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Subclinical anaplasmosis infection on Ettawa Goats from Kaligesing, Purworejo, Central Java, Indonesia

Infeksi anaplasmosis subklinis pada Kambing Ettawa dari Kaligesing, Purworejo, Jawa Tengah, Indonesia

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ABSTRACT

Small ruminant anaplasmosis is a blood-parasitic disease that can cause reproductive and economic problems, especially in Ettawa crossbred. In Indonesia, especially in Central Java Province, the study of this pathogen is still limited. This study examines subclinical anaplasmosis in Ettawa crossbred goats from central Java, Indonesia. Twenty adult goats underwent comprehensive physical and laboratory examinations. The physical exams assessed factors like sex, body temperature, heart rate, respiration, and rumen function. Laboratory testing involved blood smears to detect Anaplasma species and complete blood counts using a hematology analyzer. The results showed that 8 goats were positive for Anaplasma, with 2 samples resembling A. phagocytophilum and 6 resembling A. marginale. Females were more likely to be infected. Infected goats did not exhibit significant changes in vital signs compared to healthy goats. However, infected goats had lower red blood cell counts, hemoglobin, and hematocrit, as well as higher white blood cell counts, indicating subclinical anemia. This is the first study to demonstrate the impact of subclinical Anaplasma infection on Ettawa crossbred goats in this region, highlighting the potential for production losses from this overlooked disease.

ABSTRAK

Anaplasmosis adalah penyakit parasit darah yang dapat menyebabkan masalah reproduksi dan ekonomi, terutama pada kambing Peranakan Ettawa. Di Indonesia, khususnya di Provinsi Jawa Tengah, kajian patogen ini masih terbatas. Penelitian ini mengkaji anaplasmosis subklinis pada kambing peranakan Ettawa dari Jawa Tengah, Indonesia. Dua puluh kambing dewasa dilakukan pemeriksaan fisik dan laboratorium yang komprehensif. Pemeriksaan fisik menilai faktor-faktor seperti jenis kelamin, suhu tubuh, detak jantung, pernapasan, dan fungsi rumen. Pengujian laboratorium melibatkan apusan darah untuk mendeteksi spesies Anaplasma dan menghitung darah lengkap menggunakan automatic hematology analyzer. Hasil penelitian menunjukkan bahwa 8 ekor kambing positif Anaplasma, dengan 2 sampel terdeteksi A. phagocytophilum dan 6 sampel A. marginale. Kambing betina memiliki tingkat infeksi lebih tinggi dibanding jantan. Kambing yang terinfeksi tidak menunjukkan perubahan yang signifikan pada tanda-tanda vital dibandingkan dengan kambing yang sehat. Namun, kambing yang

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Anaplasmosis
Peranakan ettawa,
Kambing
Kaligesing
Subklinis

terinfeksi memiliki jumlah sel darah merah, hemoglobin, dan hematokrit yang lebih rendah, serta jumlah sel darah putih yang lebih tinggi, menunjukkan anemia subklinis. Ini adalah studi pertama yang menunjukkan dampak infeksi Anaplasma subklinis pada kambing kawin silang Ettawa di wilayah ini, menyoroti potensi kerugian produksi dari penyakit yang diabaikan ini.

INTRODUCTION

Ettawa crossbred (EC) goats are superior local goats that have great potential to meet the increasing market demand for both meat and milk (Cyrilla et al., 2016). The excellence of genetic quality and high productivity make EC goats an important asset in advancing the goat farming industry in Indonesia. Kaligesing District in Purworejo Regency is known as the center for producing the most PE goat seeds in Central Java province (Mudawamah et al., 2021). The development of PE goat livestock in this region not only has an impact on improving the economy of local farmers but also contributes to national food security because Central Java Province has 20.6% of the national goat population (Mudawamah et al., 2021; Rasyid et al., 2020). The livestock subsector in Purworejo is important for the local economy but is less competitive than other sectors, so there is a need for a strategy to increase farmers' income and develop the PE goat farming subsector. Small ruminant farms need to increase prevention as early as possible to reduce losses due to the impact of reproductive-related diseases (El-Raghi & Hashem, 2022).

