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Investigation of tick-borne pathogen in goats, case study in Samigaluh, Kulon Progo, Yogyakarta

Investigasi tick-borne patogen pada kambing, study kasus di Samigaluh, Kulon Progo, Yogyakarta

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ABSTRACT

Tick-borne pathogens of the genus *Theileria* and *Anaplasma* has been widely distributed and infect small ruminant in tropical and subtropical countries. Several species are considered highly pathogenic, while some cause subclinical infections in small ruminants. The distribution data of theileriosis and anaplasmosis in Samigaluh, Kulon Progo remain scarce and poorly understood. Therefore, further investigations are required to control this tick-borne disease. This study aimed to investigate the occurrence of theileriosis and anaplasmosis in domestic goats in Samigaluh, Kulon Progo, Western Yogyakarta. A total of 53 blood samples were collected from domestic goats. Blood sample was drawn from the jugular vein individually and kept in an EDTA tube. Thin blood smear was stained by Giemsa and observed under microscope for genus-level identification. As a result, it was found that theileriosis 12/53 (23%), anaplasmosis 25/53 (47%) and mixed infection 6/53 (11%) were detected in the present study.The conclusion of this study is that the incidence of Anaplasma sp. infection in goats is greater than Theileria sp. infection in Samigaluh, Kulon Progo. Although all domestic goats were asymptomatic, the finding of this study may shed light on the distribution of theileriosis and anaplasmosis infecting domestic goats in Samigaluh, Kulon Progo

ABSTRAK

Theileria sp. dan Anaplasma sp. merupakan agen penyakit diperantarai caplak, yang dilaporkan telah menyebar secara luas dan menginfeksi ruminan kecil di beberapa negara tropis dan subtropis. Beberapa spesies dapat menjadi agen membahayakan dan menyebabkan infeksi subklinis pada hewan ruminansia kecil. Data distribusi penyakit akibat Theileria dan Anaplasma di Samigaluh, Kulon Progo masih jarang diteliti dan sedikit dipahami. Oleh karena itu, penelitian lebih lanjut mengenai patogen ini perlu dilakukan untuk mendukung pengendalian penyakit yang disebabkan caplak ini. Penelitian ini bertujuan untuk mendeteksi kejadian penyakit pada kambing lokal yang disebabkan oleh Theileria sp. dan Anaplasma sp. di Samigaluh, Kulon Progo, bagian barat Yogyakarta. Sebanyak 53 sampel darah diambil dari vena jugularis kambing lokal dan disimpan dalam tabung EDTA. Preparat apus darah menggunakan Giemsa dan diamati dibawah mikroskop untuk identifikasi genus Theileria dan Anaplasma.

Kata kunci:
Anaplasma
Kambing lokal
Theileria
Tick-borne pathogen

Hasil penelitian menunjukkan bahwa terdeteksi theileriosis 12/53 (23%), anaplasmosis 25/53 (47%) dan infeksi campuran 6/53 (11%). Kesimpulan dari penelitian ini adalah kejadian infeksi Anaplasma sp. pada kambing lebih besar daripada infeksi Theileria sp di Sidoharjo, Samigaluh, Kulon Progo, meskipun semua kambing tidak menunjukkan gejala klinis. Data hasil penelitian ini dapat digunakan sebagai acuan distribusi theileriosis dan anaplasmosis di Samigaluh, Kulon Progo

INTRODUCTION

Theileriosis and anaplasmosis are tick-borne diseases that are transmitted by ixodid ticks (Nguyen, Tiawsirisup, and Kaewthamasorn 2020). These pathogens are spread by ticks and cause an economic impact on small ruminants due to their infection. *Theileria* has been reported to infect large animals such as cattle and buffalo (Thompson and Goodrich 2018). On the contrary, *Theileria* sp. infecting small ruminants such as goats in Indonesia remains scarce and poorly understood. Previous studies have revealed three *Theileria* spp. to cause high mortality in goats as follows: *T. lestoquardi, T. luwenshuni* and *T. uilenbergi*, while other three species: *T. separata, T. ovis* and *T. recondite*, are recognized as less pathogenic agent (Hassan et al. 2015; Metwally et al. 2021; Nasreen et al. 2020). According to Mohsin et al. (2022), the clinical signs of infected animals consist of fever, anemia, weight loss, and even death.

