT	55	392
MS1	F1: CAAGTTGCAAGTTCAGTGTTTGA, R1: GATGAATATGCATCCATTGATGTT	58	843
	F2: TTGTAAATCGACCAAATGTGCTAT, R2: GGACATAAACCCTAATTAATGTAAC	58	676
MS3	F1: CAAGCACTGTGGTTACTGTT, R1: AAGTTA GGGCATTTAATAAAAATTA	55	702
	F2: GTTCAAGTAATTGATACCAGTCT, R2: CTCATTGAATCTAAATGTGTATAA	55	537
MS4	F1: GCATATCGTCTCATAGGAACA, R1: GTTCATGGTTATTAATTCCAGAA	55	965
	F2: CGA AGTGTACTACATGTCTCT, R2: GGACTTTAATAAGTTACCTATAGT	55	885
MS7	F1: GTTGATCGTCCAGATGGAATT, R1: GACTATCAGTATTACTGATTATAT	55	684
	F2: CAATAGTAAAGGAAGATGGTCA, R2: CGTCGCTTTGTTTCATAATCTT	55	471

CONCLUSION

In conclusion, past studies show that a Malayan sun bear can be infected with four gastrointestinal parasites: *Ancylostoma sp.*, *Trichuris sp.*, *Baylisascaris transfuga* and *Enterocytozoan bienneusi*. Most studies in the past, including a recent study in 2019, use floatation methods to identify the gastrointestinal parasites from the faecal sample of the Malayan sun bear. The only study made in 2016 used a molecular approach via PCR to identify the presence of *Enterocytozoan bienneusi* inside the gastrointestinal tract of a captive Malayan sun bear at Chengdu Zoo, China. This review is essential as it provides information on the types of gastrointestinal parasites that infect the Malayan sun bear. It also provides information on the effect of the gastrointestinal parasites on the sun bears. This review can be a valuable reference for veterinarians and conservationists as it will help them determine the anti-parasitic treatment to be used if the bears were infected by the parasites mentioned in this review and maintain their welfare in rehabilitation centres.



ACKNOWLEDGMENTS

The authors would like to express gratitude to the Universiti Teknologi Mara (UiTM) for providing the facilities for this research.

AUTHOR'S CONTRIBUTION

Elden Zoumin carried out the research and wrote and revised the article. Siti Sarayati Hj Abdul Mawah, Lo Chor Wai, Farnidah Jasnien and Eldem Zoumin conceptualized the central research idea and provided the theoretical framework. Lo Chor Wai and Elden Zoumin designed the research. At the same time, Farnidah Jasnien and Siti Sarayati Hj Abdul Mawah supervised the research progress, anchored the review and revisions and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

- [1] Scotson, L., Fredriksson, G., Augeri, D., Cheah, C., Ngoprasert, D., & Wai-Ming, W. (2017). *Helarctos malayanus* (errata version published in 2018). The IUCN Red List of Threatened Species 2017.
- [2] Asher, C. (2016). Malayan sun bear: bile trade threatens the world's smallest bear. Mongabay, August, 1–12. <https://news.mongabay.com/2016/08/malayan-sun-bear-bile-trade-threatens-the-worlds-smallest-bear/>
- [3] Ray, G. (2019). Parasite load and disease in wild the extent of parasitism in nature what is the parasite load on the typical wild. *Wild Animal Suffering Research*, 1–25.
- [4] Lane, C. (1916). The genus *Ancylostoma* in India and Ceylon. *Indian Journal of Medical Research*, 4(1), 74.
- [5] Baylis, H. A., & Daubney, R. (1922). Report on the parasitic nematodes in the collection of the zoological survey of India. *In Memoirs of the Indian Museum* (Vol. 7, Issue 4, pp. 264–347).
- [6] Rogers, L. L., & Rogers, S. M. (1976). Parasites of bears: A review. *Bears: Their Biology and Management*, 3, 411.