A common, yet often overlooked disease affecting goats is blood parasitic infection. A blood parasite that often attacks goats is Anaplasma spp. (A. Khan et al., 2019; Rahman, Faruque, Rahman, & Chowdhury, 2022). Anaplasmosis has spread throughout the world and caused huge economic losses to large and small ruminant farm animals. Anaplasmosis in goats causes mild clinical symptoms, but in the event of an acute infection, it can cause depression, muscle weakness, decreased milk production, weight loss, miscarriage, severe anemia and jaundice (Alessandra & Santo, 2012; Stuen, 2020; Yousefi, Rahbari, Shayan, Sadeghi-dehkordi, & Bahonar, 2017). Anaplasma spp. is a gram-negative intracellular obligate bacterium that is transmitted by ticks to vertebrate hosts, including humans. In wild and domestic animals, this bacterium infects hematopoietic cells causing persistent infections. Goats infected with Anaplasma spp. will become reservoirs and transmit the disease to other livestock through vector intermediaries over a long period of time (Almoheer et al., 2022; Fthenakis & Papadopoulos, 2018; Lacasta et al., 2020). In Indonesia, especially in Central Java Province, the study of this pathogen is still limited. Limited research has been conducted on the prevalence and impact of Anaplasma spp. infection in goats in this region. Further investigation is needed to fully understand the epidemiology and disease burden of anaplasmosis among the significant goat population in Central Java.

This study aims to determine the incidence of Anaplasma spp. infection on EC goats in Kaligesing district, Purworejo Regency, Central Java Province. Information on the health status of goats is expected to increase farmers' awareness of the selection of broodstock and good livestock management to reduce the risk of economic losses. Animal health workers are also expected to be able to control cases of subclinical anaplasmosis in the livestock center area.

MATERIALS AND METHODS

Sampling Site and Study Design

EC goats were used as sample in this study. All goats originated from Kaligesing, Purworejo, Jawa Tengah which is located at -7.74459724008656 South Latitude and 110.12815672009724 East Longitude with an altitude of 250m above sea level (Figure 1). Sample examination was carried out at the Laboratory of the Department of Clinical Pathology of FKH UGM from December 2023 to March 2024.

Sample Collection

Blood samples were randomly collected without consideration of the sex and age. Samples were calculate using a website-based calculator, namely EpiTools on the https://epitools.ausvet.com.au/freedomfinitepop website with the detect disease method based on goat population data in Kaligesing sub-district in 2019, which was 49,780 heads (Nugraheni

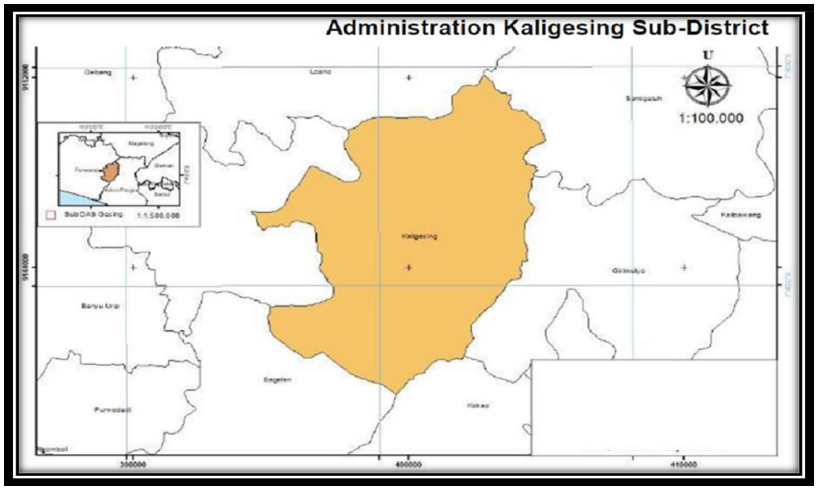


Figure 1. Map of Kaligesing sub-district (Wiratmoko & Yuda, 2019)

et al., 2023), anaplasmosis prevalence 15.75% (Rahman et al., 2022), 95% confidence level and 0.9 sensitivity test. The results of the calculation of the goat samples needed for this study are 20 samples. All samples were screened for the presence of Anaplasma sp. infection. A total of 2-3 mL of individual blood samples were drawn from the jugular vein of individual goats followed by Rahman et al., 2022. Blood samples were kept in EDTA tube collection and were subjected to thin blood smear microscopic examination.

Clinical Examination

The examination of goats begins with comprehensive anamnesis, followed by thorough observation of the visible clinical symptoms. The physical examination includes sex, measurements of body temperature, heart rate, respiratory rate, and rumen motion. All data and information obtained are recorded in detail in medical records (Abdisa, 2017).

Blood Smear Examination

Thin blood smear samples were prepared and fixed with methanol before stained by Giemsa (MerckTM). An individually stained blood smear was observed using emersion oil under 1000x magnification lens of a microscope Olympus BX51 for the presence Anaplasma sp. infection. A slide was considered positive when at least one Anaplasma sp. was found from a total of 1000 red blood cells according to WHO protocol. All positive samples were documented by camera Olympus DP12 in the Department of Clinical Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. A negative blood smear sample was evaluated at least two times by two trained technicians for reliable negative results confirmation (Rosyadi et al., 2022).