Anaplasmosis is a tick-borne disease caused by an obligate intracellular bacterium, *Anaplasma* sp., a member of the Anaplasmatacae family within the order of Rickettsiales (Long et al. 2022). Infected animals are recognized to have weight loss, high fever, decreased milk production, abortions, and even death (Wei et al. 2020). There are six *Anaplasma* species have been recognized as follows: *Anaplasma ovis, A. marginale, A. centrale, A. platys, A. bovis* and *A. phagocytophilum*. Among them, *A. ovis* has been reported as the most pathogenic, while A. marginale less pathogenic in small ruminants (Cabezas-Cruz et al. 2019). To date, Human-pathogenic Anaplasma species have been identified, including *A. capra, A. ovis, A. phagocytophlum*, and *A. platys*. However, a previous study pointed out human granulocytic anaplasmosis has been reported to be caused by *A. phagocytophlum* (Bakken and Dumler 2015). In contrast, another *Anaplasma* species in small ruminants are less attention and poorly understood, although they have been detected in the vector (ticks) and small ruminants. There have been few reports about theileriosis and anaplasmosis in Samigaluh, Kulon Progo the region in Yogyakarta province and they remain scarce. This finding provides an update about the occurrence of theileriosis and anaplasmosis in this area. Therefore, this study aims to investigate the prevalence of *Theileria* sp. and *Anaplasma* sp. in goats in Sidoharjo, Samigaluh, by microscopic examination.

MATERIALS AND METHODS

Sampling site and study design

This study was conducted by a Cross-sectional study design. Blood samples were collected from small and medium-sized goat farms and smallholder farmers in Sidoharjo, Samigaluh, Kulon Progo, Yogyakarta. It is in Menoreh hills, close to Purworejo and Magelang border of Central Java. This area is surrounded by clove and hardy plants as the most predominant vegetation. Map of sample collection as presented in Figure 1.

Figure 1. Map of sample collection in this study created by Google earth pro version 7.3.6

Sample collection

According to the data obtained from the department of agriculture and food, Kulon Progo, the domestic goat's population is estimated

to be 711 animals in Sidoharjo, Samigaluh, Kulon Progo, consisting of 185 males and 526 females (data up to date September 2022). In the present study, a total of 53 goat blood samples were collected in November 2022 from Sidoharjo, Samigaluh. The number of samples was calculated based on the detection of disease formula implemented in epidemiological tools freely accessed at https://epitools.au-svet.com.au/. All measurements are based on a 0.95 confidence level and 0.9 sensitivity test. Therefore, 33 samples were required as the sample size in this study based on population size. However, we increased the number of samples to 53 to increase the possibility of finding positive samples. All samples were screened for the presence of tick-borne pathogens *Theileria* sp. and *Anaplasma* sp. infection. Location, date of collection and data on goat information regarding age, history of tick infestation, and other symptoms were recorded. A total of 3 mL of individual blood samples were drawn from the jugular vein of individual goats. Blood samples were kept in EDTA tube collection and were subjected to thin blood smear microscopic examination.

Thin blood smear examination

Thin blood smear samples were prepared individually. Each blood smear was fixed with methanol before then stained by ten times Giemsa (MerckTM) dilution in aquadest buffer. An individually stained blood smear was observed using emersion oil under 1000x magnification lens of a microscope Olympus BX51 for the presence of *Theileria* sp. and *Anaplasma* sp. infection. A slide was considered positive when at least one *Theileria* sp. or *Anaplasma* sp. was found from a total of 2000 red blood cells according to WHO protocol (WHO, 2015). All positive samples were documented by camera Olympus DP12 in the department of Parasitology, 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.

