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PARASITE INFECTIONS IN POULTRY ENVIRONMENTS (CASE REPORT ON *GALLUS DOMESTICUS* ENDOPARASITE)

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Abstract

Infection of nematode and cestode worms in chickens can cause decreased egg production, weight loss, growth problems, weakness. The objective of this case study is to look at how an epidemiological approach, clinical symptoms, anatomical pathology investigation, and laboratory testing might help with disease diagnosis. The chicken was found from Banjar Tanggahan Tengah, Bangli, Bali with the type of native chicken (*Gallus domesticus*), at least 3 months old with the protocol number 369 / N / 20. Samples taken were brain, trachea, lungs, liver, heart, spleen, kidneys, proventriculus, intestines, and feces. Laboratory examinations carried out include histopathology, microbiology, and parasitology. The results of the pathological examination of the case showed that there was bleeding in the lobes of the lungs and liver, heart, brain, and trachea, there was edema, the small intestine and cecum had bleeding and mucosal enteritis, the spleen was depleted, the proventriculus was inflamed. Microbiology laboratory test results with the culture of organ samples identified by *Escherichia coli* bacteria. The results of fecal and microscopic parasite examinations found eggs and worms of the nematodes *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria caudinflata*, *Tetrameres americana*, cestode *Raillietina* sp., And oocyst *Eimeria* sp. the protozoan digestive system. It is recommended that farmers improve cage sanitation and maintenance management for better poultry environments. Thus, the chickens would not easily infect by endoparasites.

Keywords: Endoparasites; *Gallus domesticus*; Pathological examination.

1. Introduction

Gallus domesticus is a type of local poultry that has been cultivated and can be found throughout Indonesia. The management method of raising native chickens in the community is generally done traditionally or semi-intensively and extensively. The pattern of caring for *Gallus domesticus* in the environment and roaming around the coop makes native chicken feed uncontrollable and cleanliness is guaranteed. These maintenance habits can cause health problems for native chickens, such as endoparasite infections (Pranoto et al., 2019).

Endoparasites can usually attack the digestive tract of chickens, the causes include protozoa, viruses, bacteria, trematodes, cestodes, and nematodes (Hadi & Soviana, 2000). One of the most common infecting chickens is the helminth parasite (worm) called Helminthiasis or worms. Helminthiasis is a disease caused by the infection of two or more parasitic worms in the animal's body and can affect all ages of chickens. Nematode worms (*Ascaridia galli*, *Heterakis gallinarum*, *Tetrameres americana*, and *Capillaria caudinflata*), cestodes (*Raillietina* sp.), And Protozoa (*Eimeria* sp.) Are the types that most often infect native chickens (Kelly et al., 2015).

The spread of endoparasites to poultry can be through contaminated feed, water, and livestock equipment (Parede et al., 2005). *Gallus domesticus* can be infected with endoparasites through food, due to their omnivore eating habits. Worm parasites in the body of native chickens will absorb the nutrients needed for the growth of native chickens (Sudaryani, 2007). Infection with nematode and cestode worms in chickens can cause decreased egg production, weight loss, growth problems, weakness (Loliwu & Thalib, 2012). Helminthiasis cases in native chickens are generally sub-acute but can cause weight loss. Therefore, chickens become weak and thin and can even cause death (Levine, 1995). The presence of parasites in the body of native chickens can also cause damage to certain organs.

Cestodes are flatworms that resemble ribbons, are white or yellowish, and are segmented. Different tapeworm species can cause various pathological changes. Therefore, it is necessary to identify worm species in certain cases (Pranoto et al., 2019). The spread of cestode worms in chickens is greatly influenced by the presence of an intermediate host. Cestode worm eggs that are eaten by the intermediate host will hatch in the digestive tract. Worms that live in the digestive tract will take food by absorbing food juices from their host in the intestinal mucosa. If the infection rate is severe enough, the landlady will experience marked hypoglycemia and hypoproteinemia.

Nematodes are also called roundworms because these parasites are round, unsegmented, and are equipped with a smooth cuticle. In nematodes, male worms can usually be distinguished by size, males have a smaller body size than female worms besides that in the tail, in males near the anal canal there is a bulge called penial setae which is used for copulation, while females are absent (Soulsby, 1986). Nematodes that have a direct life cycle go through 4 developmental stages to become adults. Adult nematodes that live in the body of infected chickens will produce eggs that are released along with feces, and then these eggs will develop further to form embryos (Tabbu, 2000).

Infections of parasite species are common in chickens (Thapa et al., 2015; Wuthijaree et al., 2017) and they also occur in the outdoor condition of chicken breeding (Stehr et al., 2019). Environmental factors are very important and very complex factors, which are dominant in the ecology of nematode parasites such as season, rainfall, temperature, sunlight, and geographical conditions (Dina et al., 2021). The climatic conditions in Bali Province can be divided into two, namely wet and dry climates. Wet climates can be seen from the high rainfall per year, while dry climates have lower levels of rainfall. The highest rainfall occurs from October to April, while the rest, namely April to October, usually has low rainfall (Purnama, 2009). The average rainfall in each regency/city throughout Bali was recorded that Bangli area had the highest rainfall reaching 3,321.5 mm during 2017. This geographical condition is what supports the spread of parasites

Infections with parasites can have immuno-modulating effects and alter the host's immune response to other disease (Daş et al., 2021). Helminthiasis infection can cause enteritis and bleeding in the digestive tract mucosa (Urquhart et al., 1996). Any mucosal inflammation is generally followed by indigestion, absorption of nutrients such as electrolytes, vitamins (Anwar & Zia-ur-Rahman, 2002), minerals (Gabrashanska et al., 1999), and secretion of substances that play a role in the process of digestion of food. Worm infection also causes degeneration and necrosis of the epithelial cells of the small intestine villi and crypt, and a decrease in the surface area of the small intestine villi (Zalizar et al., 2008). Worm infections are chronic in nature which can cause disease symptoms slowly (FAO, 1998). Worm infection usually does not cause mortality but causes morbidity (Darmawi et al., 2007).

Apart from worm infections, other events that can inhibit the growth of native chickens are caused by the presence of protozoa in the blood and digestion of the chickens. Protozoa in the digestive system or Coccidiosis are widely reported as a disease that causes great losses because it results in growth retardation, weight loss, carcass quality, decreased egg production, and death (Michels et al., 2011). This parasite is divided into several species with different degrees of pathogenicity (Gilbert et al., 2011). Among the various kinds of protozoa *Eimeria* sp. in chickens, *Eimeria tenella* and *Eimeria necatrix* were known to be the most pathogenic species (Joyner & Long, 1974). The development of *Eimeria* sp. In small intestinal mucosal epithelial cells causes epithelial cell damage and inflammatory reactions occur. Damage to the epithelial cells will cause nutrient absorption to be not optimal in order to be able to reduce the antibodies of infected chickens. The decreased immunity of chickens will cause complications of the disease. This study aims to determine the causative agent in

animal cases with protocol number 369 / N / 20. Thus, a definitive diagnosis can be established.

2. Methods

This research was conducted in October 2019. In the case of chickens with the protocol number 369 / N / 20 are *Gallus domesticus* with an age of \pm 3 months, the animals were put to sleep using cardiac embolism and necropsy to observe changes in organs and then collected to become samples in laboratory tests as shown in Table 1. The specimens used in laboratory examinations were taken from the organ changing, namely:

Table 1. The type of sample used in each laboratory

Laboratorium	Sample
1. Pathology	Brain, Trachea, Lungs, Heart, Intestine, Proventriculus, Spleen, Liver, Kidney
2. Bacteriology and Mycology	Liver, Intestine and Brain
3. Parasitology	Feces, Worms

The method used in the data collection stage was through direct field surveys by conducting interviews with animal owners to obtain signalment and anamnestic data from sick animals, clinical examination of animals, and observing the surrounding environment. Necropsy was carried out in the Necropsy Room of the Faculty of Veterinary Medicine (FKH), Udayana University. As a supporting diagnosis, histopathological observations were made in the Pathology Laboratory of the Faculty of Veterinary Medicine, Udayana University, while to confirm the diagnosis, specimen testing was carried out in the Virology, Bacteriology and Mycology, and Parasitology Laboratory of Udayana University.

2.1. Field examination

The first thing to do in field examination was anamnesis, which was carried out to obtain information from the owner of the animal regarding the condition of the animal, medical history, population size, number of sick animals, duration of illness, disease history, and the animal raising system. Clinical examination was conducted by approaching the animal owner whose address is Banjar Tanggahan Tengah, Bangli, Bali.

Clinical examination was carried out by observing the clinical signs seen in case chickens. Observed clinical signs are used to determine the sample to be collected for further
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examination. The next step was an epidemiological examination, this step aims to analyze the relationship between hosts, agents, and the environment. Epidemiological fingerprint data were collected by direct interviews with animal owners and observation of the environment where they were kept.

2.2. Pathology laboratory testing

Chickens were necropsied for their organs to be taken as examination samples. During the necropsy process, observations were made for changes in the organs which were then recorded in the protocol and documented. Organ samples that change were cut to a size of 1x1x1cm including the brain, trachea, lungs, heart, intestines, ventricles, spleen, liver, kidneys which are then stored in sample pots and fixed by adding a solution of Neutral Buffer Formalin (NBF) 10% until the organs were submerged.

The fixed organ was inserted into the tissue cassette and the dehydration process was carried out, namely by sequentially immersing in alcohol with a concentration of 70%, 80%, 90%, 96%, absolute ethanol I, absolute ethanol II each for 2 hours to remove water from within the network. The next step was clearing, which was the process carried out to remove alcohol from the tissue by immersing it in a solution of toluene I, toluene II, xylol I, and xylol II for 2 hours each. The tissue was removed from the tissue cassette, then the network was ready to be blocked by embedding the set, which previously had the position of the network settings, then poured liquid paraffin until the entire tissue is submerged and allowed to freeze. The blocks that had been frozen were then sectioned using a microtome with a thickness of 4-5 microns. The piece was carefully placed on the surface of the water in a water bath then placed on the slide, the preparation was ready for staining.