Hemogram Analysis

Routine blood tests are carried out using the VETSCAN® HM5 Hematology Analyzer including the number of erythrocytes, hemoglobin, MCV, MCH, MCHC, hematocrit and leukocyte (Rosyadi et al., 2021).

Data and Statistical Analysis

Qualitative data, namely the results of clinical examinations and blood smears, are presented descriptively, while routine blood quantitative data is tested statistically using the chi-square (x2) test with Statistical Product and Service Solution (SPSS) software version 23 for windows and Ms. Office Excel 2021. $P<0.05$ indicates significant differences.

RESULTS AND DISCUSSION

Microscopic examination was used to identify and classify each pathogen according to its developmental stage. Examination of EC goat blood samples showed the presence of piroplasm located in the edge of red blood cells corresponding to the characteristics of Anaplasma marginale, and small intracellular inclusions (morulae), which appear as clusters of dot-like basophilic granules inside neutrophils corresponding to the characteristics of Anaplasma phagocytophilum. Furthermore, the characteristics of Anaplasma marginale are characterized by a single dark blue spot inside a red blood cell located in the center or at the edge of the blood cell (Parodi et

al., 2022) while Anaplasma phagocytophilum is characterized by the formation of morulae in neutrophils, dark blue to purplish-gray spots is in a fluid-filled chamber (vacuole) surrounded by a membrane. These speckles consist of one to several subunits in membrane-lined vesicles, although they are not obvious to microscopic observations, the size of the specks seen under a light microscope is directly related to the number of subunits present. Unlike Howell-Jolly bodies, Anaplasma spp is generally not perfectly round, and is mostly smaller (Alyemni, Uppal, Nwaoduah, & Ly, 2016; Duguma, 2016).

Based on microscopic examination, the results showed 8 (40%) goats were positive of Anaplasma spp. consist of 2 (10%) samples similar with A. phagocytophilum and 6 (30%) samples similar with A. marginale characteristics. The prevalence of anaplasmosis in EC goats in Kaligesing is lower than the prevalence of goat anaplasmosis in Samigaluh, Kulon Progo, which is 47% but higher than the results in Malaysia (2.7%) and Philippines (38.4%) (Galon et al., 2022; Nugraheni et al., 2023; Ola-Fadunsin et al., 2018). This may be due to differences in climate conditions, management practices, and other epidemiological factors (Dey et al., 2020).

The risk of female goats being infected with Anaplasma is greater than that of male goats according to other studies (Table 1) (Biswas et al., 2023; Galon et al., 2022). Female goats are more susceptible with Anaplasma spp. infection due to long periods of pregnancy, birth and lactation (A. Khan et al., 2019; Ogbaje, Mandabsu, & Obijiaku, 2018). Female cattle also experience stress due to milking and reproductive hormonal changes (Stuen, 2020). Furthermore, goats that are reared in semi-intensive systems often have more opportunities to encounter ticks and infected animals which increases the risk of transmission (Biswas et al., 2023; A. Khan et al., 2019).

Table 1. Incidence of Anaplasmosis on PE Goat based on sex

Status	Parameter	Anaplasma (+)	Anaplasma (-)
Sex	Male	0 (0%)	3 (15%)
	Female	8 (40%)	9 (45%)

The parameters in the clinical examination showed that there can be differences between goats that had anaplasmosis and normal ones. While anaplasmosis in goats may not always present clear clinical symptoms (Nugraheni et al., 2023), it can still lead to subtle changes that are detectable through careful examination. Some studies have reported that infected goats may exhibit increased temperature, heart rate, and respiration rate compared to uninfected animals, even if the symptoms are not overtly severe (Memon, 2019; Mota-Rojas et al., 2021; Stuen, 2020).

Goat with Anaplasma spp. positive did not show significant increases in temperature, heart rate, or respiration rate compared to normal goats (Table 2). In fact, these clinical parameters remained largely within the normal range, suggesting that subclinical anaplasmosis infections may not always present with overt clinical signs in affected goats. These results are in accordance with a study on goats in Pakistan and Europe (Rahman et al., 2022; Stuen, 2020). Contrary to the findings, other studies have reported that goats infected with Anaplasma spp. can exhibit increased temperature, heart rate, and respiration rate compared to uninfected animals. While subclinical anaplasmosis infections may not always present with severe clinical signs, the affected goats may still show subtle physiological changes detectable through careful examination. The differences in clinical manifestations could be influenced by various factors such as the stage of infection, the virulence of the Anaplasma strain, and the individual host immune response (Khan et al., 2019; Naeem et al., 2023).

Rumen motion parameters in EC goats infected with Anaplasma spp. are still within the normal interval. Contrary to the findings, some studies from Korea (6.6%), Ethiopia (7%), German (2-5%) have reported that goats infected with Anaplasma spp. can exhibit decreased rumen motility compared to uninfected animals. The reduced rumen movement may be a result of the physiological impacts of the subclinical anaplasmosis infection on the gastrointestinal tract of the affected goats (Lee et al., 2015; Mihaylenko et al., 2019). The examination of normal clinical anaplasmosis occurs because anaplasma infections in small ruminants are often subclinical

in nature so they do not show any real clinical symptoms except in stressful conditions (Biswas et al., 2023). Differences in prevalence in age, sex, race and clinical symptoms in goats infected with anaplasma are highly determined by the condition of the goats (Ogbaje et al., 2018).

Table 2. The Relationship between Goat Anaplasmosis and Body Temperature, Heart Rate and Respiration Rate

Parameter	Anaplasma (+)	Reference*	Result
Temperature (oC)	39.1	38-39.70	Normal
Heart rate (x/min)	96.25	70-90	Increased
Respiration Rate (x/min)	32.88	15-30	Increased
Rumen motion (x/5 minutes)	8	5-Oct	Normal

*(Plug et al., 2020)

The results of the examination of goat blood samples were carried out by the Mann-Whitney U test to find out if there was a significant median difference between the independent groups. Based on the test, it was known that there was no significant difference between the routine blood parameters of goats infected with Anaplasma spp. and those not infected with Anaplasma, it was shown from the significance value of > 0.05. Routine blood results are shown in the Table 3.

All blood parameters are also within the normal value range (reference interval) (Pugh et al., 2021). This suggests that anaplasmosis in EC goats does not cause significant changes in the animal's blood profile or is subclinical in nature (Hosseini et al., 2015; Khaki, et al., 2018). However, the blood parameters of goats infected with Anaplasma spp. do not always remain within the normal range. Some studies have reported significant decreases in erythrocyte count, hemoglobin, and hematocrit, as well as increases in leukocyte count in infected goats compared to uninfected animals. This indicates that subclinical anaplasmosis infection can still lead to notable hematological changes, even if the clinical symptoms are not overtly severe. The differences in clinical manifestations may be influenced by factors such as the stage of infection, the virulence of the Anaplasma strain, and the individual host immune response (Hosseini et al., 2015; Khaki et al., 2018).

Table 3. Data on routine blood tests of EC goats

Parameter	Anaplasma (+)	Anaplasma (-)	Reference*	Sig.**	Interpretation
	Interval (mean)	Interval (mean)			
RBC (106/µl)	10.95-20.53 (17.22)	11.90-20.40 (17.33)	8.00-18.00	0.85	decreased
Hb (g/dL)	6.10-11.11 (8.39)	5.71-10.11 (8.59)	8.00-12.00	0.45	decreased
Hematocrit (%)	15.47-30.21 (24.37)	15.95-30.25 (24.78)	22.00-38.00	0.46	decreased
MCV (fL)	12.10-19.10 (15.32)	13.10-18.10 (15.10)	16.00-25.00	0.70	increased
MCH (Pg)	4.61-6.10 (5.35)	4.81-5.95 (5.20)	5.20-8.00	0.17	increased
MCHC (g/dL)	31.61-37.91 (34.48)	31.25-37.12 (34.51)	30.00-36.00	0.85	Normal
WBC (103/µl)	3.50-18.99 (10.65)	4.20-15.20 (10.11)	4.00-13.00	0.61	increased

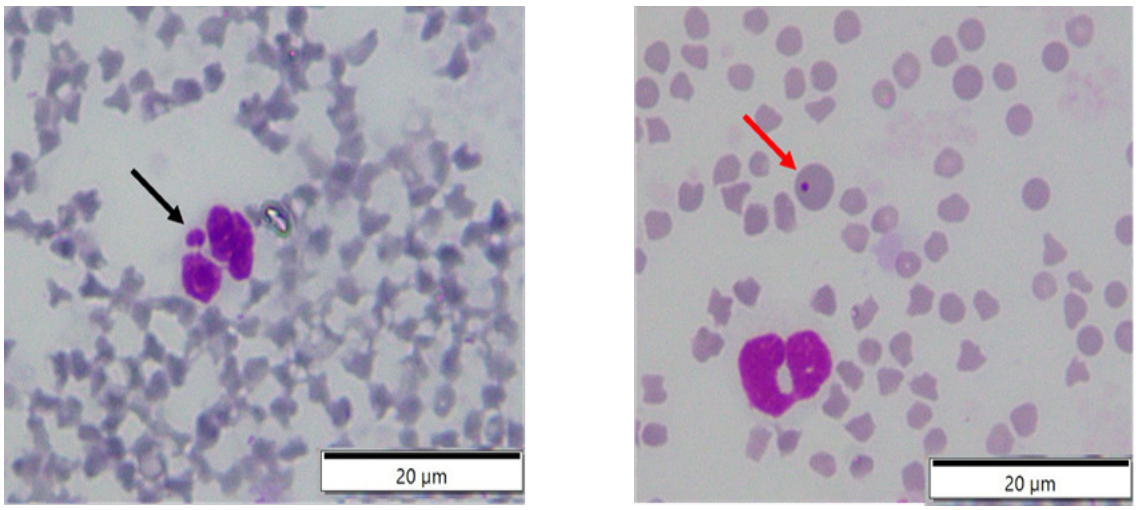
Note: *referensi interval (Pugh et al., 2021), RBC (red blood cell); Hb (hemoglobin); MCH (mean cell hemoglobin); MCHC (mean corpuscular hemoglobin concentration); MCV (mean cell volume); WBC (white blood cell). ** P<0.05 indicates significant differences.

In the other hand, descriptively, hemogram analysis revealed differences in several blood parameters between goats infected with Anaplasma spp. and uninfected goats. Infected goats showed significant decreases in erythrocyte count, hemoglobin, hematocrit and increased leukocyte count compared to normal goats. These findings are consistent with several previous studies on goats with anaplasmosis which also reported anemia and leukocytosis as common hematological alterations. Anemia can occur due to the destruction of red blood cells by the Anaplasma parasite, while leukocytosis is likely a response to inflammation and infection (A. Khan et al., 2019; Lacasta et al., 2020; Memon, 2019; Ogbaje et al., 2018).

Significant differences in hematological parameters between infected and uninfected goats indicate the hematological profile of goats can be used as a marker for subclinical anaplasmosis infection. Despite the lack of overt clinical signs, the subclinical infection can still induce notable changes in the goats' blood profile, which could be detected through careful laboratory analysis. Overall, the results suggest that subclinical Anaplasma infections are prevalent in Ettawa dairy goats in Kaligesing, Purworejo, Central.

Subclinical anaplasmosis, though often underdiagnosed and underappreciated, can have far-reaching consequences for the goat farming industry at a national level. It impacts productivity, increases susceptibility to other diseases, raises veterinary costs, and affects the economic viability of goat farming. Moreover, it can influence animal trade, food security, and the sustainability of farming practices. Addressing subclinical anaplasmosis requires a combination of improved disease surveillance, better management practices, and strategic interventions to minimize its impact on the industry. National strategies should focus on disease control, improving farm-level biosecurity, and educating farmers on the importance of managing subclinical infections to safeguard the goat farming sector and rural livelihoods (Khan, 2019; Lacasta et al., 2020; Memon, 2019).

The Anaplasma marginale and Anaplasma phagocytophilum strains examined in this study appear to have had relatively low pathogenicity, as none of the tested goats exhibited overt clinical symptoms. However, it is important to note that there can be significant variability in the pathogenic potential of these parasites. It is noteworthy that the clinical manifestations in infected animals are often influenced by a variety of factors, such as immune status, region, vectors, and farming management. The key question of whether the anaplasmosis examined in this research has the potential to infect and cause disease in humans necessitates further clarification through additional targeted investigations. Consequently, more comprehensive investigations are warranted to elucidate the precise pathogenicity of the Anaplasma strains found in EC goats, as well as their potential to cause significant disease in human populations.



Infected red blood cells in this study. (a) Anaplasma phagocytophilum (black arrow), (b) Anaplasma marginale. (red arrow).

CONCLUSIONS

In general, the results of this study indicate that subclinical Anaplasma spp. infection can occur in Ettawa crossbred goats raised in the Kaligesing area, Purworejo, Central Java. The study relied exclusively on microscopic examination, which is based on morphological assessment, to diagnose all diseases. While this approach can provide valuable initial insights, it has limitations in accurately identifying the specific species involved. To overcome these limitations and achieve more precise diagnosis, the researchers recommend conducting molecular identification techniques. Employing molecular methods would be advantageous for the future detection of anaplasmosis, particularly in the Purworejo region of Central Java, as they can offer higher specificity and sensitivity compared to traditional morphological assessments.

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