Data and statistical analysis

The number of positive samples based on the microscopic was assessed by binomial Wilson confidence interval to estimate the proportion. Data regarding the association between infected goat males and females were analyzed by the Chi-square method. All statistical analyses were evaluated using an online statistical analysis platform freely available at https://epitools.ausvet.com.au.

RESULTS AND DISCUSSION

Microscopic blood smear examination

Based on microscopic examination, each pathogen was identified and classified morphologically according to developmental stage. It was found that out of 53 samples, 12 and 25 samples were detected positive for *Theileria* sp. and *Anaplasma* sp. infection, respectively. We investigate the presence of piroplasm (*Teileria* sp.) in the red blood cells under microscopic examination. *Theileria* sp. was observed to infect red blood cells, as presented in Figure 2a. *Anaplasma* sp. infection was also identified based on morphological examination depicted in Figure 2b. Mixed infection by *Theileria* sp. and *Anaplasma* sp. was also detected in 6 goats in this study, as shown in Figure 2c.

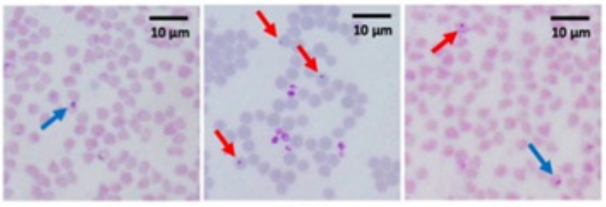

Figure 2. Infected red blood cells in this study. *Theileria* sp. (blue arrow), *Anaplasma* sp. (red arrow). (a) *Theileria* sp. (b) *Anaplasma* sp. (c) Mixed infection *Theileria* sp. and *Anaplasma* sp.

Statistical analysis results show that the sample infected by *Theileria* sp. proportion was calculated as 0.23 (0.13-0.36), while *Anaplasma* sp. infection was 0.47 (0.34-0.60) and Mixed infection was 0.11 (0.05-0.23) as shown in Table 2. The association between males and females against infection rate was evaluated statistically by Chi-square. The data statistical results showed that there were no significant differences between infected males and females (p-value>0.05), 0.2 and 0.19 for *Theileria* sp. and *Anaplasma* sp., respectively (Table 3). Therefore, this finding implies no association between infected males and females, suggesting that these pathogens could infect male or female

Table 2. Estimated proportion calculated by binomial Wilson confidence interval

Test	Number of tested	Number positive	Proportion (%)	95 % CL
Theileria sp.	53	12	23	0.13-0.36
Anaplasma sp.	53	25	47	0.34-0.60
Mixed infection	53	6	11	0.05-0.23

CL: Confidence level

Table 3. Chi-square statistical analysis of infected and uninfected goats

Sex	*Theileria* sp.		*Anaplasma* sp.	
	Infected	Uninfected	Infected	Uninfected
Male	2	13	7	8
Female	10	28	18	20
Total	12	41	25	28
Chi-square (p-value)	0.20*		0.19*	

*No significant difference (p-value greater than 0.05)

goats without any tendencies. Theileriosis and anaplasmosis could be affected by many factors. For instance, biting arthropods and colostrum transfer have been reported previously as possible routes of this disease transmission. Although there was no report of tick infestation in this area, *Theileria* and *Anaplasma* could be transmitted by mechanical transfer (Hammer et al. 2016). In the present study, we found a higher infection rate with *Anaplasma* sp. than with *Theileria* sp.

Identifying the occurrence of theileriosis in goats is essential for the control of theileriosis in goats. A previous study was conducted to investigate the most common *Theileria* sp. in sheep in Iran. It was found that *T. ovis* is the most predominant species in southwest Iran while *T. lestoquardi* and *T. annulata* infection was detected in a lesser proportion of sheep (Jalali et al. 2014). In the present study, we identified *Theileria* sp. based on morphology by microscopic examination in the stained blood smear we confirmed the genus of *Theileria*. Our limitation is we did not use the molecular technique for *Theileria* sp. detection. However, microscopic examination can be used to investigate the parasite development stage inside the red blood cell and estimate the parasite burden (Gebrekidan et al. 2020). Microscopic examination was unable to determine *Theileria* up to the species level because it relies on their morphological characteristics in the stained Giemsa blood smear. It is also less sensitive when the parasite burden is too low and time-consuming. Consequently, an advanced molecular method should be conducted in order to improve the sensitivity and specificity of *Theileria sp*. detection in goats.

On the other hand, anaplasmosis has been studied in domestic goats in northern China. The investigations conducted in rural areas of northern China noted that 48.9% (44/90) of goats were positively infected by *A. phagocytophilum* screened by PCR assay (Zhang et al. 2014). Moreover, anaplasmosis was detected in sheep in Shanxi and Mongolia, which was caused by *A. capra* infection (Peng et al. 2018; Yang et al. 2017). It should be noted that both *A. phagocytophilum* and *A. capra* were identified as human pathogens, as reported by (Khatat et al. 2016; Peng et al. 2021). *Anaplasma ovis* and *A. marginale* have also been reported by Rahman et al. (2022) to infect goats in Bangladesh. Although we are unable to differentiate *Anaplasma* at the species level, however, our study provides the current data on theileriosis and anaplasmosis occurrence in domestic goats. Moreover, our study revealed the mixed infection *Theileria sp*. and *Anaplasma sp*. in domestic goats in Sidoharjo, Samigaluh, Kulon Progo district.

Theileria sp. and *Anaplasma sp*. investigated in the present study likely did not have high pathogenicity since all the tested goats did not show clinical signs. There is a variation in pathogenicity among these parasites. It should

be noted that the clinical sign of the infected animals is considered to depend on animal welfare and immune status (Čobádiová et al. 2013). Whether anaplasmosis investigated in this study could infect humans is essential to be clarified. Therefore, further studies should be performed in order to determine the pathogenicity of Anaplasma infected in goats as well as in humans.

CONCLUSIONS

In conclusion, the finding of this study suggests that tick-borne pathogens *Theileria* sp. and *Anaplasma* sp. in this study were detected in domestic goats in Samigaluh, Kulon Progo, Yogyakarta. The present study has revealed mixed infection between *Theileria* sp. and *Anaplasma* sp. in domestic goats. However, since all diagnosed diseases in this study are based on microscopic examination, which relies on morphological examination, molecular identification should be conducted for species-specific confirmation. Therefore, the molecular technique would be helpful in theileriosis and anaplasmosis detection in the future, particularly in Kulon Progo.

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REFERENCES

Bakken, Johan S., and Johan S. Dumler. 2015. "Human Granulocytic Anaplasmosis." *Infectious Disease Clinics of North America* 29(2):341–55.

Cabezas-Cruz, Alejandro, Mélanie Gallois, Mélanie Fontugne, Eléonore Allain, Myriam Denoual, Sara Moutailler, Elodie Devillers, Stephan Zientara, Marc Memmi, Alain Chauvin, Albert Agoulon, Muriel Vayssier-Taussat, and Christophe Chartier. 2019. "Epidemiology and Genetic Diversity of Anaplasma Ovis in Goats in Corsica, France 06 Biological Sciences 0604 Genetics." *Parasites and Vectors* 12(1). doi: 10.1186/s13071-018-3269-7.

Čobádiová, Andrea, Katarina Reiterová, Markéta Derdáková, Silvia Špilovská, Ľudmila Turčeková, Ivana Hviščová, and Vladimir Hisira. 2013. "Toxoplasma Gondii, Neospora Caninum and Tick-Transmitted Bacterium Anaplasma Phagocytophilum Infections in One Selected Goat Farm in Slovakia." *Acta Parasitologica* 58(4). doi: 10.2478/s11686-013-0171-5.

Gebrekidan, Hagos, Piyumali K. Perera, Abdul Ghafar, Tariq Abbas, Robin B. Gasser, and Abdul Jabbar. 2020. "An Appraisal of Oriental Theileriosis and the Theileria Orientalis Complex, with an Emphasis on Diagnosis and Genetic Characterisation." *Parasitology Research* 119(1).

Hammer, Jade Frederick, Cheryl Jenkins, Daniel Bogema, and David Emery. 2016. "Mechanical Transfer of Theileria Orientalis: Possible Roles of Biting Arthropods, Colostrum and Husbandry Practices in Disease Transmission." *Parasites and Vectors* 9(1). doi: 10.1186/s13071-016-1323-x.

Hassan, Mehdi Aghamohammad, Afshin Raoofi, Samad Lotfollahzadeh, and Javad Javanbakht. 2015. "Clinical and Cytological Characteristics and Prognostic Implications on Sheep and Goat Theileria Infection in North of Iran." *Journal of Parasitic Diseases* 39(2). doi: 10.1007/s12639-013-0318-1.

Jalali, Seyedeh Missagh, Zohreh Khaki, Bahram Kazemi, Sadegh Rahbari, Parviz Shayan, Mojgan Bandehpour, and Seyedeh Parastoo Yasini. 2014. "Molecular Detection and Identification of Theileria Species by PCR-RFLP Method in Sheep from Ahvaz, Southern Iran." *Iranian Journal of Parasitology* 9(1).

Khatat, Sarah Elhamiani, Hamid Sahibi, Mony Hing, Ismail Alaoui Moustain, Hamid El Amri, Mohammed Benajiba, Malika Kachani, Luc Duchateau, and Sylvie Daminet. 2016. "Human Exposure to Anaplasma Phagocytophilum in Two Cities of Northwestern Morocco." *PLoS ONE* 11(8). doi: 10.1371/journal.pone.0160880.

Long, Sarah S., Charles G. Prober, Marc Fischer, and David Kimberlin. 2022. "Principles and Practice of Pediatric Infectious Diseases." in Principles and Practice of Pediatric Infectious Diseases, 6th Edition. Elsevier.

Metwally, Dina M., Reem Alajmi, Muslimah N. Alsulami, Isra M. Al-Turaiki, Rewaida Abdel-Gaber, Afrah F. Alkhuriji, Haleema H. Albohiri, Khalil Mohamed, Hanadi B. Baghdadi, Manal F. El-Khadragy, Guillermo T. Isaias, and Saeed El-Ashram. 2021. "Identification of Theileria Spp. In Sheep and Goats from Jeddah, Saudi Arabia, Using Molecular Techniques." *PeerJ* 9. doi: 10.7717/peerj.12596.

Mohsin, Mohsinullah, Khalid Hameed, Mustafa Kamal, Asad Ali, Naseem Rafiq, Tahir Usman, Wali Khan, Ansar Ahmed Abbasi, Rahmat Ullah Khan, and Gulalai Jamal Yousafzai. 2022. "Prevalence and Risk Factors Assessment of Theileriosis in Livestock of Malakand Division, Pakistan." *Journal of the Saudi Society of Agricultural Sciences* 21(4). doi: 10.1016/j.jssas.2021.09.002.

Nasreen, Adil Khan, Sadaf Niaz, Mir Hassan shah, Aisha Khan, Haroon Ahmed, Irfan Khattak, Jehan Zeb, Huma Naeem, Muhammad Adeel Hassan, Mehmet Can Ulucesme, Sezayi Ozubek, and Munir Aktas. 2020. "Molecular Detection of Small Ruminant Piroplasmosis and First Report of Theileria Luwenshuni (Apicomplexa: Theileridae) in Small Ruminants of Pakistan." *Experimental Parasitology* 212. doi: 10.1016/j.exppara.2020.107872.

Nguyen, Anh H. L., Sonthaya Tiawsirisup, and Morakot Kaewthamasorn. 2020. "Low Level of Genetic Diversity and High Occurrence of Vector-Borne Protozoa in Water Buffaloes in Thailand Based on 18S Ribosomal RNA and Mitochondrial Cytochrome b Genes." *Infection, Genetics and Evolution* 82(February):104304. doi: 10.1016/j.meegid.2020.104304.

Peng, Yongshuai, Chenyang Lu, Yaqun Yan, Ke Shi, Qian Chen, Cong Zhao, Rongjun Wang, Longxian Zhang, Fuchun Jian, and Changshen Ning. 2021. "The First Detection of *Anaplasma Capra*, an Emerging Zoonotic *Anaplasma* Sp., in Erythrocytes." *Emerging Microbes and Infections* 10(1). doi: 10.1080/22221751.2021.1876532.

Peng, Yongshuai, Kunlun Wang, Shanshan Zhao, Yaqun Yan, Haiyan Wang, Jichun Jing, Fuchun Jian, Rongjun Wang, Longxian Zhang, and Changshen Ning. 2018. "Detection and Phylogenetic Characterization of Anaplasma Capra: An Emerging Pathogen in Sheep and Goats in China." *Frontiers in Cellular and Infection Microbiology* 8(AUG). doi: 10.3389/fcimb.2018.00283.

Rahman, Mizanur, Md Rayhan Faruque, Md Mizanur Rahman, and Mohammed Yousuf Elahi Chowdhury. 2022. "Epidemiology and Molecular Detection of Anaplasma Spp. in Goats from Chattogram District, Bangladesh." *Veterinary Medicine and Science* 8(3). doi: 10.1002/vms3.775.

Thompson, Belinda S., and Erin L. Goodrich. 2018. "Miscellaneous Infectious Diseases." in Rebhun's Diseases of Dairy Cattle: Third Edition.

Wei, Wei, Jie Li, Ya Wei Wang, Bao Gui Jiang, Hong Bo Liu, Ran Wei, Rui Ruo Jiang, Xiao Ming Cui, Lian Feng Li, Ting Ting Yuan, Qian Wang, Lin Zhao, Luo Yuan Xia, Jia Fu Jiang, Ye Feng Qiu, Na Jia, Wu Chun Cao, and Yan Ling Hu. 2020. "Anaplasma Platys-Like Infection in Goats, Beijing, China." *Vector-Borne and Zoonotic Diseases* 20(10). doi: 10.1089/vbz.2019.2597.

Yang, Jifei, Zhijie Liu, Qingli Niu, Junlong Liu, Rong Han, Guiquan Guan, Muhammad Adeel Hassan, Guangyuan Liu, Jianxun Luo, and Hong Yin. 2017. "A Novel Zoonotic Anaplasma Species Is Prevalent in Small Ruminants: Potential Public Health Implications." *Parasites and Vectors* 10(1). doi: 10.1186/s13071-017-2182-9.

Zhang, Lijuan, Hong Liu, Bianli Xu, Zhilun Zhang, Yuming Jin, Weiming Li, Qunying Lu, Liang Li, Litao Chang, Xiuchun Zhang, Desheng Fan, Minghua Cao, Manli Bao, Ying Zhang, Zengzhi Guan, Xueqin Cheng, Lina Tian, Shiwen Wang, Huilan Yu, Qiang Yu, Yong Wang, Yonggen Zhang, Xiaoyan Tang, Jieying Yin, Shijun Lao, Bin Wu, Juan Li, Weihong Li, Qiyi Xu, Yonglin Shi, and Fang Huang. 2014. "Rural Residents in China Are at Increased Risk of Exposure to Tick-Borne Pathogens Anaplasma Phagocytophilum and Ehrlichia Chaffeensis." *BioMed Research International* 2014. doi: 10.1155/2014/313867.