The last process is routine staining with Hematoxylin-Eosin staining. The preparations were immersed in xylol I, II, and III then dehydrated with ethanol I and II for 5 minutes each, rinsing with distilled water for 1 minute. Soak the preparation in the Hematoxylin solution for 15 minutes, then rinse it with running water. Immediately dip into acid alcohol for 5 dips, and rinse with distilled water. Check color differentiation under a microscope, the color should not be pale. Then, dip 5 times in ammonium or lithium carbonate until the preparation is bright blue and rinse with distilled water for 15 minutes. Soak the preparations in eosin solution for 4 minutes, then dehydrate in alcohol with a concentration of 70%, 80%, 96%, absolute ethanol for 3 minutes each, and immersed in xylol I, II, and III for 3 minutes each. The next process is mounting, namely closing the preparation with a glass cover, the

preparation is removed from the xylol solution in a wet state and given an adhesive liquid (entelan DPX) then covered with a glass cover. The results of the staining can be seen under a microscope at 100x and 400x magnifications. Furthermore, the microscopic changes found were recorded (Berata et al., 2011; Muntiha, 2001).

2.2.1. Bacteriology and Mycology laboratory examination

The first thing to do in this part was isolating bacteria on Nutrient Agar (NA) media, the general media used for bacterial cultivation was Nutrient Agar (NA) media. Isolation of bacteria was done by injuring samples of the lungs and intestines with sterile scissors. Then the liquid was taken with sterile ossa and rubbed on the surface of the culture media using the streak culture method. It was then incubated for 24 hours at 37°C in an incubator. Colony growth on the media was observed macroscopically to see the size, shape, elevation, edge/margin of the growing colony.

Colonies that grew on the culture media were taken with sterile ossa and smeared on a glass object then dropped with distilled water then flattened on the surface of the glass object and fixed. After fixation, the smear was dripped with 2% crystal violet solution and left for 2 minutes, then washed with running water. The next step was dripped with iodine solution and let stand for 2 minutes, then wash with running water. After that, it was dripped with 95% alcohol and left for 30 seconds then washed with running water. Then stained with safranine solution for 1 minute, then washed with running water. The results of the staining can be seen under a microscope at 1000x magnification aided by emersion oil. Gram-positive bacteria stained purple because they absorb crystal violet dye, while Gram-negative bacteria stained red because they absorb the safranine dye.

2.2.2. Isolation of bacteria on Eosin Methylene Blue Agar (EMBA) differential selective media

Take the bacterial colonies that grew on Nutrient Agar (NA) media which had the same macroscopic characteristics as the bacterial colonies which were stained with Gram using cold sterile osse. Then wipe with a streak culture technique on the surface of the EMBA media. Incubate the media in an incubator at 37°C for 24 hours.

The catalase test was carried out to determine the presence of catalase enzyme activity, by taking the suspected bacterial colony on NA media with sterile ossa and smearing it on a

glass object then dropping 3% H₂O₂. A positive result is indicated by the presence of gas bubbles.

2.3. Biochemical test and Carbohydrate fermentation

Planting germs on Triple Sugar Iron Agar (TSIA) media to determine whether or not the ability of bacteria to ferment carbohydrates, H₂S, and gas production. Germination in TSIA was carried out utilizing the germ colony taken from the EMBA using a sterile needle, then inserted into the upright part of the medium then scratched on the oblique part of the medium, then the medium was incubated for 24 hours at 37°C. Carbohydrate fermentation was indicated by a color change on the TSIA medium from red to yellow. H₂S production was characterized by a change in the color of the media to black. The presence of gas can be observed by the presence of gas bubbles and cracks in the media or the media being lifted upwards.

The next step was planting on Sulfide Indole Motility (SIM) media to determine the nature of germs in producing H₂S, indole, and determine the movement of germs (motility). Germination on SIM media was carried out by a colony of bacteria from TSIA media which was taken using a sterile needle then inserted into the upright part of the medium, then the media was incubated for 24 hours at 37°C. H₂S production was characterized by black media. Indole production can be seen after 3 drops of Erlich / Kovac's reagent into the media, if the indole is positive, a red ring will form on the surface of the media. If the germs are motile, a blur will appear on the puncture mark.

Planting on Methyl Red (MR) media to determine the nature of germs in producing single or mixed acids and acetyl methyl carbinol (C₄H₈O₂). The test was carried out by taking the colony from TSIA media with sterile ossa and then immersing it in the media. The media were incubated for 24 hours at 37°C. After incubation, the media was dripped with MR reagent. A positive test result is indicated by the formation of red color on the surface of the media.

Planting on Simmons Citrate Agar (SCA) media to determine the nature of germs in using citrate as a carbon source or not. The colony of bacteria from TSIA media was taken using sterile ossa and then rubbed on the surface of the medium starting from the base to the same end on the SCA medium. Then incubated for 24 hours at 37°C. A positive result is indicated by a change in the color of the media from green to blue.

The confectionery test includes testing for glucose and lactose using a liquid medium with a Durham tube inside. This test is conducted to determine the presence of carbohydrate fermentation. The test was carried out by taking the colony on TSIA media with sterile ossa and then immersing it in each medium. The media were incubated for 24 hours at 37°C. Positive results if the media changes color while the presence of gas production can be observed if the Durham tube contains gas bubbles.

2.4. Parasitology laboratory examination feces examination and worm identification

Stool examination aims to diagnose the presence or absence of infection with worm eggs and cysts or protozoan oocysts in chickens. There are two methods, namely the direct method and the concentration method. Worm identification was carried out microscopically by observing the worm samples.

The direct (native) method was done by taking feces as big as a matchstick, placing it on a glass object then dropping it with distilled water and homogenizing it. Furthermore, the crude fiber was removed and the glass object is covered with a glass cover and then observed under a microscope (Dwinata, 2017).

The concentration method was done firstly by doing an examination of feces using the sedimentation method was carried out by means of feces as big as pecan seeds mixed with water to a concentration of 10% (3 g of feces + 30 ml of water) and stirring until homogeneous. The mixture was filtered and collected with a centrifuge tube to a scale of $\frac{3}{4}$ tube. Then the liquid was centrifuged at 1500 rpm for 5 minutes. Then throw away the sediment, stir evenly and take a little then place it on the object-glass. Perform an examination under a microscope with a magnification of 100x and 400x, then identify it (Dwinata, 2017).

The next step was an examination of feces with the flotation method, this step was carried out by means of faeces as big as pecan seeds mixed with water to a concentration of 10% (3 grams of feces + 30 ml of water) and stirring until homogeneous. The mixture was filtered and collected with a centrifuge tube to a scale of $\frac{3}{4}$ tube. Then the liquid was centrifuged at 1500 rpm for 5 minutes. Then the material was removed, then the precipitate was added with a floating solution (saturated NaCl) up to the $\frac{3}{4}$ scale of the tube. The mixture was stirred until homogeneous and centrifuge at 1500 rpm for 3 minutes. After that, the tube was removed and placed on the test tube rack in an upright position. Add more float solution (saturated NaCl) drop by drop with a Pasteur pipette until the surface of the liquid was

convex (not to be spilled). Let stand 1 minute, touch the glass cover on the surface of the floating liquid, and affixed to the object-glass. Furthermore, it was examined under a microscope with a magnification of 100x and 400x. Then identification was carried out (Dwinata, 2017).

2.5. Worm examination

Examination of worms was carried out microscopically, by identifying samples in the form of preparations. Preparations for worms are made by placing the sample worms on a glass object and sandwiching them between other glass objects. The preparations were then dehydrated using graded alcohol (70%, 80%, and 95%) for 1 hour each. After that, the preparations are stored in a methanol solution. Observations were made by covering the worm preparations with a glass object, then examined under a microscope for identification.

3. Results and Discussions

3.1. Results

Chickens are traditionally raised by releasing them around the owner's house during the day and being caged at night. The chickens that are raised have never been vaccinated. In figure 1, it shown the chickens that used as sample, it raised whitout vaccination. The feed that is given is in the form of maize, foraging for himself, and drinking water from supplied water by a government company. The chicken started showing symptoms 2 days before the necropsy. From the information obtained from interviews with chicken owners, the chicken used as a case looked thin, limp, dull feathers, decreased appetite, and diarrhea. Chickens have never been given worming or other medication before.



Figure. 1 Chicken used as a case

The chicken used in the case was observed to show symptoms of illness for 2 days. With initial symptoms of not eating, diarrhea, and weakness. Chickens with the same age as case chickens show more rapid growth. When observed and examined, the chicken looks limp, feathers dull, and inactive. There was a slight discharge in the nose and the chickens did not show any neurological symptoms. In epidemiological studies, the factors that need to be known are the host, environment, and agents. These three factors play an important role in the occurrence of a disease. The host, in this case, was a native chicken aged ± 3 months.

According to the environmental observations, the environment where the chicken owner lives is a house with a yard still in the form of land or a large yard surrounded by many trees. Chickens are traditionally raised or released around the owner's house. The number of chickens raised was 35. The traditional way of raising chickens is that they are released in the yard of the house. Of the 35 chickens that experienced a similar incident, 9 were. Chickens are fed with corn every day or find their own food.

According to information from the owner, this chicken has never been vaccinated, has never been given worm medicine and other treatments. Since the clinical signs of decreased appetite, thinness, weakness, and diarrhea, the chicken, in this case, was suspected of being a parasitic disease, because of the time of pain and clinical signs seen and the findings of nematodes and cestodes in the small intestine and cecum, epidemiological data of chicken can be seen in table 2.

Table 2. Epidemiological data of chicken cases.

Population	Sick Chicken	Dead Chicken
35	9	0
Mortality	$= \frac{\text{dead chicken}}{\text{chicken at risk}} \times 100\%$ $= \frac{0}{35} \times 100\% = 0\%$	
Morbidity	$= \frac{\text{sick chicken}}{\text{chicken at risk}} \times 100\%$ $= \frac{9}{35} \times 100\% = 25,71\%$	
Case Fatality Rate	$= \frac{\text{dead chicken}}{\text{sick chicken}} \times 100\%$ $= \frac{0}{18} \times 100\% = 0\%$	

Calculation result shows that:

- a. The percentage of the mortality rate is: 0%
- b. The percentage of morbidity rate is: 25,71%
- c. The percentage of case fatality rate is: 0%

3.1.1. Pathology examination results

To confirm the diagnosis, it is necessary to perform a necropsy to see the changes that occur in the internal organs of the chicken and take organ specimens for further examination in the laboratory.

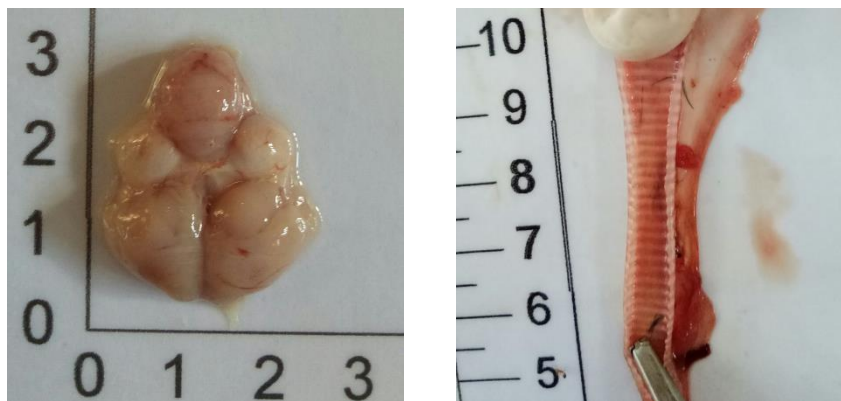


Figure 2. (a) Brain (left), looks normal; (b) Trachea (right), after incision looks normal

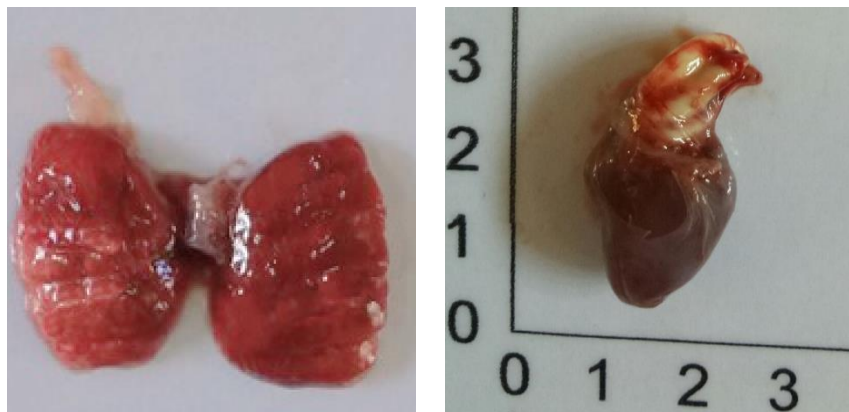


Figure 3. (a) Lungs (left), looks normal; (b) Heart (right), looks normal

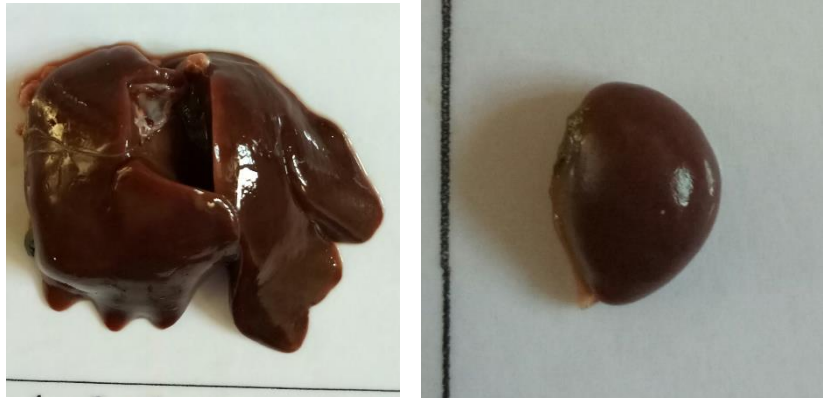


Figure 4. (a) Liver (left) looks normal; (b) Spleen (right), looks normal.



Figure 5. (a) Kidney (left), seen normal; (b) Proventriculus (right), visible tetramers (shown by arrow)



Figure 6. Ventriculus, visible inflammation

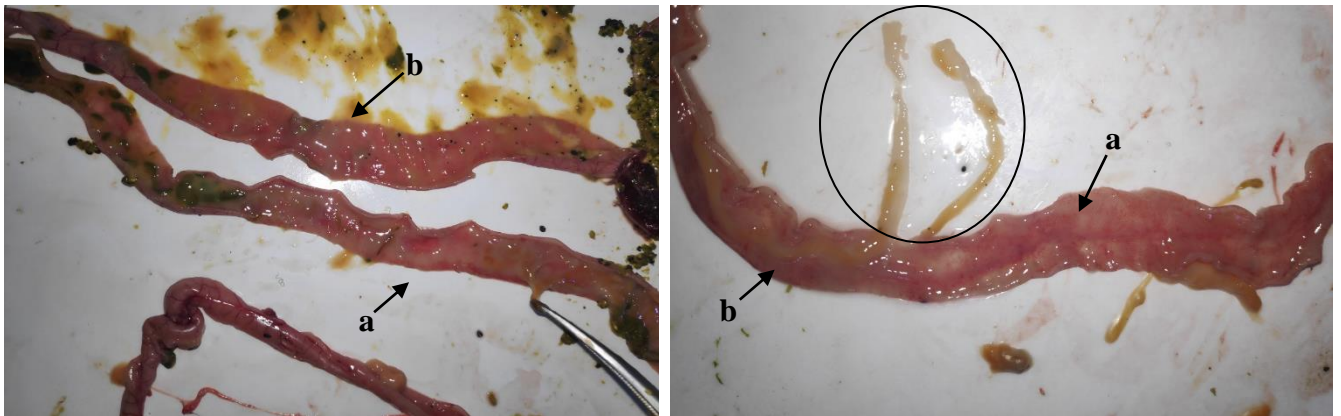


Figure 7. (a) Small Intestine (left, found worm); (b) Large intestine (right, found worm)



Figure 8. Trachea, (a) Congestion in the trachea (H&E: 100X); (b) Edema of the trachea (H&E: magnified 100X); (c) Mucosa erosion of the trachea (H&E: magnified 100X)

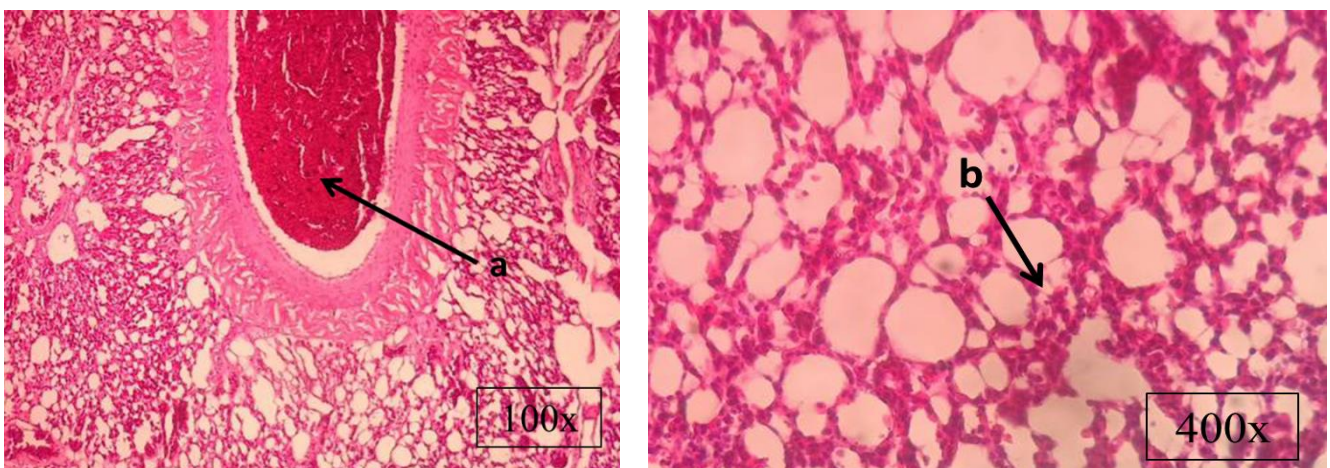


Figure 9. (a) Congestion of the lungs (H&E: magnified 100X); (b) Bleeding (H&E: magnified 400X).

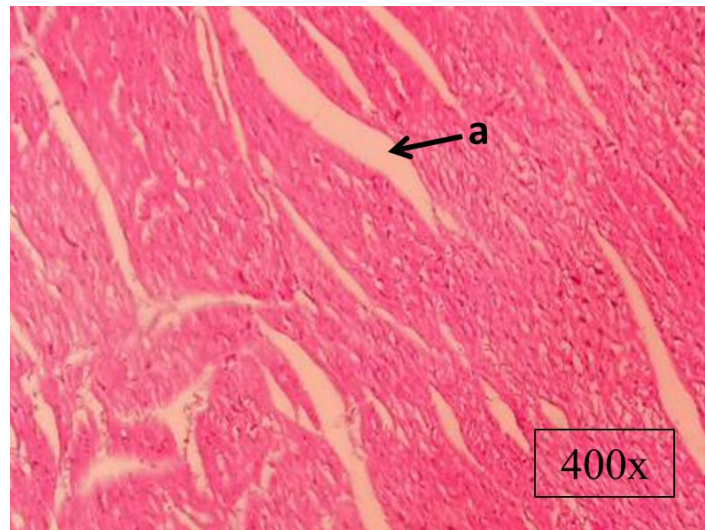


Figure 10. Heart Myocardial edema, Edema of the heart muscle (H&E: magnified 400X).

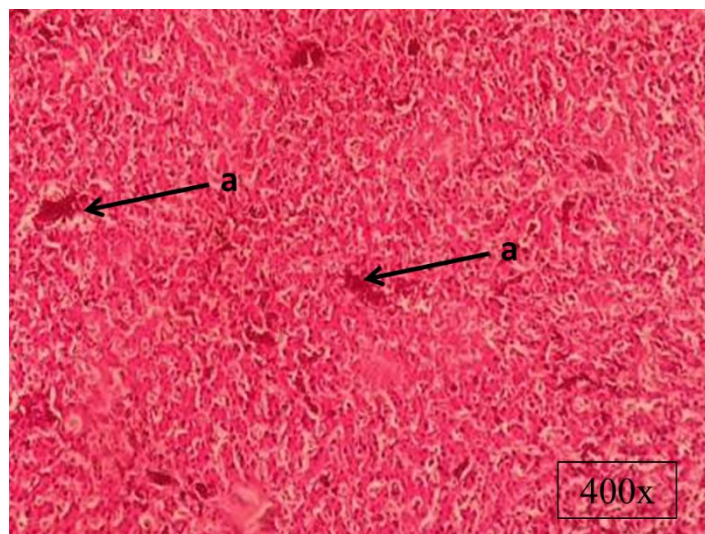


Figure 11. Liver (a) Bleeding (H&E: magnified 400X).

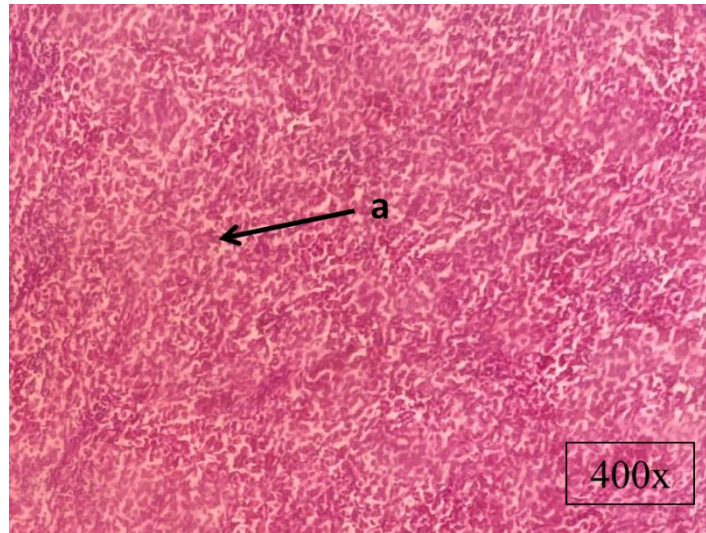


Figure 12. Spleen (a) Depletion on spleen (H&E: magnified 400X).

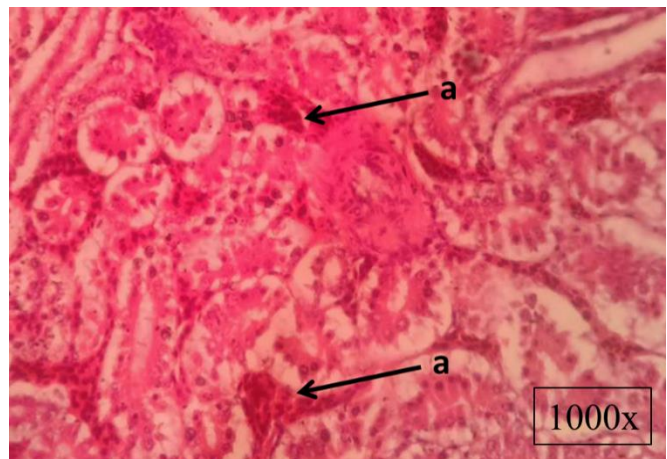
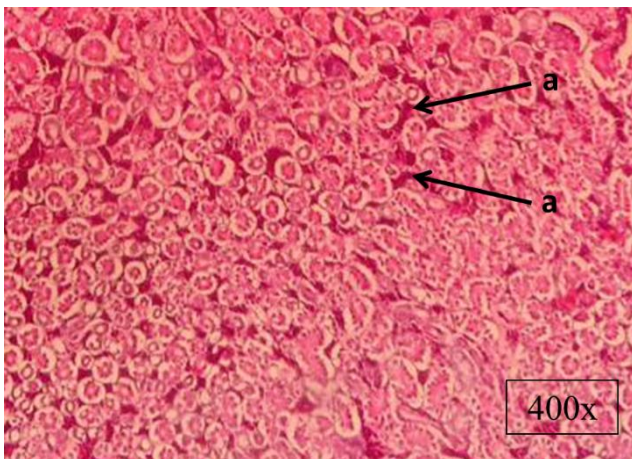


Figure 13. Kidney (a) Bleeding on tubulus (H&E: magnified 400X and 1000X).

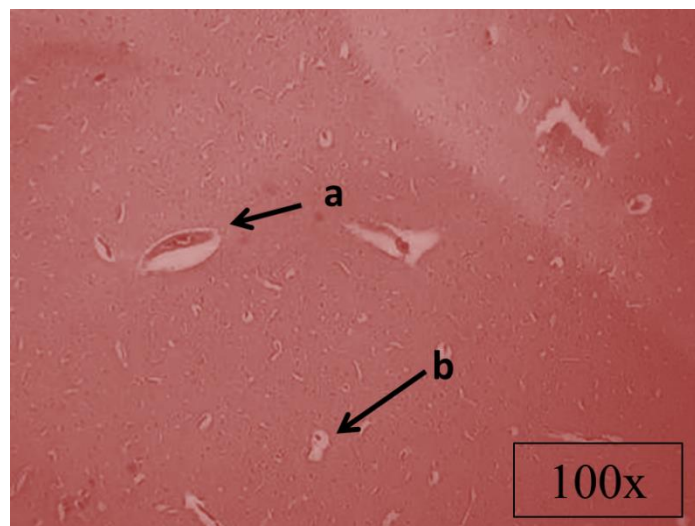


Figure 14. Brain (a) Perivascular edema and congestion, degeneration of neurons (H&E: magnified 100X).

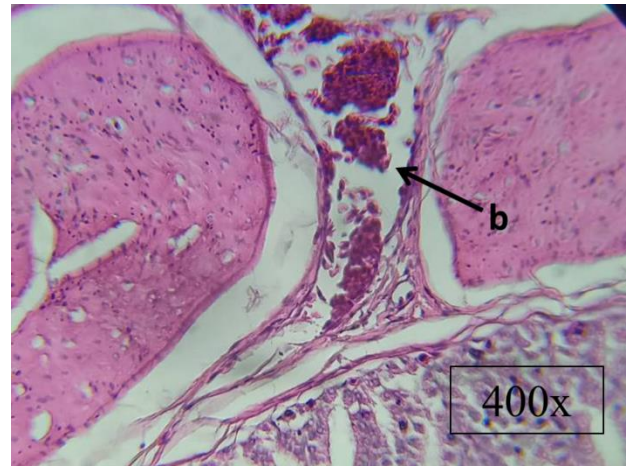
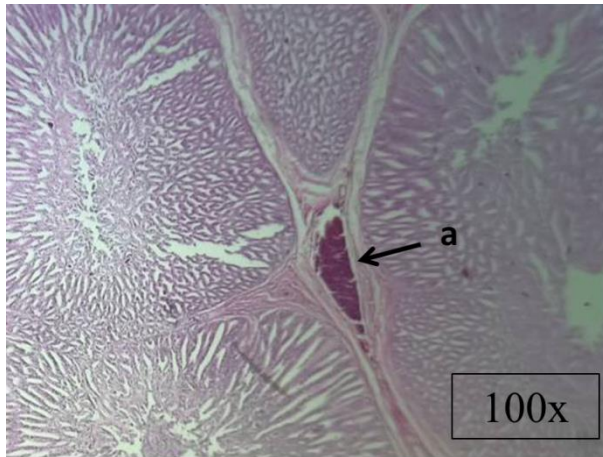


Figure 15. Proventrikulus (a) Congestion; (b) Bleeding (H&E: magnified 400X and 1000X)

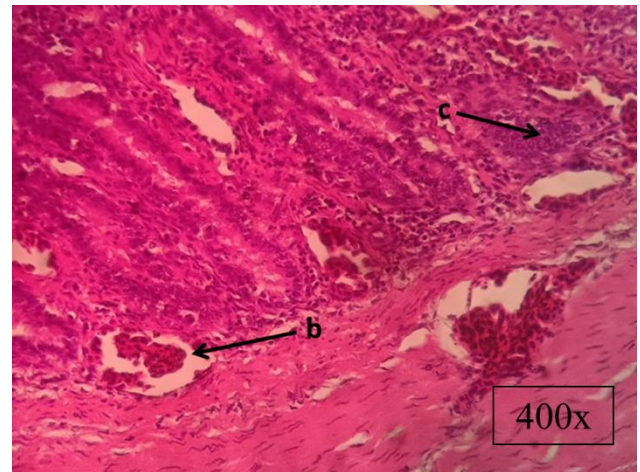
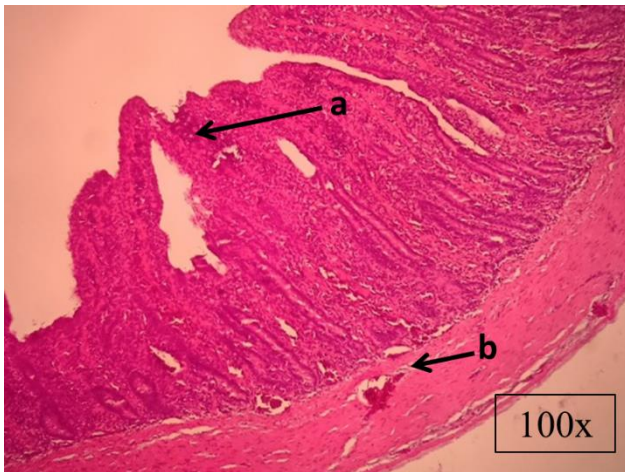


Figure 16. Small Intestine (a) Necrosis of the intestinal epithelium (H&E: magnified 100X); (b) Hemorrhage (H&E: magnified 100X and 400X); (c) Inflammation of the submucosa (H&E: magnified 400X)

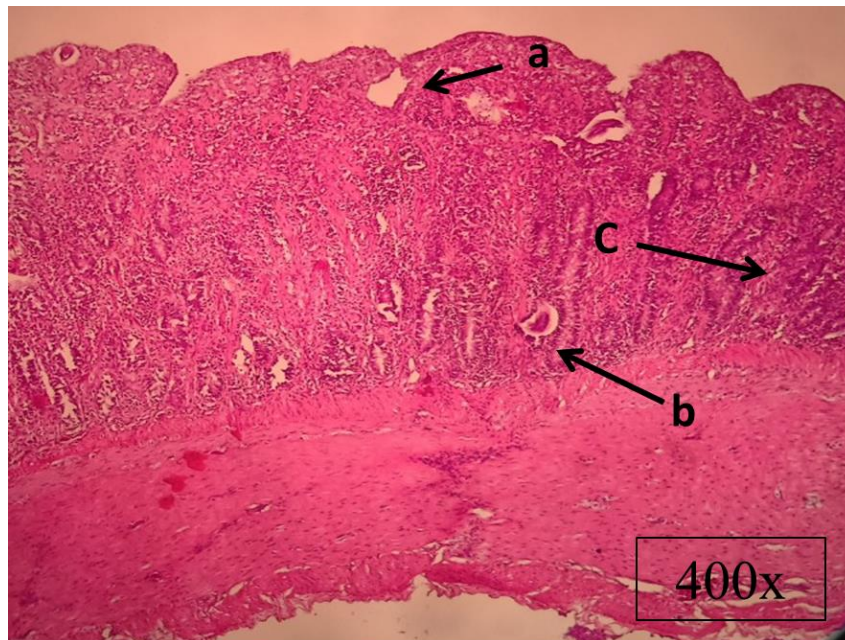


Figure 17. Small Intestine (a) Necrosis of the intestinal epithelium (H&E: magnified 100X); (b) Hemorrhage (H&E: magnified 100X and 400X); (c) Inflammation of the submucosa (H&E: magnified 400X)

Based on Pathology Examination Results shown on Figure 2a and 2b brain and trachea are normal; Figure 3 shows lungs and heart also in normal condition; Liver, spleen and kidney normal shown on Figure 4 and 5a; The proventriculus in figure 5b which found tetramers; In the ventriculus (Figure 6) can be found visible inflammation; In the small intestine and large intestine, worms can be found as shown on Figure 7.

At 400x magnification histology, in the trachea has congestion, edema, mucoso erosion shown on Figure 8; the lung on Figure 9 shown that has congestion and bleeding; Edena was found in the heart myocardial and heart muscle (figure 10); Figure 11 shown bleeding occurs in the liver; in spleen (Figure 12) depletion occurs; there also kidney bleeding on tubules (Figure 13); presence of brain perivascular edema and congestion degeneration of neurons shown on Figure 14; On figure 15, proventrikulus has congestion and bleeding; as Figure 16 and Figure 17 shown small intestine found necrosis of the intestinal epithelium, hemorrhage, and inflammation of the submucosa.

3.1.2. Microbiological examination results

The microbiological examination results (Table 3) observed Planting media in Nutrient Agar (NA), Gram stain, Bacterial planters on Eosin, Methylene Blue Agar (EMBA) media, Catalase Test, Triple Sugar Iron Agar (TSIA) Biochemical Test, Biochemical Test of Sulfide Indole Motility (SIM), Methyl Red (MR) Biochemical Test, Simon Citrate Agar (SCA) Test, Glucose Test, Lactose Test, and this examination is accompanied by an explanation.

Table 3. Results of microbiology laboratory tests

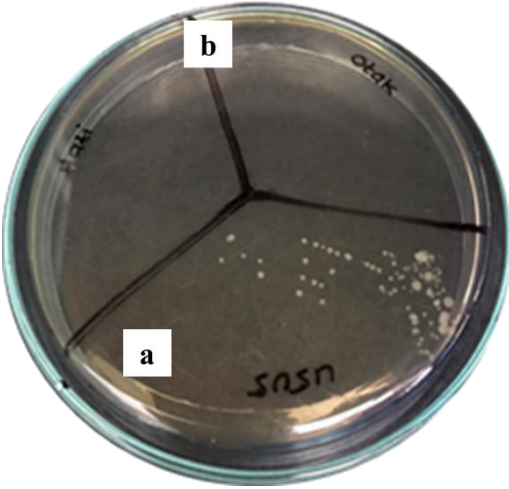
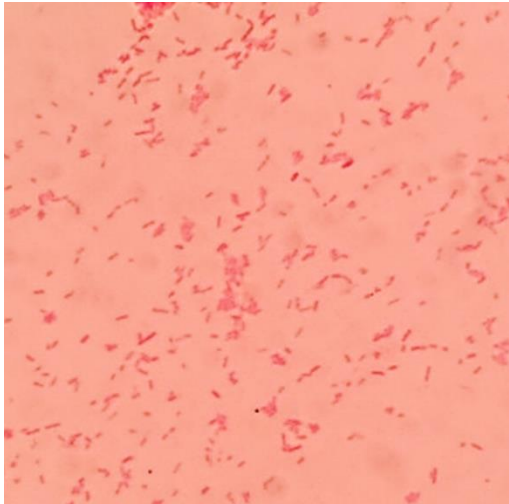
Figure	Explanation
1.	<p>Planting media in Nutrient Agar (NA) (Culture: intestines, lungs, and brain)</p>
	<p>a. Intestines there are growing colonies, morphology: round shape, convex elevation, white color with smooth surface, ± 1-3 µm colony diameter.</p> <p>b. Heart and Brain In the liver and brain, there is no visible growth of bacteria in accordance with the strike line.</p>
2.	<p>Gram stain</p>
	<p>From the results of the gram stain of the intestinal culture samples, it is seen that the bacteria in the form of short rods are colored red.</p> <p>Conclusion: Gram-negative cocobacil.</p>


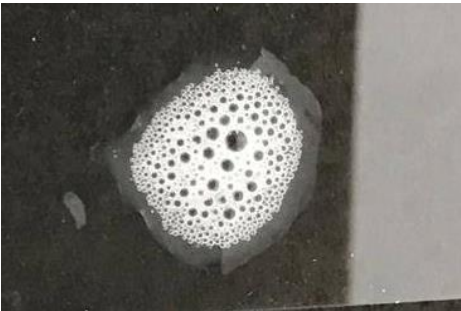
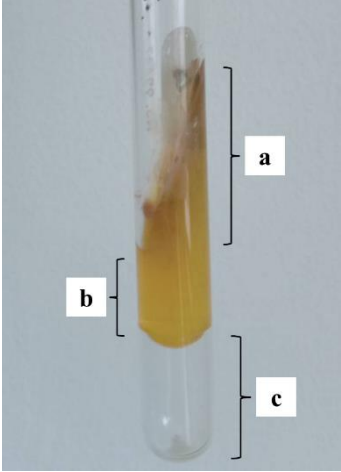
Figure	Explanation
<p>3.</p> 	<p>Bacterial planters on Eosin Methylene Blue Agar (EMBA) media</p> <p>In the EMBA media the intestinal samples were cultured to grow colonies of metallic green, round shape with flat edges, and a convex surface with a diameter of $\pm 1-3 \mu\text{m}$.</p>
<p>4.</p> 	<p>Catalase Test</p> <p>The catalase test for intestinal culture samples showed a positive result (+). This is indicated by the presence of bubbles after the bacterial colony is smeared on a glass object that has been dripped with 3% H_2O_2.</p>
<p>5.</p> 	<p>Triple Sugar Iron Agar (TSIA) Biochemical Test</p> <p>a. Slant (slant): changes color from red to yellow (acid), indicating the bacteria are acidic.</p> <p>b. Erect plane (butt): change color from red to yellow (acid) indicates the bacteria are acidic.</p> <p>c. The media has an air cavity indicating it is producing gas (+).</p> <p>The media is not black, meaning that it does not produce H_2S.</p>

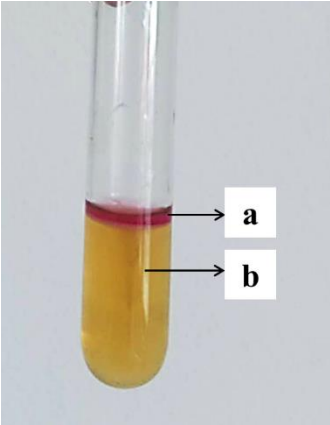
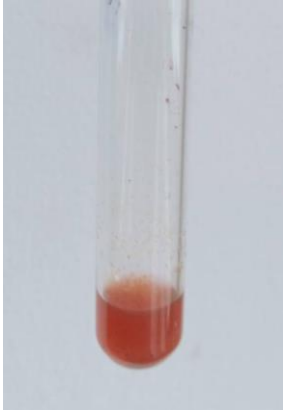

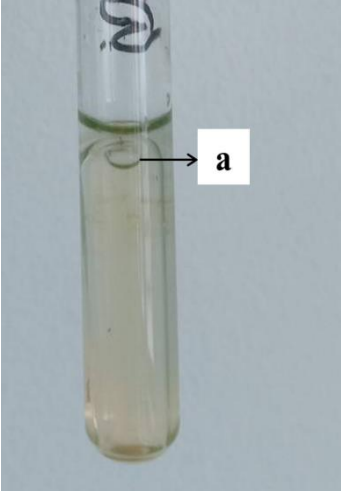
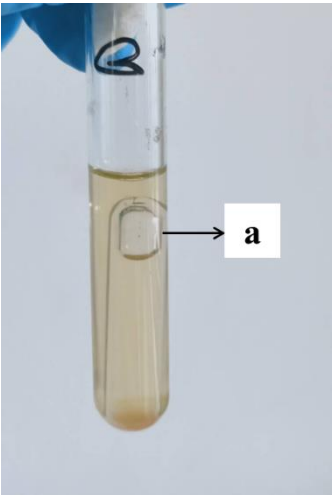
Figure	Explanation
6.	Biochemical Test of Sulfide Indol Motility (SIM)
	<p>a. Indole positif (+), indicated by the presence of a red ring after being tethered with Kovac's reagent.</p> <p>b. Motility positive (+), indicated by the presence of turbidity in the puncture area.</p> <p>H₂S is negative (-), there is no change in color to black.</p>
7.	Methyl Red (MR) Biochemical Test
	<p>MR (+), there is a change in color to red after being tethered with Methyl Red reagent.</p> <p>This shows that the bacteria ferment glucose and produce a stable acid.</p>
8.	Simon Citrat Agar (SCA) Test
	<p>SCA (-), no discoloration of the SCA media.</p> <p>This shows that bacteria cannot use citrate as a carbon source.</p>

Figure	Explanation
9. 	Glucose Test Glucose test changes the color of the media from blue to yellow. a. The presence of gas in the durham tube indicates that the glucose result is positive (+).
10. 	Lactose Test The Lactose test changes the color of the media from blue to yellow. a. The presence of gas in the Durham tube indicates that the lactose result is positive (+).

3.1.3. Parasitology examination results

On parasitology examination found nematode and cestode worms, photos and sizes of worms found can be seen in the Table 4.

Table 4. Findings of nematode worms

Nematode



Result:

Types: *Ascaridia galli*, *Heterakis gallinarum*, and *Capillaria caudinflata*

Color: Milky white

Average size: 4mm - 3cm

Total: 133 individuals

Predilection: Small intestine and cecum



Result:

Type: *Tetrameres americana*



Color: Dark brown

Average size: \pm 3mm

Total: 3 tails

Predilection: Proventriculus

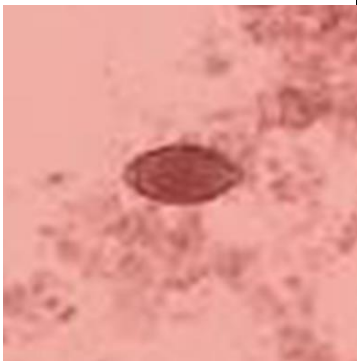

Table 5. Findings of cestode worms

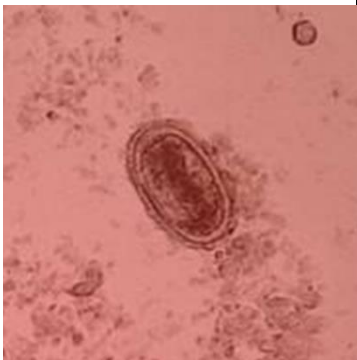

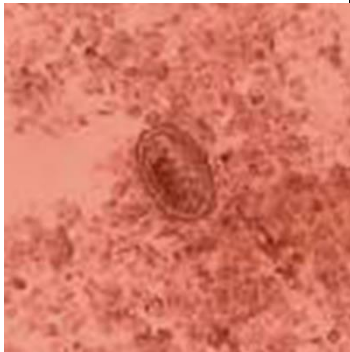
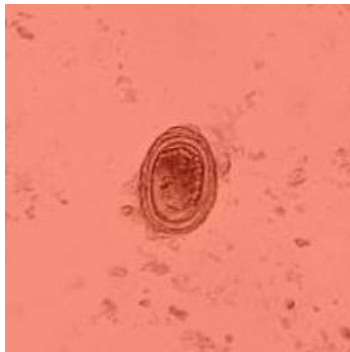
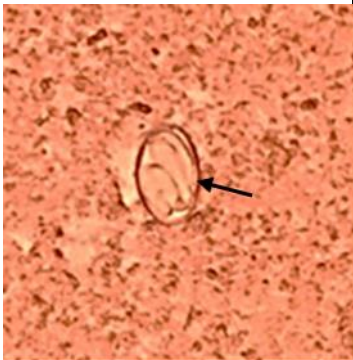



Cestode	
	
<p>Result : Type: Tapeworm (<i>Raillietina</i> sp.) Color: Milky white Average size: ± 2-5cm Quantity: 7 (skolek: 1) Predilection: Small intestine</p>	

3.1.4. Qualitative examination of stool

On the qualitative examination of stool found eggs from worms which can be enlarged in the Table 6.

Table 6. Fecal qualitative examination results (magnified 100X)

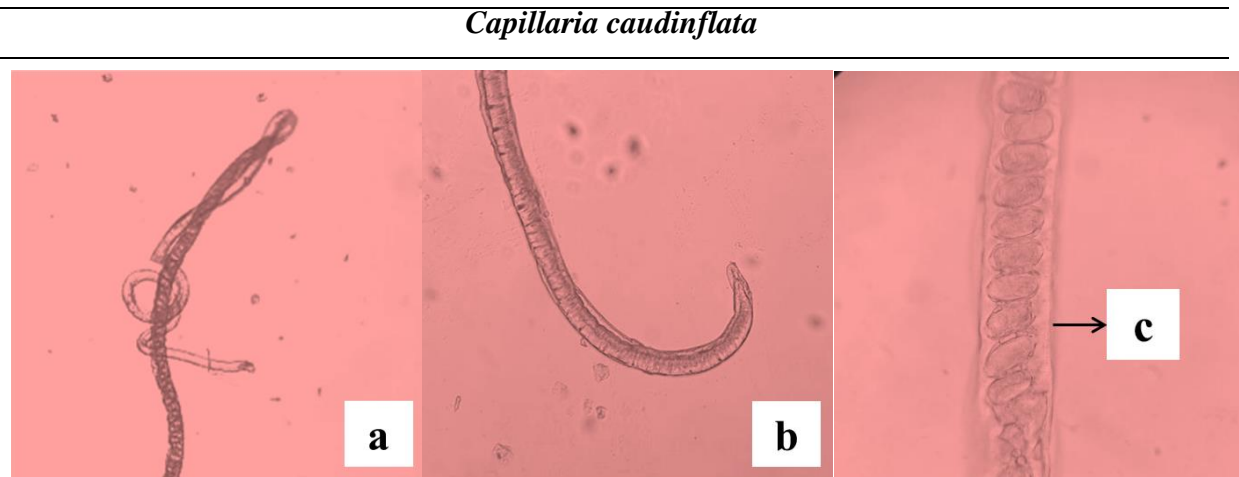
Sedimentation	Floating	Explanation
		<p>Type: <i>Capillaria caudinflata</i> Average size: 43-60 µm (length) - 20-27 µm (width) Shape: lemon-like - there is a bipolar plug / transparent plug protruding at each end (arrow) - thick, brown, smooth walls - Grain, unsegmented</p>

		<p>Type: <i>Heterakis gallinarum</i> Average size: 63-75 μm (length) - 36-48 μm (width) Shape: oval like a capsule - thick walls, smooth - not segmented - larger than the eggs of <i>Ascaridia galli</i></p>
		<p>Type: <i>Ascaridia galli</i> Average size: 75-80 μm (length) - 45-50 μm (width) Shape: oval - wall consists of 3 thick and smooth layers (middle layer is most developed) - not segmented - smaller in size than <i>Heterakis gallinarum</i> eggs</p>
		<p>Type: <i>Tetrameres americana</i> Average size: 33-65 μm (length) - 25-45 μm (width) - Shape: oval, - Transparent, thick walls - there is a larva (arrow)</p>
		<p>Species: <i>Eimeria</i> sp. oocysts are not sporulating Average size: 33-65 μm (length) - 25-45 μm (width) - Form: ovoid - Layered walls</p>

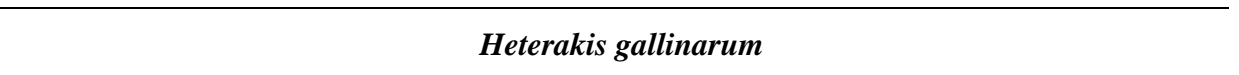
3.1.5. Microscopic Examination

On microscopic examination found *Capillaria caudinflata*, *Heterakis gallinarum*, *Ascaridia galli*, *Tetrameres americana*, *Raillietina* sp. which can be seen in the Table 7.

Table 7. Microscopic examination results of worms (magnified 100X)



- a. Head
 - b. Tail
 - c. Worm body that contains eggs
- shape: like thread, very fine



a. Head

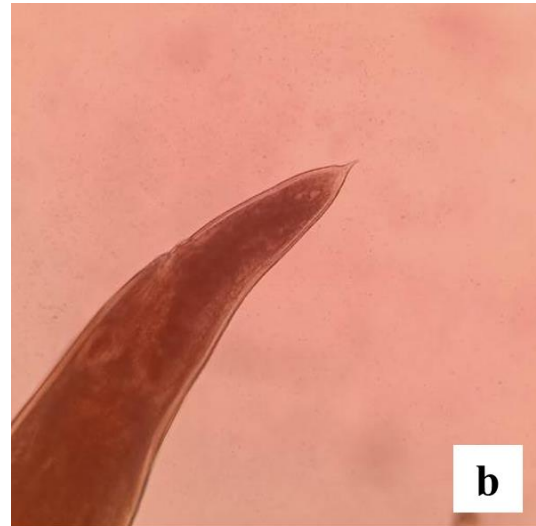
b. Tail

round shape

Length: male 3-4 mm; the female is 8-15mm long

Has 3 lips

Ascaridia galli



a. Head

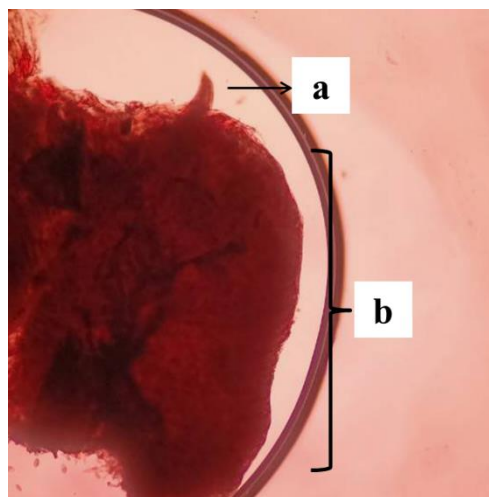
b. Tail

Shape: gilig

male body length: 51-76 mm; females: 72-116 mm.

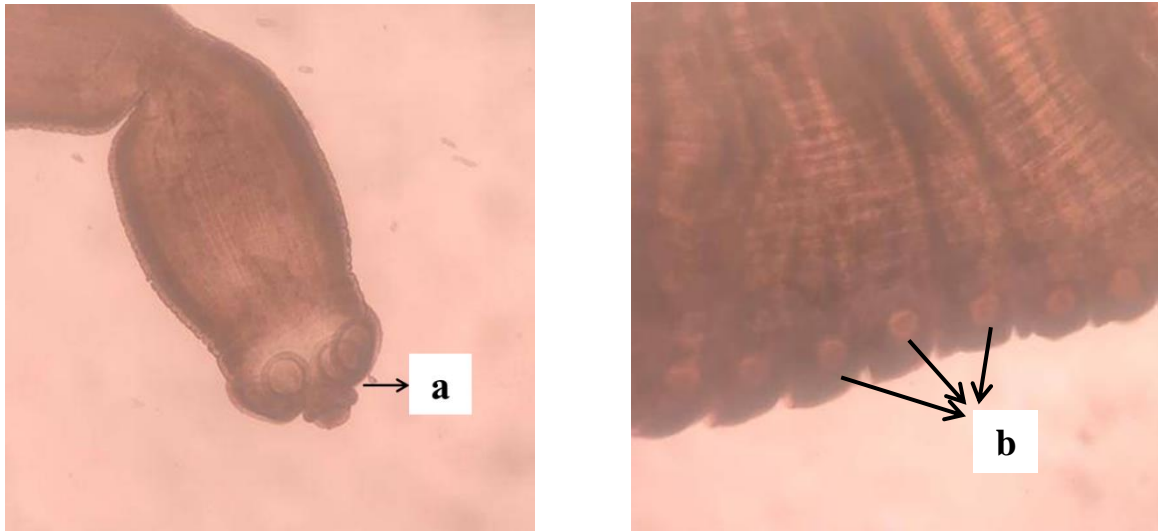
has 3 lips

Tetrameres americana



- a. head / tail
 - b. body
- female
shape: round or globular (conical anterior and posterior)

Raillietina sp.



- a. Skolek
Surrounded by four suckers
- b. Gravid proglottid: porus genitalis (arrow)

3.2. Discussion

In confirming the diagnosis of a disease, it is necessary to combine the epidemiological triangle consisting of three factors, namely the agent, host, and environment, associated with the findings of clinical symptoms, changes in anatomical pathology, changes in histopathology, and laboratory examinations. Hence, a native chicken can be diagnosed with protocol number 369 / N / 20 experienced Helminthiasis accompanied by Coccidiosis, with suspicion of blood or bacterial protozoa infection based on the results of organ histopathology examination. The diagnosis was confirmed when the nematode worm, *Tetrameres americana*, was found. in the proventriculus (Table 4), *Ascaridia galli* and *Capillaria caudinflata* in the intestine, *Heterakis gallinarum* in the cecum, and cestode worms *Raillietina* sp. in the small intestine can be seen in Table 5, oocysts *Eimeria* sp. on the examination of the floating test stool, total of worms found can be seen in Table 8.

Table 8. Number of worms found

	Worm types	Total
Nematode	<i>Ascaridia galli</i> , <i>Heterakis gallinarum</i> , <i>Capillaria caudinflata</i>	133
	<i>Tetrameres americana</i>	3
Cestode	<i>Raillietina</i> sp.	7

Based on information from the chicken owners during the field survey, it was found that the number of chickens kept by the chicken owners was 35, 9 of which showed symptoms of illness. Symptoms shown 2 days earlier include decreased appetite, look weak, dull hair, stunted growth, with diarrhea. Traditional maintenance methods that are never vaccinated make native chickens have the potential for disease because they interact with disease-carrying hosts outside the cage environment. Based on epidemiological data, epidemiological calculations are obtained and produce results, namely 25.7% morbidity, 0% mortality, 0% case fatality rate. The low case fatality rate is due to parasite infections which are usually sub-acute and rarely cause death (Loliwu & Thalib, 2012).

Necropsy in the case of animals was carried out to determine changes that occur in the organs by pathological anatomy. Specimen collection aims to further examine the cause of the disease by conducting laboratory tests. In the observation of anatomical pathology, the brain, heart, liver, lungs, spleen, and kidneys did not appear to have significant changes. In the proventricular organ, there is *Tetrameres* sp. seen on the walls of the proventriculus, there is inflammation of the ventriculus, along with the intestines bleeding especially in the cecum and experiencing enteritis hemorrhagia, nematode and cestode worms are also found along the intestine. In this case, bleeding in the intestine occurs due to a worm infection that is along the intestine. Intestinal worms can cause severe damage during migration to the tissue phase of the larval development stage, such as in the intestinal mucosal lining where bleeding occurs or hemorrhagic enteritis (Tabbu, 2000).

Histopathological observations showed that the trachea had congestion, edema, and erosion of the mucosa. The lungs are bleeding and congested. The liver and kidneys bleed, the heart has mild myocardial edema, the spleen has follicular depletion. The histopathological examination of the intestine shows bleeding, hemorrhage, necrotic erosion

of the villi, and inflammation of the intestinal submucosa. Inflammation is a vascular response, one of which is marked by the dilation of blood vessels. The number of capillaries in the lung parenchyma is dilated resulting in a red color of the lung organs (Cheville, 2006). Bleeding in the organs may also be caused by various things including viruses, bacteria, fungi, protozoa, worms, and others (Berata et al., 2011).

Examination in the microbiology laboratory uses the liver, brain, and intestines. This examination is performed to identify the presence or absence of secondary bacterial infection in these animals. As a result of implanting the liver and brain on NA media, there were no colonies that grew according to the strike or OSA line. Intestinal bacteria grew on NA media. Colonies with round bodies, 1-3 μm in size, milky white in color, were then fertilized on EMBA selective media. The colonies that grew on EMBA media were metallic green. The test was then continued with biochemical tests and gram staining. The results of the identified gram colony staining were red bacterial colonies and short bacilli. The red color indicates that the bacteria are gram-negative because they are unable to retain the methyl purple dye in the gram stain. Biochemical test by fertilizing on Triple Sugar Iron Agar (TSIA) media with positive results with a change in color to yellow, which means the bacteria ferment lactose and sucrose, the acid butt part also changes color which means the bacteria are acidic, and TSIA media shows gas formation.

The Methyl Red (MR) test changes color to red, after dropping the MR reagent, which means a positive result (+); In the Sulfide Indole Motility (SIM) test, there is a blur in the needle puncture area which indicates that motile bacteria (+) changes color to red after being dripped with Kovac's reagent (a red ring is formed), which means positive indole (+). The citrate test using Simon Citrate Agar (SCA) media reacts negatively, which means that these bacteria do not use citrate as a carbon source, in the sugar test with glucose and lactose media, the positive bacteria ferment glucose and lactose, and the Catalase test (+) which means there is the activity of the enzyme catalase which breaks down H_2O_2 . This character is in accordance with the character of the biochemical test of members of the genus *Escherichia* (Holt, 1994). These bacteria are normal flora in the digestive tract.

Examination at the parasitology laboratory was carried out by observing using fecal samples and identifying worms that had been microscopically collected from the proventriculus and intestines of native chickens. In the proventriculus, there were 3 *Tetrameres americana* worms included in the nematode worms and supported by the discovery of worm eggs in the sedimentation test, while in the intestine, 133 worms were

found in the form of roundworms (nematodes) which were difficult to identify macroscopically, after microscopic observation and support. Through the sedimentation and buoyancy feces test, 3 different types of worms were found based on their morphology, namely *Ascaridia galli*, *Capillaria caudinflata*, and *Heterakis gallinarum*.

Ascaridia galli is the largest worm in nematodes in poultry. *Ascaridia galli* predicts chicken intestines. The adult worm *Ascaridia galli* has a semitransparent morphology that is white. On the anterior part of the acing *Ascaridia galli* there is a mouth that is equipped with three lips, one on the dorsal side and the other two on the lateroventral part. The size of adult male worms is 51-76 mm long and adult female worms are 72-116 mm. Male worms have a preanal sucker and two spicula measuring 1-2.4 mm, while female worms have a vulva in the middle of the body. *Ascaridia galli* eggs are oval, smooth-walled, non-segmented, and measure 73–92 μm (length) x 45–57 μm (width), life cycle of *Ascaridia galli* can be seen in Figure 18.

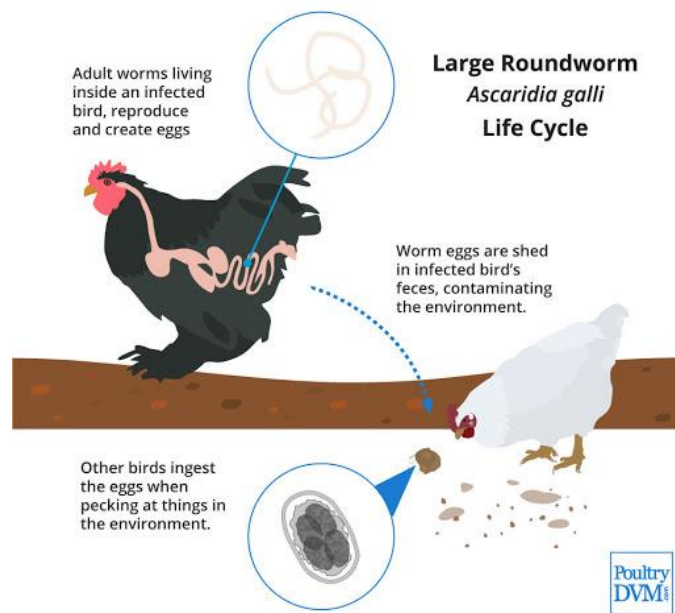


Figure 18. Life Cycle *Ascaridia galli*
(Source: IDVM Animal, 2020)

Heterakis gallinarum worms are cecum worms that are commonly found in chickens and birds. Morphologically, male worms have preanal suction edges, and the speculum is the same size. Female worms have a vulva located in the middle of the body. Male worms are 3-4 mm long, 120-470 μm in diameter, 0.85-2.80 mm long right spiculum and 0.37-1.10 μm left spiculum while female worms are 8-15mm long.

The morphology of *Heterakis gallinarum* worms is white with smooth and elongated tails. The morphology of eggs is oval-shaped like a capsule, thick-walled measuring 63-75 µm (length) x 36-48 µm (width). *Heterakis gallinarum* worms have a direct life cycle involving chickens as hosts. *Heterakis gallinarum* eggs come out with chicken feces. At optimal temperature (22 ° C), eggs will turn infective in 12-14 days and remain infective for years in the soil. Once ingested by the host, the embryonic eggs hatch into larvae, the second stage in the duodenum, and are passed on to the cecum. The development will be completed in the lumen, but some larvae will enter the mucosa and settle. The prenatal period is 24-30 days. Intermediate hosts are earthworms and house flies because they can swallow eggs, which remain inactive until the larvae are eaten by chickens, life cycle of *Heterakis gallinarum* can be seen in Figure 19.

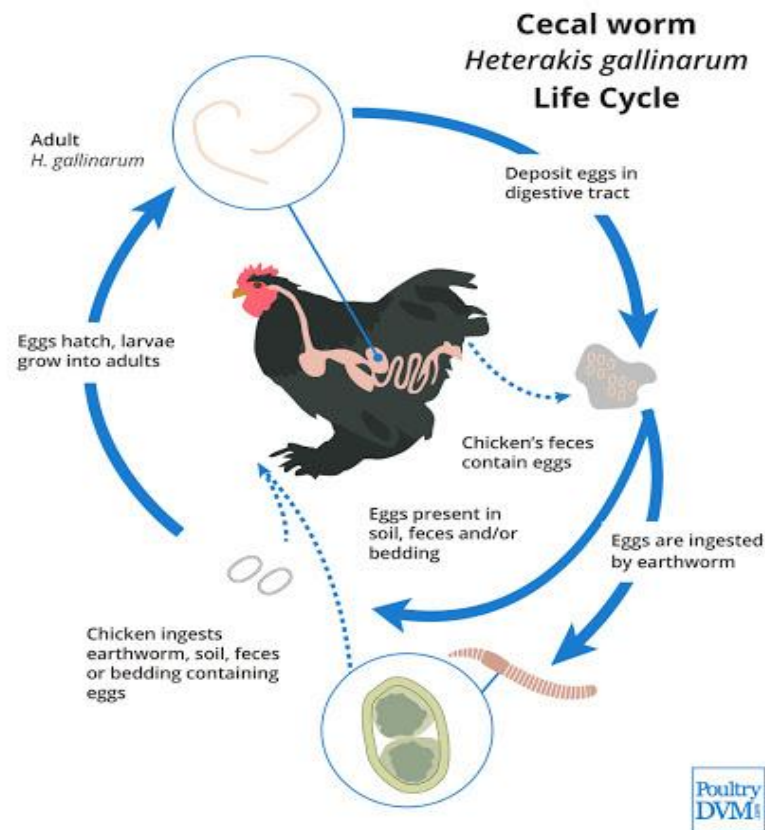


Figure 19. *Heterakis gallinarum* Life Cycle

(Source: IDVM Animal, 2020)

Capillaria caudinflata has a slender overall body. The body is capillary and has a simple mouth. The female worm's vulva is close to the end of the esophagus. Sometimes these worms have a curriculum that is always there. Male worms 11-15 mm long, 10-25 mm

female. This egg has two plugs at both ends with an egg size of 43-70 μm (length) x 21-30 μm (width). The life cycle of *Capillaria caudinflata* is indirect. Eggs that come out with feces are swallowed by earthworms and develop to the infective stage after 2-3 weeks. The prenatal period for these worms is about 3-4 weeks, life cycle of *Capillaria caudinflata* can be seen in Figure 20.

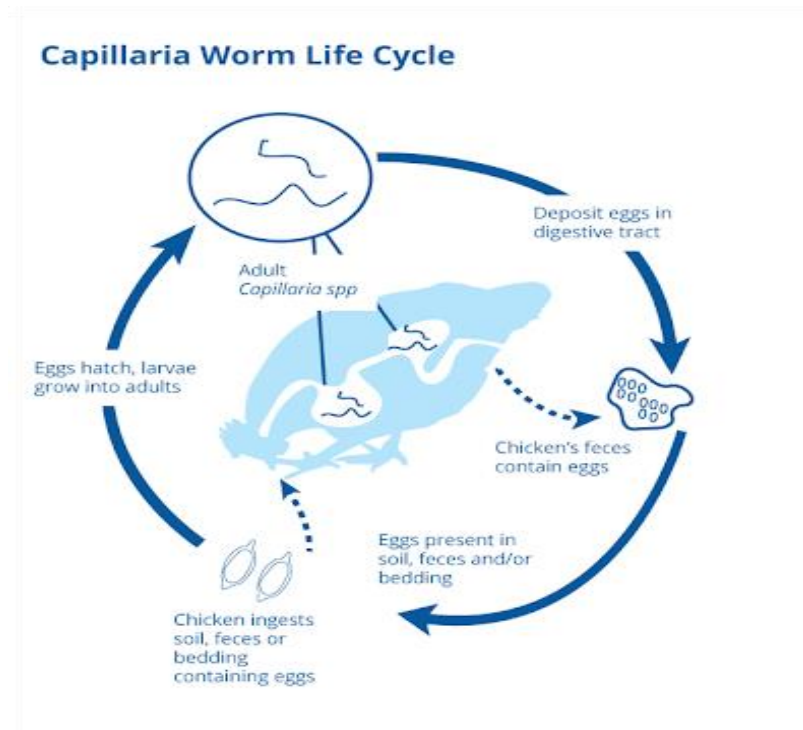


Figure 20. Life Cycle of *Capillaria caudinflata*

(Source: IDVM Animal, 2020)

Furthermore, *Tetrameres americana* worms were also found in the proventriculus walls, with the morphology of the malignant female worms, namely globular or round board with brownish red color. While male worms have a shape like nematode worms in general, resemble threads and are white, accompanied by a spiculum that is not as large. The morphology of egg-filled, thin-walled embryos when excreted with feces, measuring 42-50 μm (length) x 24 μm (width). Male worms are 5.0-5.5 mm long with a diameter of 116-133 μm , with a spiculum 290-312 μm and 100 μm in length. The female worm is 3.5-4.5 mm long and 3 mm wide. The anterior and posterior ends protrude about 0.9 mm towards the outside of the round part of the body. The intermediate hosts of the worm *Tetrameres americana* are insects such as grasshoppers and cockroaches, life cycle of *Tetrameres americana* can be seen in Figure 21.



Figure 21. Life Cycle of *Tetrameres americana*
(Source: Ziela, 1999)

Infection with nematode worms in the body of chickens can have a detrimental effect on the survival of the chickens. This impact can affect the digestive system and metabolism of the chicken body. This is caused by the activity of worms as parasites in the body. Such as that caused by *Capillaria caudinflata* worm infection which will dig up the mucosa resulting in thickening of the intestinal wall and enlargement of the mucosal glands which can cause desquamation. Chicken cases with protocol number 369 / N / 20 experienced indigestion and slow growth, these symptoms are due to *Capillaria caudinflata* worm infection which can cause emolition, diarrhea, hemorrhagic enteritis, and even death. This is related to lesions in the digestive tract which interfere with digestion, feed absorption, and underperformance of native chickens. Losing in chickens can also be caused by infection with *Ascaridia galli* worms, which can cause weight loss which is directly related to the number of worms present in the chicken's body (Permin, Nansen, Bisgaard, & Frandsen, 1998). In severe infections, intestinal obstruction can occur, large amounts of *Ascaridia galli* infection can lead to decreased blood sugar levels, increased uric acid, atrophy of the thymus, growth disorders, and increased mortality. The impact of *Tetrameres americana* worm infection, on the proventriculus wall will be enlarged due to the predilection of worms, thus narrowing the lumen.

In the intestine, the cestode worms *Raillietina* sp. Were also found, but in the fecal test, there were no sedimentation or floating eggs of the worms, this probably happened because the tapeworm reproductive system in the gravid proglottid section will only release eggs within a certain time when it is ready. *Raillietina* sp. Worms is a tapeworm that can reach 25cm in length. Has a sucker and a hooked rostellum and unilateral genital pore that is anterolateral to mediolateral. The prenatal period of *Raillietina* sp. is 3-4 weeks (FAO, 1998). The life cycle of cestode worms starts from the proglottid segments or eggs that are released by poultry through feces. The infective worm eggs contained in the segment will be eaten by the intermediate host (ant) for several days. The eggs then develop into cysticercoids in the cavity of the intermediate host body.

Poultry that ingests the contaminated intermediate host will be infected, after digestion, the cysticercoids will release the young tapeworms attached to the intestinal wall. The time between infection and the first egg release is 2-3 weeks. The life cycle of tapeworms generally passes through an intermediate host, the role of the intermediate host which makes tapeworms easy to spread widely. Eggs that come out with feces will be active in the environment. Therefore, they can then be eaten and develop in the body of the intermediate host. Clinical symptoms due to cestode worms in chickens are influenced by the feed status or nutritional status of the livestock, the number of infections, and the age of the chickens. In young chickens, it is usually indicated by weight loss, loss of appetite, stunting, and diarrhea (Rismawati, Yusfiati, & Mahatma, 2013), enteritis, diarrhea, and death if there is a lot of worm intensity in the intestine (FAO, 1998).

In addition to worm eggs in the floating test, oocysts were also found, this indicates an infection of the digestive tract protozoa. Protozoa infection of the digestive tract in chickens is caused by the species *Eimeria* sp. Protozoa in the digestive system cause histopathological changes in the intestine in the form of bleeding, erosion of the intestinal villi. Breeding of *Eimeria* sp. in the small intestine mucosal epithelial cells can cause epithelial cell damage and an inflammatory reaction. The cells that collect around the lesion will increase the permeability of the small intestine blood vessels resulting in hemorrhage of the pericedesis. Several species of *Eimeria* sp. forming colonies in the small intestine which contain hundreds of merozoites. The merozoites will develop and invade deeper into the lamina propria layer. Moreover, when the merozoites are released from the colony there will be severe erosion of the small intestinal mucosa. Erosion of the small intestine mucosa causes nutrient absorption to be not optimal (Akoso, 1993). Absorption of nutrients that are not optimal in a sustainable

manner will cause a decrease in the immunity of these birds. According to research by [Akhtar et al. \(2015\)](#) chickens infected with *Eimeria* sp. experienced an increase in lymphocytes, heterophils, monocytes, and eosinophils compared to uninfected chickens.

In the results of the histopathological observation of the native chicken cases with the protocol number 369 / N / 20, almost all of them experienced bleeding, outside of the digestive organs which were caused by helminth infection. Inflammation is a vascular response, one of which is marked by the dilation of blood vessels ([Cheville, 2006](#)). Inflammation and bleeding can be caused by many possibilities, one of which is the presence of infectious agents such as viruses, bacteria, and parasites. Inflammation can also be caused by exposure to chemicals, extreme temperatures, animal stress, trauma, and surgery ([Berata et al., 2011](#)). In this case, it is suspected that inflammation in other organs such as the liver, lungs, kidneys, and spleen is caused by other agents besides parasites such as bacteria or viruses, because many worm infections in the native chicken body can have an impact on the decline in the chicken's immunity, making it easier for the agent. other diseases to infect the body. In parasitic relationships, immune suppression occurs either by immune cells, soluble suppressants, or both or by temporary or permanent damage to elements of the immune system ([Muneer, Farah, Newman, & Goyal, 1988](#)). Endoparasitic infection can cause failure to increase antibody titers against viral agents, which is frequently observed in poultry ([Kurkure et al., 2006](#)). This explains the complications of the disease that may occur because it is still needed for further observation.

Based on the results, it was explained that endoparasitic infection can be caused by several factors, such as food factors and how to raise chickens. Chicken is an omnivorous animal so it does not select the food to eat ([Marhiyanto, 2006](#)). In addition, endoparasite infection can also be influenced by endoparasitic distribution factors, namely the interaction between the host and endoparasite individuals, host compatibility, and the level of host immunity to endoparasites ([Kusumamihardja, 1992](#)). [Firman \(2011\)](#) stated that the maintenance of chickens with traditional (extensive) systems as well as under-paid rearing systems can result in a very large possibility of endoparasite infection. This is because the feed eaten by chickens cannot be completely controlled. The environment that is not considered is also a hotbed for host disease vectors. In prevention efforts, it is recommended that owners pay more attention to environmental cleanliness and chicken coops with good control with a combination of preventive treatment and optimal cage management including cage sanitation ([Tabbu, 2000](#)). The development of environmental hygiene has an impact on the DOI: <https://doi.org/10.7454/jessd.v4i1.1083>

development of the quality of life of poultry, which is a good quality of life conditions in poultry could prevent infection from endoparasites, therefore by maintaining the quality of the living environment of the poultry house is in line with reducing the risk of exposure to endoparasitic infections.

4. Conclusion

Based on the history, clinical symptoms, epidemiology, anatomical pathology, histopathological examination, and microbiological examination results, it can be concluded that chicken cases with protocol number 369 / N / 20 experienced Helminthiasis caused by nematode worms (*Ascaridia galli*, *Heterakis gallinarum*, *Capillaria caudinflata*, and *Tetrameres americana.*), cestodes (*Raillietina* sp.), and Coccidiosis by protozoa (*Eimeria* sp.) with suspected secondary infection by other agents such as bacteria, viruses, or blood protozoa due to histopathological examinations of organ preparations which are dominated by internal bleeding of native chickens. On postmortem examination as definitive diagnosis, nematode and cestode worms were found in the proventriculus and intestine.

It is recommended that farmers improve cage sanitation and maintenance management. Thus, the chickens are not easily infected by endoparasites. The limitation of this research is that the data collection is still less diverse and recommendations for future research can carry out many experimental tests with existing samples and it should be noted for a more effective way of collecting worms.

Acknowledgment

We would like to express our gratitude to the people who helps in this research.

Author Contribution

Phebe Amadea Tanuwijaya contributed ideas for choosing background, methods, data collection, and data analysis. David Febraldo carried out method, presentation of results, discussions, and bibliography.

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