- [7] Mahannop, A., Keittivuti, A., & Mahannop, P. (1984). Survey of intestinal parasitism in the exotic animals at Dusit Zoo, Thailand. Eleventh Annual Veterinary Conference Bangkok, Thailand, 12-14 Dec 1984, 351–353. <https://agris.fao.org/agris-search/search.do?recordID=TH9121313>
- [8] Jenantika, P. U., Fahrimal, Y., Sayuti, A., Studi, P., Dokter, P., Fakultas, H., Hewan, K., Syiah, U., Parasitologi, L., Fakultas, V., Hewan, K., Syiah, U., Medan, T. M., & Madu, B. (2019). Identifikasi parasit gastrointestinal pada Beruang madu (*Helarctos malayanus*) di Taman Margasatwa Medan. *Jurnal Ilmiah Mahasiswa Veteriner*, 3(3), 142–148.
- [9] Global Health Division of Parasitic Disease and Malaria. (2020a). Parasites - Hookworm. Centers for Disease Control and Prevention. <https://www.cdc.gov/parasites/hookworm/index.html>
- [10] Canavan, W. P. N. (1929). Nematode parasites of vertebrates in the Philadelphia zoological garden and vicinity. *Parasitology*, 21(1–2), 63–102
- [11] Sapp, S. G. H., Gupta, P., Martin, M. K., Murray, M. H., Niedringhaus, K. D., Pfaff, M. A., & Yabsley, M. J. (2017). Beyond the raccoon roundworm: The natural history of non-raccoon *Baylisascaris* species in the New World. *International Journal for Parasitology: Parasites and Wildlife*, 6(2), 85–99.
- [12] Sato, H., Une, Y., Kawakami, S., Saito, E., Kamiya, H., Akao, N., & Furuoka, H. (2005). Fatal *Baylisascaris larva migrans* in a colony of Japanese macaques kept by a safari-style zoo in Japan. *Journal of Parasitology*, 91(3), 716–719.
- [13] Štrkolcová, G., Goldová, M., Šnábel, V., Špakulová, M., Orosová, T., Halán, M., & Mojžišová, J. (2018). A frequent roundworm *Baylisascaris transfuga* in overpopulated brown bears (*Ursus arctos*) in Slovakia: a problem worthy of attention. *Acta Parasitologica*, 63(1), 167-174.
- [14] Szczepaniak, K., Listos, P., Lopuszynski, W., Skrzypek, T., & Kazimierczak, W. (2012). Granulomatous peritonitis in a European brown bear caused by *Baylisascaris transfuga*. *Journal of Wildlife Diseases*, 48(2), 517-519.
- [15] Wang, T., Xie, Y., Zheng, Y., Wang, C., Li, D., Koehler, A. V., & Gasser, R. B. (2018). Parasites of the giant panda: a risk factor in the conservation of a species. *Advances in parasitology*, 99, 1-33.
- [16] Borecka, A., Gawor, J., & Zieba, F. (2013). A survey of intestinal helminths in wild carnivores from the Tatra National Park, southern Poland. *Annals of Parasitology*, 59(4).
- [17] Costa, H., Hartasánchez, R., Santos, A. R., Camarão, A., Cruz, L., Nascimento, M., ... & de Carvalho, L. M. M. (2022). Preliminary findings on the gastrointestinal parasites of the brown bear (*Ursus arctos*) in the Cantabrian mountains, Spain. *Veterinary Parasitology: Regional Studies and Reports*, 28, 100681.
- [18] Liza, F. T., Mukutmoni, M., & Begum, A. (2020). Gastrointestinal Parasites of Captive Asiatic Black Bear in Three Zoological Parks of Bangladesh. *Bangladesh Journal of Zoology*, 48(1), 119-125.
- [19] Global Health Division of Parasitic Disease and Malaria. (2020b). Trichuriasis. Centers for Disease Control and Prevention. <https://www.cdc.gov/parasites/whipworm/>
- [20] Li, W., Deng, L., Yu, X., Zhong, Z., Wang, Q., Liu, X., ... & Peng, G. (2016). Multilocus genotypes and broad-host-range host range of *Enterocytozoon bienersi* in captive wildlife at zoological gardens in China. *Parasites & Vectors*, 9(1), 1-9.
- [21] Meggitt, F. J. (1931). On Cestodes Collected in Burma. Part II. *Parasitology*, 23(2), 250–263.
- [22] Schaul, J. C. (2006). *Baylisascaris transfuga* in captive and free-ranging populations of bears (Family: Ursidae) [The Ohio State University]. In Ohio State University.

- https://etd.ohiolink.edu/apexprod/rws_etd/send_file/send?accession=osu1163172292&disposition=attachment
- [23] Pare, G., & Kitsiou, P. (2016). Methods for Literature Reviews. In F. Lau & C. Kuziemy (Eds.), *Handbook of eHealth Evaluation: An Evidence-based Approach* [Internet] (pp. 157–180). University of Victoria. <https://www.ncbi.nlm.nih.gov/books/NBK481583/>
- [24] Tankeshwaya, A. (2022). Formal Ether Sedimentation techniques. *Microbe Online*. <https://microbeonline.com/formal-ether-sedimentation-techniques/>
- [25] Uga, S., Tanaka, K., & Iwamoto, N. (2010). Evaluation and modification of the formalin-ether sedimentation technique. *Trop Biomed*, 27(2), 177-84.
- [26] Haller, J. (2021). The Veterinary Nurse's Guide to Fecal Flotation Techniques. *Today's Veterinary Nurse*. <https://todaysveterinarynurse.com/clinical-pathology/the-veterinary-nurses-guide-to-fecal-flotation-techniques/>
- [27] Caetano-Anollés, D. (2013). Polymerase Chain Reaction. In *Brenner's Encyclopedia of Genetics: Second Edition* (Vol. 5). Elsevier Inc. <http://dx.doi.org/10.1016/B978-0-12-374984-0.01186-4>
- [28] Smith, M. (2022). Polymerase chain reaction. National Human Genome Research Institute. <https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction>