

Identification of endoparasites in cow faeces in the working area of the Maros Veterinary Center

Icha Ayunita Kahby¹, Syarif Hidayat Amrullah^{1*}, Dirhamzah¹, Hadi Purnama Wirawan²

¹Department of Biology

Faculty of Science and Technology, Universitas Islam Negeri Alauddin Makassar
H. M. Yasin limpo Street No. 36, Gowa, South Sulawesi, Indonesia. 92118.

²Balai Besar Veteriner Maros

DR. Ratulangi Street, Maros, South Sulawesi, Indonesia. 90514

*E-mail: syarifhidayat.amrullah@uin-alauddin.ac.id

Abstrak: Sapi merupakan salah satu hewan ternak yang banyak dimanfaatkan oleh manusia untuk memenuhi kebutuhannya seperti daging dan susu. Namun kondisi peternakan yang kurang bersih dapat menjadi faktor penyebab penurunan produktivitas ternak yang diakibatkan oleh infeksi parasit. Penelitian ini bertujuan untuk mengetahui jenis endoparasit, gejala yang ditimbulkan, cara mengidentifikasi dan kekerabatan antar jenis endoparasit yang ditemukan pada feses sapi yang diamati. Metode yang digunakan dalam penelitian ini ada dua metode yaitu metode apung (uji flotasi) dan metode sedimentasi. Hasil yang didapatkan dari 21 sampel feses sapi terdapat 7 sampel yang positif untuk masing-masing metode dengan 5 spesies yang berbeda yaitu *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp., *Paramphistomum* sp., dan *Eimeria* sp.. Setiap spesies akan menimbulkan gejala yang hampir sama seperti terjadi penurunan nafsu makan, penurunan berat badan, terlambatnya pertumbuhan, gangguan metabolisme tubuh, diare, dan beberapa gejala klinis lainnya yang dapat mengakibatkan turunnya produktivitas ternak. Kunci determinasi digunakan dalam menentukan filum dan kelas dari spesies yang didapatkan berdasarkan ciri-cirinya, hasil yang didapatkan yaitu spesies *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp. berada dalam filum yang sama Nematelminthes dan kelas Nematoda, spesies *Paramphistomum* sp. berada dalam filum Platyhelminthes dari kelas Trematoda, dan spesies *Eimeria* sp. berada dalam filum Protozoa dari kelas Sporozoa. Pohon filogeni digunakan untuk melihat tingkat kekerabatan dari suatu spesies, seperti pada hasil yang didapatkan bahwa *Strongyloides* sp., *Oesophagostomum* sp., dan *Cooperia* sp. berada satu cabang monofiletik karena berada dalam filum Nematelminthes dan polifiletik dengan *Paramphistomum* sp. dan *Eimeria* sp. karena spesies *Paramphistomum* sp. berada dalam filum Platyhelminthes serta spesies *Eimeria* sp. berada dalam filum Protozoa.

Kata Kunci: endoparasit, feses sapi, kekerabatan, penyakit ternak, pohon filogeni

Abstract: Cows are one of the livestock animals that are widely used by humans to fulfill their needs such as meat and milk. However, unclean farming conditions can be a factor in reducing livestock productivity due to parasitic infections. This study aims to determine the type of endoparasite, the symptoms caused, how to identify them, and the kinship between types of endoparasites found in the faeces of cows observed. This study used the buoyancy method (flotation test) and the sedimentation method. The results obtained from 21 cow faecal samples showed there were seven positive samples for each technique with five different species, namely *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp., *Paramphistomum* sp., and *Eimeria* sp. Each species will cause almost the same symptoms, such as decreased appetite, weight loss, late growth, metabolic disorders, diarrhoea, and several other clinical symptoms that can result in decreased livestock productivity. The key to the determination is used in determining the phylum and class of the species obtained based on their characteristics; the results obtained are species *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp. are in the same phylum Nematelminthes and class Nematoda, species *Paramphistomum* sp. is in the phylum Platyhelminthes of the class Trematoda, and the species *Eimeria* sp. is in the phylum Protozoa of the class Sporozoa. Phylogeny trees are used to see the level of kinship of a species, as in the results obtained that *Strongyloides* sp., *Oesophagostomum* sp., and *Cooperia* sp. is one monophyletic branch

because it is in the phylum Nematelminthes and polyphyletic with *Paramphistomum* sp. and *Eimeria* sp. due to the species *Paramphistomum* sp. is in the phylum Platyhelminthes as well as the species *Eimeria* sp. is in the phylum Protozoa.

Keywords: endoparasites, cow feces, kinship, livestock diseases, phylogenetic tree

INTRODUCTION

Ruminants are a class of vertebrate animals characterized by having jaws, legs with an even number of hooves, and hollow horns. They feed their young by breastfeeding and have a unique digestive system that involves chewing and regurgitating food, known as food. The stomach of ruminants consists of four distinct compartments, specifically the rumen, reticulum, omasum, and abomasum. Cattle are the dominant type of ruminant livestock found in the tropics. This ruminant animal has many advantages and a higher economic value than other livestock. Farmers often acquire cattle in the harvest season and then sell them in the growing season (Majawati & Matatula, 2018; Kamilah & Wulandari, 2019)

Cows are pets that humans raise to obtain meat, milk, employment, and other human needs. This creature feeds on grass and has a digestive system consisting of three stomachs, so it is classified as a ruminant animal. Cattle farming produces meat and milk, makes manure, and creates new job prospects. Cattle offer superior advantages and yield higher profits than other livestock categories. (Crisdayanti & Erina, 2020). The livestock business is accessible to develop because it is more attractive. Two types of cattle in Indonesia are dairy and broiler. The health of cattle is essential to increase livestock productivity. Livestock diseases can decrease livestock productivity, which means unhealthy livestock. (Susilo et al., 2020).

A parasite is an organism that lives on another species, known as a host. The hospital provides financial support to the parasites that inhabit it. Parasites inhabit specific anatomical locations within the bodies of their hosts, and these parasites can potentially hinder the progress of animal husbandry, especially concerning population growth and livestock productivity. This parasite is a hazardous disease for livestock. Parasites are classified into ectoparasites and endoparasites based on their habitat. Ectoparasites are parasites that inhabit the outer surface of the host's body. The parasite obtains nutrients by introducing haustorium into the actively dividing host cell. Ectoparasites include leeches, ciliates, flagellates, monogenea, copepods, isopods, and branchiura. Endoparasites are organisms that reside in the body of another organism, such as a cow, and obtain necessary nutrients by eating the host's food. Some species of worms can cause damage to the intestines and trigger various physiological responses in the body. These parasites only reduce the body mass of adult and juvenile animals and do not cause immediate death. Endoparasites include multiple parasites, including digea, cestodes, nematodes, acantocephala, coccidia, microsporidia, and amoeba (Tolistiawaty et al., 2016; Zalizar & The Ranch, 2017; Istirokah, 2019).

Cows can contract gastrointestinal parasitic infections. Gastrointestinal parasites usually deplete hospital nutrients, consume hospital tissue, or exploit cells in the digestive organs to fulfil their life cycle. Parasitic worms infest the digestive tract of ruminant animals, including cows, buffaloes, goats, sheep, pigs, horses, and other mammals. The aetiology of this disorder is caused by helminth infections that attack the gastrointestinal tract. Although livestock mortality is not always a direct result, it is essential to note that this condition can lead to financial setbacks such as reduced animal weight, impaired

meat quality, decreased value of offal and skins, and decreased overall livestock productivity, including labour input (Nugraheni et al., 2018; Pima et al., 2023).

The veterinary industry strives to prevent the transmission of worms before infection occurs proactively. A practical method of detecting the presence of helminth eggs is to find them in the faeces. The goal is to detect worm infestations, especially those affecting the digestive system, in a quick, easy, and efficient way. Preventing the development of helminth eggs that undergo reproduction in the body of animals and turn into worms can effectively inhibit the occurrence of the disease (Kutbi et al., 2020).

Based on the background description, research was carried out which aimed to determine the types of endoparasites, the symptoms they cause, how to identify them and the relationships between the types of endoparasites found in the cow feces that were observed. The research results can be a reference for preventive measures against disease infections in livestock, especially those caused by endoparasites, so that they can maintain the health quality of livestock and will have a positive effect on increasing livestock productivity.

RESEARCH METHODS

This research was conducted at the Parasitology Laboratory of the Balai Besar Veteriner Maros. The tools used are a faecal stirrer (mortar), filter, Pasteur pipette, microscope, scale, centrifuge, centrifuge tube, plastic pot bottle, plastic spoon, tube rack, timer, preparation glass, cover glass, and stirring rod. The ingredients used are saturated NaCl, cow faeces, 0.1% *methylene blue*, alcohol, and tissues.

Research methods include: **(1) Sample preparation**, the stool sample to be worked on is arranged on the testing table according to the EPI and specimen numbers. In contrast, the unworked stool sample is stored in a refrigerator or given a preservative such as 10% formalin to prevent the development of worm egg larvae; **(2) Test solution preparation**, the manufacture of saturated NaCl begins with prepared NaCl and aqua dest. Then, NaCl is added in aqua dest and *a water bath* for two hours at a temperature of 57°C. After that, it is cooled at room temperature until the moment it will be used. Making a 0.1% methylene blue solution *begins with the preparation* of 0.1 grams of methylene blue and aqua dest. Next, *methylene blue* is dissolved in 100 ml of aquades and stirred until well mixed; **(3) Testing process**, using the flotation test (Buoyancy method) and sedimentation method; and **(4) Phylogeny tree making**, before making a phylogeny tree, search for species names on the NCBI website. Next, species names are chosen based on partial mitochondrial genes or partial gene sequences. Then press FASTA, and the order in which FASTA appears is copied. After all FASTA from each species is copied, the MEGA11 application is opened. Then, all FASTA is opened in the MEGA11 application, and the cutting process is carried out. After that, a phylogeny tree was made in the application of MEGA11 with FASTA that had been cut before, and then the level of kinship between species was based on the branches contained in each species.

Flotation test technique (Buoyancy method) is carried out in the following stages: the necessary tools and materials have been collected. Next, the faeces are weighed, weighing 2 grams and then transferred into plastic containers. Next, a saturated NaCl solution of 30 ml is put and stirred until well mixed. Next, the faeces are filtered, and the resulting filtrate is transferred into a 15 ml centrifuge tube. This process is carried out continuously and sequentially until the entire stool sample runs out. After all samples are prepared, a centrifuge tube with faecal filter findings is placed on a centrifuge scale to ensure balance. The mixture is further centrifuged at 1500 revolutions per minute for five

minutes. After centrifugation, the tube is taken, and NaCl is added gradually until it reaches maximum capacity. Next, place the cover glass over the tube's opening and leave it undisturbed for 5 minutes. Next, a cover glass is placed on the object glass, followed by observing it using a microscope with a magnification of 100x.

Sedimentation test technique (sedimentation method) is carried out in the following stages: The necessary tools and materials have been collected and arranged. Next, the faeces are weighed, weighing 2 grams and then transferred into plastic containers. Next, a saturated NaCl solution of 30 ml is put and stirred until well mixed. Next, the impurities are filtered, and the resulting filtrate is collected in a 15 ml centrifuge tube. This process is carried out continuously and sequentially until the entire stool sample runs out. After all samples have been made, the centrifuge tube that holds the faecal filter results is placed on the centrifuge scale to ensure balance. Next, the sample was centrifuged at 1500 revolutions per minute for five minutes. Next, the supernatant is extracted, leaving only the sediment at the tube's bottom. After the precipitate is extracted using a pipette, it is further deposited on the glass of the object. Next, a solution containing 0.1% methylene blue is introduced, stirred until it reaches a homogeneous consistency, and then covered with a cover glass. Furthermore, it is examined using a microscope with a magnification of 100x.

RESULT AND DISCUSSION

Samples in the form of cow faeces taken are active samples taken directly during *surveillance* in the Sinjai area. Balinese, Limousin, and Simental cattle are the most common cattle raised in the area. Stool samples were taken from Balinese cows estimated to have adolescence to adulthood, with 21 samples. Based on the results of the observation and identification of 21 cow faecal samples using the flotation test and sedimentation test, which can be seen in Table 1 and Figure 1.

Table 1. Results of identification of endoparasites in cow faeces samples

Sample Number	Flotation Test	Sedimentation Test
1	-	-
2	<i>Strongyloides</i> sp.	-
3	<i>Eimeria</i> sp.	-
4	-	-
5	-	-
6	-	-
7	<i>Oesophagostomum</i> sp.	-
8	-	<i>Paramphistomum</i> sp.
9	-	<i>Paramphistomum</i> sp.
10	<i>Eimeria</i> sp.	-
11	-	<i>Paramphistomum</i> sp.
12	<i>Oesophagostomum</i> sp.	<i>Paramphistomum</i> sp.
14	-	<i>Paramphistomum</i> sp.
15	-	-
21	<i>Eimeria</i> sp.	-
31	-	<i>Paramphistomum</i> sp.
34	-	-
45	<i>Cooperia</i> sp.	-
46	-	-
50	-	-
51	-	<i>Paramphistomum</i> sp.

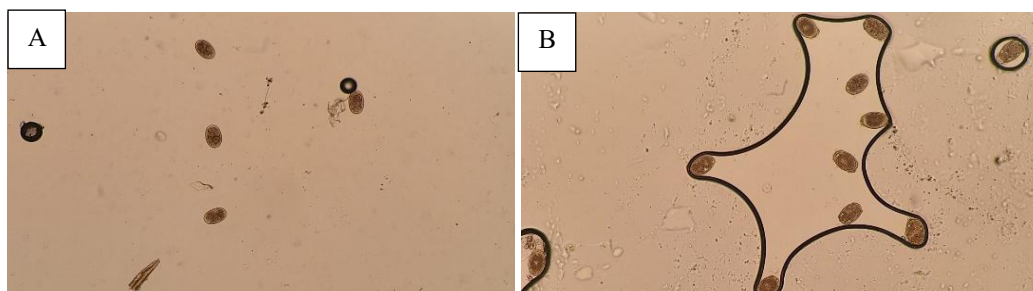


Figure 1. Results of identification of endoparasites in cow faeces; A. Flotation test, and B. Sedimentation test

Based on research results, the examination of parasitic worm eggs in cow faeces that had been carried out at the BBVet Maros Parasitology Laboratory found that seven out of 21 samples were declared infected with parasitic worms. This result can be seen in Table 1 in the flotation test obtained by *Strongyloides* sp., *Oesophagostomum* sp., and *Cooperia* sp., which are species of worm eggs from the Nematode group and *Eimeria* sp., from the Protozoa group. In the sedimentation test, *Paramphistomum* sp. is a species of worm eggs from the Trematod group.

Nematode worms, sometimes known as earthworms, inhabit the digestive tract and take in food by consuming juice, blood, body fluids, or body tissues. Excessive amounts of worms can cause intestinal disorders or cause various body reactions due to the toxins they produce. This helminth infection causes a decrease in livestock productivity characterized by reduced body weight, stunted growth, decreased milk supply in lactating animals, and weakened immune response. Indications of helminthic attacks include emaciation, non-shiny fur, reduced appetite, diarrhoea (primarily occurs in the rainy season), and rapid death of young animals (Khozin & Kutbi et al., 2020).

Strongyloides sp. is a parasite that attacks many Bali cows. These organisms inhabit the small intestine, feed on blood, and have oval-shaped eggs 40–85 µm long with smooth walls that house larvae. This worm has excellent strength and is much smaller than the rhabditiform oesophagus. The tail of the male worm is conical and shortened. The length of male worms ranges from 700 to 825 microns. They have solid and curved spiculums and gubernaculum that measure 20 microns long and 2.5 microns wide. The length of female worms ranges from 640 to 1200 microns. Infections caused by parasitic worms (Dina et al., 2021) *Strongyloides* sp. can cause anaemia, emaciation, delayed growth, metabolic problems, and decreased endurance. These infections also result in economic losses (Thamsborg et al., 2017).



Figure 2. (A) *Strongyloides* sp. worm eggs (Suastini et al., 2021); (B) Adult worms *Strongyloides* sp.

According to ITIS (*Integrated Taxonomic Information System*) *Strongyloides* sp. can be classified as follows:

Kingdom : Animalia
 Phylum : *Nemathelminthes*
 Classis : Nematodes
 Order : Rhabditida
 Family : Strongyloidae
 Genus : *Strongyloides*
 Species : *Strongyloides* sp.

Humpworm (*Oesophagostomum* sp.) is a worm that lives in the colon of cattle, sheep, goats, and pigs, and its larvae form humpbacks. Adult worms have pronounced corona radiata and small buccal capsules. The size of the female is 13.8–19.8 mm, and the size of the male is 11.2–14.5 mm. The blastomere that meets the egg and the elliptical egg has a thin outer shell and measures 70-76 x 36-40 microns. The larvae form a humpback in the large and small intestine, but the adult form is present only in the large intestine, sometimes in the cecum (appendix). One of the gastrointestinal nematodes (Dalimunthe et al., 2023) is the worm *Oesophagostomum* sp., which is more accurately classified into humpback worms because the symptom is the onset of humpback in the colon. Animals affected by this parasite do not show pronounced clinical symptoms despite weight loss; the faeces are black, soft with mucus, and sometimes contain fresh blood. Signs of chronic cow disease include diarrhoea, black stools, reduced appetite, low body weight, anaemia, low levels of albumin and protein in the blood, and swelling. (Pradana et al., 2015).



Figure 3. (A) *Oesophagostomum* sp. worm eggs (Dalimunthe et al., 2023); (B) Adult Worm *Oesophagostomum* sp.

According to ITIS (*Integrated Taxonomic Information System*) *Oesophagostomum* sp. can be classified as follows:

Kingdom : Animalia
 Phylum : *Nemathelminthes*
 Classis : Nematodes
 Order : Strongylida
 Family : Strongyloidae
 Genus : *Oesophagostomum*
 Species : *Oesophagostomum* sp.

Cooperia sp. is a nematode that lives in the intestines of ruminants. Although familiar, these worms are not very dangerous. Its head appears inflated or bulb-like because the cuticle at its anterior end is widened. Both spiculums are short and robust, with one end ending and usually have a serrated widening in the centre that resembles a wing. Gubernaculum does not exist. Behind the middle of the body is the vulva of the female worm. Its life cycle resembles *Trichostrongylus* (Sihombing & Mulyowati, 2018). *Cooperia* sp. has about 20 types, with adult male worms 4-9 mm long and female worms

5-9 mm long. The eggs are oval, the blastomere is indistinct, the shell is thin, and its size is $71-83 \times 28-35$ microns. Tainted food or drink infects worms that live in the intestines of cows. Symptoms of this worm infection are diarrhoea, dehydration, and weight loss (Tolistiawaty & Widjaja, 2021).

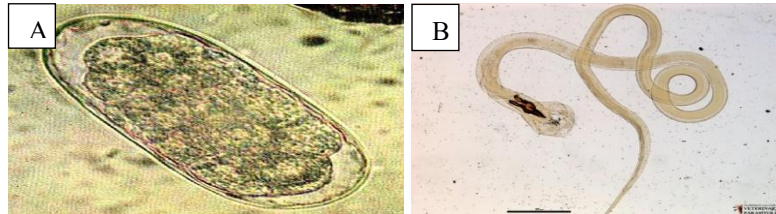


Figure 4. (A) Eggs of *Cooperia* sp. (Sihombing & Mulyowati, 2018); (B) Adult worms *Cooperia* sp.

According to ITIS (*Integrated Taxonomic Information System*) *Cooperia* sp. can be classified as follows:

Kingdom : Animalia
 Phylum : *Nemathelminthes*
 Classis : Nematodes
 Order : Rhabditida
 Family : Cooperidae
 Genus : *Cooperia*
 Species : *Cooperia* sp.

Eimeria sp. is a vital protozoan that induces coccidiosis, resulting in considerable morbidity and mortality, especially in calves up to one year old. Various species of *Eimeria* are commonly found in cattle, with varying degrees of pathogenicity. Some species are highly pathogenic, others are less dangerous, and others are nonpathogenic. Based on morphometry, there are two species of coccidia known as *Eimeria auburnensis* and *Eimeria bovis*. *E. auburnensis* is a species with low pathogenicity and rare manifestations of clinical symptoms. *E. bovis* is one of the most dangerous bovine coccidia. At first, the mucosa experiences thickening and swelling accompanied by the appearance of small red or purple spots or widespread bleeding. Subsequently, the mucosa becomes injured and detached. Damage to the submucosa is also possible. Gastrointestinal (Indraswari et al., 2017) protozoan infections in Bali cattle have symptoms that vary based on the number of species infected. Even if only one species infects, the clinical symptoms may not be noticeable. This remains true even though the infecting species does not cause disease (Arsyitahlia et al., 2019). Symptoms include diarrhoea or dysentery, accompanied by tenesmus and increased body temperature, and are often fatal. Gamont causes significant pathological changes in the cecum, colon, and last 0.3 meters of the terminal ileum (Regina et al., 2018).

According to ITIS (*Integrated Taxonomic Information System*) *Eimeria* sp. can be classified as follows:

Kingdom : Protist
 Phylum : Apicomplexa
 Classis : Sporozoa
 Order : Eucoccidae
 Family : Eimeriidae
 Genus : *Eimeria*
 Species : *Eimeria* sp.



Figure 5. *Eimeria* sp. (Arsyitahlia et al., 2019)

Trematode worms belonging to the phylum *Platyhelminthes*, class Trematoda, suborder Prosostomata, and order Digenea are commonly found in cattle. In the order Digenea, there are several families, including Dicrocoeliidae, which include the genus *Eurytrema*; Fasciolidae, which includes the genus *Fasciola*; Paramphistomidae, which includes the genus *Paramphistomum*; and Schistosomatidae, which includes the genus *Schistosoma*. All species of digenean are parasitic trematodes found in ruminants. The body of the trematode is dorsoventrally flattened and non-segmented, and its shape resembles a leaf. This worm has a large enough thickness to inhabit a humid or humid environment. The suction cavity or oral cavity is located at the front of the body, while the acetabulum or ventral cavity is the outermost area of the body. The acetabulum is located in the anterior part of the body, but its exact location may differ depending on the trematode species (Kurnia et al., 2022).

Paramphistomum sp. is a trematode worm that is thick and flattened, resembling *Fasciola* sp. and *Eurytrema* sp. It has an acetabulum, a suction tube located in the stomach, and a small mouth suction tube. *Paramphistomum* sp. has testicles in the anterior ovary and a straight digestive tract (Firdayana, 2016) *Paramphistomum* sp. eggs have dimensions of 113-175 microns in length and 73-100 microns in width. At the same time, adult worms measure 5-13 mm long and 2-5 mm wide. *Paramphistomum* sp. eggs are stored in a humid environment along with faeces. *Paramphistomum* sp. eggs take at least four weeks at 17°C to develop into miracidia and utilize snails as intermediate hosts. *Paramphistomum* sp. infection develops through the intestinal phase and rumen phase. In the intensity phase, juvenile worms cause bleeding, inflammation and erythema in the duodenum and abomasum. In the rumen phase, the worm modifies the rumen epithelium, inhibiting reabsorption. Immature worms (Kurnia et al., 2022) of the *Paramphistomum* species can penetrate the submucosa, causing inflammation of the intestines, cell death, and erosion of the mucosal villi (Pradana et al., 2015).



Figure 6. *Paramphistomum* sp. worm eggs (Kurnia et al., 2022)

According to ITIS (*Integrated Taxonomic Information System*) *Paramphistomum* sp. can be classified as follows:

Kingdom : Animalia
 Phylum : *Platyhelminthes*
 Classis : Trematodes
 Order : Plagiorchiida
 Family : Paramphistomatidae
 Genus : *Paramphistomum*
 Species : *Paramphistomum* sp.

Identification is an essential step in the classification of biological organisms. Identification refers to procedures for ascertaining or differentiating the specific classification of an entity. Some identification techniques include obtaining verification from a specialist, comparing specimens, or utilizing tools such as identification or determination keys. The determination key consists of a series of questions that can be asked to ascertain the nomenclature of the organism whose classification to ascertain. To identify and categorize creatures into taxonomic groups, the crucial factor is the systematic description of the properties of living things, arranged hierarchically from broad to more detailed features. The dichotomous key, the most basic form of determination, consists of pairs of information displaying contrasting qualities (Utami et al., 2015; Izza et al., 2018).

Here are the determination keys of the types of endoparasites found in cow faeces that have been observed:

1. a. Unicellular 2 (Protozoa)
 b. Multicellular 4
2. a. Has locomotor Rhizopods
 b. No locomotor 3 (Sporozoa)
3. a. Causes of Coccidia disease *Eimeria* sp.
 b. Causes of malaria *Plasmodium* sp.
4. a. Has a body cavity 6a (Platyhelminthes)
 b. It has no body cavity 5 (Nemathelminthes)
5. a. Having embryos *Strongyloides* sp.
 b. Does not have embryos 6b
6. a. Has a Blastomere 7b
 b. Does not have a blastomere *Cooperia* sp.
7. a. Has an operculum *Paramphistomum* sp.
 b. Does not have an operculum *Oesophagostomum* sp.

After Darwin and Lamarck's strong theory of evolution, the classification of phylogenetic systems became known worldwide. It is a classification system of living things adapted based on evolutionary processes and based on kinship relationships between organisms. Phylogeny explains the correlation between a species and its ancestors and the evolutionary relationships between creatures. The term (Subari et al., 2021). "*Phylogen*" in Greek refers to a tribe or race, while "*Genesis*" denotes origin or source. Phylogenetic research aims to determine the classification and infer phylogenetics from related data obtained by comparing organism character variables (Fietri et al., 2021). Fiolgeni trees can be used to find relationships between living things on Earth. The phylogeny tree consists of two words, meaning the arrangement of points and branches.

Two points are connected by one branch. Dots represent part of its taxonomy. Phylogeny, conversely, means showing evolutionary relationships among a group of living things. In addition, a phylogeny tree is a collection of species connected through evolutionary kinship. Two methods exist to create a phylogeny tree: manually simple and using (Octavia & Badruzsaufari, 2021) software. One method used is to compare DNA sequences. Another method is to use software, namely Clustal W (Subari et al., 2021).

Based on Figure 7, the degree of kinship between the five species found is not much different. The level of kinship can be seen from the branches in the phylogeny tree, such as the closest level of kinship in the species *Oesophagostomum* sp. and *Cooperia* sp. *Strongyloides* sp. is in a monophyletic branch. This can be because these three species belong to the same phylum, *Nemathelminthes*. However, it can be seen that *Oesophagostomum* sp. and *Cooperia* sp. are in one branch, so it can be concluded that these two species have a close level of kinship. This is because the parasites *Oesophagostomum* sp. and *Cooperia* sp. attack in the same place, namely the large intestine and reticulum, while *Strongyloides* sp. only attack the small intestine, especially on the intestinal mucosa. Guna et al. (2014) reported that *Strongyloides* ransom has the potential to cause damage to the intestinal mucosa. Severe infections can cause bloody diarrhoea, anaemia, emaciation, and sudden death. *Strongyloides* worm eggs are elliptical and have thin walls enclosing the larvae. The primary mode of transmission in lactating calves is through the transmission of larvae through milk.

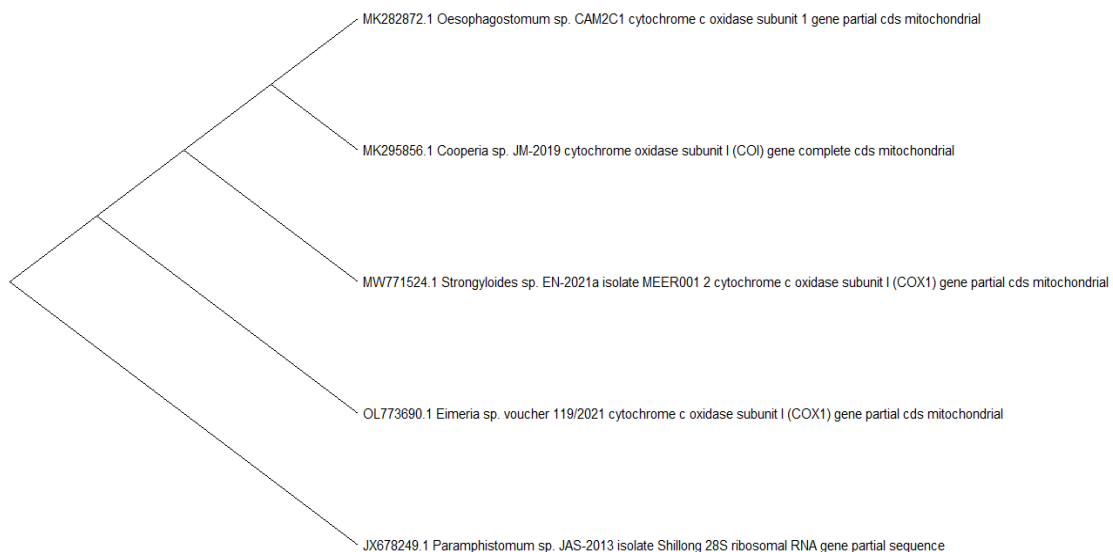


Figure 7. Phylogenetics tree of types of endoparasites present in cow feces

Next in line is *Eimeria* sp., as it belongs to the category of *Protozoan* organisms, which indicates a reasonably close kinship. This is also evidenced because these *protozoan* parasites target the small intestine, similar to *Strongyloides* sp., and follow a similar pattern of infection. According to Arsyitahlia et al. (2019), *Eimeria* sp. reproduces inside epithelial cells of the small intestinal mucosa, resulting in damage to epithelial cells and inflammatory responses. The accumulation of inflammatory cells around the lesion will increase the permeability of blood vessels in the small intestine, causing peridiapedesis bleeding. Bleeding in the small intestine results in blood in the stool.

Eimeria species build colonies in the small intestine consisting of many merozoites. Merozoites mature and penetrate further into the lamina propria, releasing merozoites from the colony and causing extensive erosion of the small intestine's mucous membrane. Mucosal erosion in the small intestine leads to suboptimal nutrient absorption and subsequent dehydration. The occurrence of death occurs within four to six days after infection.

In contrast, *Paramphistomum* sp. shows significant differences from the other four species, likely due to its classification within the *Platyhelminthes* group. The worm first invades the duodenum and then moves to the rumen. Lestari et al. (2017) reported that the worm *Paramphistomum* sp. prefers the duodenum, while the adult worm *Paramphistomum* sp. prefers the rumen and reticulum. In the small intestine, the juvenile worm *Paramphistomum* will attach and go to the intestine's mucous membrane. Inside the rumen and reticulum, these worms will attach and take blood from the surface of the mucosa.

CONCLUSION

Based on research, it can be concluded that in the flotation and sedimentation tests of 21 cow feces samples, seven samples had positive results or the discovery of parasitic worm eggs. Worm eggs obtained as many as five species, namely *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp., *Eimeria* sp., and *Paramphistomum* sp.. Each species will cause almost the same symptoms, such as decreased appetite, weight loss, late growth, impaired body methodism, diarrhoea, and several other clinical symptoms that can result in decreased livestock productivity. The key to the determination is used in determining the phylum and class of the species obtained based on their characteristics; the results obtained are species *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp. are in the same phylum Nematelminthes and class Nematoda, species *Paramphistomum* sp. are in the phylum Platyhelminthes of class Trematoda, and the species *Eimeria* sp. is in the phylum Protozoa of class Sporozoa. Phylogeny trees are used to see the level of kinship of a species, as in the results obtained that *Strongyloides* sp., *Oesophagostomum* sp., *Cooperia* sp. are monophyletic because they are in the phylum Nematelminthes and polyphyletic with *Paramphistomum* sp. and *Eimeria* sp. due to the species *Paramphistomum* sp. is in the phylum Platyhelminthes and the species *Eimeria* sp. is in the phylum Protozoa.

REFERENCES

- Arsyitahlia, N., Ardana, I. B. K., & Apsari, I. A. P. (2019). Prevalence of *Eimeria* spp. infection in broiler given feed without antibiotic growth promoters (AGP) in Tabanan Regency, Bali. *Indonesia Medicus Veterinus*, 8(2), 186-192. <https://doi.org/10.19087/imv.2019.8.2.186>.
- Dalimunthe, K., Tambunan, E. P. S., and Syukriah, S. (2023). Identifikasi dan prevalensi telur cacing endoparasit pada feses kambing di Kecamatan Kotarih Kabupaten Serdang Bedagai Sumatera Utara. *BEST: Journal of Biology Education, Science, and Technology*, 6(1), 260–266. <https://doi.org/10.30743/best.v6i1.6321>.
- Depison, D., Crisdayanti, S., Gushairiyanto, G., & Erina, S. (2020). Identifikasi karakteristik morfometrik sapi bali dan sapi brahman cross di Kecamatan Pamenang Barat Kabupaten Merangin. *Jurnal Peternakan Sriwijaya*, 9(2), 11-20. <https://doi.org/10.33230/JPS.9.2.2020.11945>.
- Dina, P. A., Apsari, I. A. P., & Dwinata, I. M. (2021). Prevalence of strongyl type Nematoda infection in bali cattlein wet and drought low land of Bali Province. *Indonesia Medicus Veterinus*, 10(1), 125–133. <https://doi.org/10.19087/imv.2021.10.1.125>.
- Fietri, W. A., Rasak, A., & Ahda, Y. (2021). Analisis filogenetik ikan tuna (*Thunnus* spp.) di perairan Maluku Utara menggunakan COI (Cytochrome Oxydase I). *Bioma*, 6(2), 31-39. <https://doi.org/10.20956/bioma.v6i2.14053>.

- Indraswari, A. A. S., Suwiti, N. K., & Apsari, I. A. P. (2017). *Eimeria auburnensis* and *Eimeria bovis* of protozoa gastrointestinal infected on female bali cattle in Nusa Penida. *Buletin Veteriner Udayana*, 9(1), 112–116. <https://doi.org/10.21531/bulvet.2017.9.1.112>.
- Istirokah, Y. (2019). Identifikasi Telur Cacing Parasit Usus Pada Feses Sapi di Dusun Tanjung Harapan Desa Bojong Kecamatan Sekampung Udik Lampung Timur (Sebagai Alternatif Sumber Belajar Peserta Didik Pada Sub Materi Invertebrata). Lampung: UIN Raden Intan Lampung.
- Izza, F. R., Retnoningsih, A., & Pukan, K. K. (2018). Pengembangan kunci determinasi tumbuhan hasil eksplorasi Hutan Wisata Guci Kabupaten Tegal untuk sekolah menengah atas. *Indonesian Journal of Conservation*, 7(2), 119-130. <https://doi.org/10.15294/ijc.v7i2.19008>.
- Kamilah, S. N., & Wulandari, D. A. (2019). Jenis-jenis parasit internal pada feses sapi (*Bos* sp.) Di Desa Lempuing Kota Bengkulu. *Konservasi Hayati*, 15(1), 23–29. <https://doi.org/10.33369/hayati.v15i1.10944>.
- Kurnia, D., Lefiana, D., Sujatmiko, S., Noor, P. S., Zelpina, E., & Ardyes, R. (2022). Prevalensi trematoda gastrointestinal pada sapi di Pasar Ternak Payakumbuh. *Jurnal Vitek Bidang Kedokteran Hewan*, 12(1), 35–39. <https://doi.org/10.30742/jv.v12i1.102>.
- Majawati, E. S., & Matatula, A. E. (2018). Identifikasi telur cacing *Fasciola hepatica* pada sapi di peternakan sapi daerah Tangerang. *Jurnal Kedokteran Mediatek*, 24(68), 60-66. <https://doi.org/10.36452/jkdoktmeditek.v24i68.1703>.
- Nugraheni, Y. R., Priyowidodo, D., Prastowo, J., Rohayati, E. S., Sahara, A., & Awaludin, A. (2018). The gastrointestinal parasites cows on Progo Watershed in Yogyakarta. *Jurnal Ilmu Peternakan Terapan*, 1(2): 46-50.
- Oktafia, R. E., & Badruzaufari, B. (2021). Phylogenetic analysis of *Garcinia* spp. based on rRNA gene sequences. *Ziraa'ah*, 46(2), 259-264. <http://dx.doi.org/10.31602/zmip.v46i2.4526>.
- Pradana, D. P., Haryono, T., & Ambarwati, R. (2015). Identification of endoparasites of broilers and egg-laying chickens based on fecal examination. *LenteraBio*, 4(2), 119–123.
- Regina, M. P., Halleyantoro, R., & Bakri, S. (2018). Comparison of stool examination between ordinary sedimentation method and formol-ether sedimentation method in detecting soil-transmitted Helminth. *Jurnal Kedokteran Diponegoro*, 7(2), 527–537.
- Sihombing, F. U., & Mulyowati, T. (2018). Identification worm eggs of hookworm, *Toxocara vitulorum* on breedersfeaces's andcows feaces's in the farm cow at Karangnongko Village. *Boyalali. Biomedika*, 11(02), 76-78.
- Subari, A., Razak, A., & Sumarmin, R. (2021). Phylogenetic analysis of *Rasbora* spp. based on the mitochondrial DNA COI gene in Harapan Forest. *Jurnal Biologi Tropis*, 21(1), 89–94. <https://doi.org/10.29303/jbt.v21i1.2351>.
- Supriadi, S., Kutbi, M. K., & Nurmayani, S. (2020). Identifikasi parasit cacing nematoda gastrointestinal pada sapi bali (*Bos sondaicus*) di Desa Taman Ayu Kabupaten Lombok Barat. *Bioscientist: Scientific Journal of Biology*, 8(1), 58–66. <https://doi.org/10.33394/bioscientist.v8i1.2658>.
- Susilo, H., Abdillah, N. A., & Amelia, K. R. (2020). Identifikasi telur cacing parasite pada feses hewan ternak di Propinsi Banten. *Biodidaktika: Jurnal Biologi dan Pembelajarannya*, 15(2), 21-30. <http://dx.doi.org/10.30870/biodidaktika.v15i2.8719>.
- Tambunan, E. P. S., Syukriah, S., & Dalimunthe, K. (2023). Derajat infeksi telur cacing endoparasit pada feses kambing di Kecamatan Kotarih Kabupaten Serdang Bedagai. *BEST: Journal of Biology Education, Science, and Technology*, 6(1), 197-203. <https://doi.org/10.30743/best.v6i1.6355>.
- Thamsborg, S. M., Ketzis, J., Horii, Y., & Matthews, J. B. (2017). *Strongyloides* spp. infections of veterinary importance. *Parasitology*, 144(3): 274-284. <https://doi.org/10.1017/S0031182016001116>.
- Tolistiawaty, I., Widjaja, J., Lobo, L. T., & Isnawati, R. (2016). Gastrointestinal parasites in livestock in slaughterhouse Sigi District, Central Sulawesi. *BALABA*, 12(2), 71-78. <https://doi.org/10.22435/blb.v12i2.200>.
- Utami, S., Pradani, A. W. M., Laksana, S. D. (2015). Pengembangan kunci determinasi *Myriapoda* dalam model pembelajaran inquiry untuk meningkatkan hasil belajar taksonomi invertebrata mahasiswa Prodi Pendidikan Biologi IKIP PGRI Madiun semester genap 2013/2014. *Jurnal LPPM*, 3(1), 1-4.
- Zalizar, L. (2017). Helminthiasis saluran cerna pada sapi perah. *Jurnal Ilmu-Ilmu Peternakan*, 27(2): 116-122. <https://doi.org/10.21776/ub.jiip.2017.027.02.01>.