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Research article

Haemoparasites infection in bullfighting cattle in southern of Thailand

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Abstract

Haemoparasites affect animal health and the production leading to economic losses and may have effects on human health. In Thailand, there are few reports of infections in the bullfighting cattle. Therefore, this research aimed to determine the percentage of positive samples and distribution of haemoparasites infection in bullfighting cattle. A total of 1906 blood samples were collected from the bullfighting cattle from eight provinces in southern Thailand from 2017 to 2020. Those cattle showed the clinical signs of gasping, pale mucous membrane, haematuria, anorexia, sneezing and weakness. Micro-haematocrit centrifugation and thin blood smears with Giemsa's staining were used. The results indicated 42.50% (810/1906) of cases positive for haemoparasites infection. The most commonly found was *Theileria* spp. (37.53%, 716/1906) followed by microfilaria of *Setaria* spp. (2.68%, 51/1906), *Trypanosoma* spp. (1.21%, 23/1906), *Babesia* spp. (0.94%, 18/1906) and *Anaplasma* spp. (0.10%, 2/1906). Out of 810 positive samples, there was co-infection between *Theileria* spp. and *Setaria* spp. (1.36%, 11/810), *Theileria* spp. and *Babesia* spp. (0.99%, 8/810), and *Theileria* spp., *Trypanosoma* spp. and *Setaria* spp. (0.37%, 3/810). The distribution of haemoparasites infection in eight raising areas ranged from 39.71 to 75%, with no significantly differences. From this result, it was determined that haemoparasites were generalized distribution in southern Thailand. Therefore, there should be strict surveillance and control of movement of cattle into the fighting arena. Moreover, vector control and blood checking before fighting should be promoted.

Keywords: Bullfighting cattle, Cattle production, Haemoparasites, Risk, Southern Thailand

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INTRODUCTION

Haemoparasites mostly are transmitted to animals by blood-feeding arthropods such as mosquitoes, ticks and fleas which know as vector-borne diseases (Little, 2008). In cattle, tick is most likely to be a vector of hemoparasites diseases including anaplasmosis, babesiosis, and theileriosis (Bilgic et al., 2017). The ecology and epidemiology of diseases are affected by the interrelations between the pathogen, the host (human, animal or vector) and the environment (Harrus and Baneth, 2005). The diseases are generally found in tropical and subtropical areas, and it has been claimed that climate change and global warming will lead to greater spread because the number of vectors will increase. The ability of vectors to transmit a pathogen depends on many factors, including ecological changes either natural or human-induced, climate change, habitat destruction, and changes in population density/distribution (Kimaro et al., 2017). The climate change, the development of insecticide and drug resistance, globalisation and the significant increase in international trade, tourism and travel are also major contributors to the spread of the diseases (Harrus and Baneth, 2005, Colwell et al., 2011; Campbell-Lendrum et al., 2015). Therefore, haemoparasite are generally found in tropical and subtropical areas.

In tropical areas, haemoparasite are usually spread during the rainy season because moisture helps in the growth of vectors. In endemic areas, cattle often have passive immunity or build up immunity after infection and get rid of the infection themselves. A study in Bangladesh indicated that seasonal prevalence of ticks was highest in summer (97%) in comparison to the rainy (95%) and winter (86%) seasons. On the other hand, the seasonal prevalence of blood parasite was highest in the rainy season (45.45%) in comparison to summer (27.87%) and winter (16.55%) (Mohanta et al., 2013). The disease is most common when new animals are introduced into the herd from outside the area because of their low immunity to blood protozoan pathogens. The infected animal showed nonspecific clinical signs such as haemolytic anemia, depression, lymph node swelling, tachypnoea, dyspnoea, pneumonia, jaundice, abortion, stillbirths and metritis (Desquesnes et al., 2013). They can often be fatal in the absence of treatment. Moreover, bovine haemoparasite infection not only have significant impact on animal health but can also be transmitted to humans in such forms as as anaplasmosis, babesiosis, theileriosis, trypanosomosis (Zintl et al., 2003; Stuchin et al., 2016).

Although bullfighting cattle in southern Thailand are raised for fighting they can also be used for human consumption. Normally, bullfighting cattle are obtained by selecting calves from beef cattle lines. The farmer selects a good-looking, strong and healthy calf from a line of cattle that have won fights. They have raised together with local domestic cattle in the same field and sometime same stable. When bringing the bull fighting cattle to fight in the arena they were very close to each other. Therefore, they have many potential chances to spared the haemoparasite to other animal via the vector. Vector-borne heamoparasites affect the health of cattle all over the world, especially in tropical areas including Thailand. The most common finding of haemoparasite in cattle are *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Theileria* spp. and *Trypanosoma evansi* (Ochirkhuu et al., 2015; Ringo et al., 2018). In Thailand, overall 78.7% of beef cattle are infected with at least one of the following parasites: *Theileria orientalis*, *Babesia bigemina*, and *B. bovis* (Jirapattharasate et al., 2016). A previous study of haemoparasite infection in bullfighting cattle in southern Thailand indicated only the seroprevalence of *T. evansi* 22.60% (40/177), (Kamyingkird et al., 2020). However, there are no reports of other haemoparasite infection in bull fighting cattle. Therefore, this study aimed to investigate haemoparasite infection in bullfighting cattle in southern Thailand. It is expected that the results of the study will be used in planning for disease control, prevention of disease between bullfighting cattle and domestic cattle, as well as to control the movement of animals for fighting.

MATERIALS and METHODS

Animal and sample collection

A total of 1906 bullfighting cattle selected for this research were from eight provinces: Songkhla, Pattalung, Satun, Nakhon Si Tham, Trang, Yala, Narathiwat and Pattani. Blood sample collection and physical examination were done at the Large Animal Hospital, Faculty of Veterinary Science, Prince of Songkla University, Thailand, from Jan 2017 to August 2020. The ID, breeding area and clinical signs were recorded.

This study was conducted on all 1906 animals at the animal hospital of Prince of Songkla University under Animal Ethics of Thailand (Approval no. U1-07133-2560).

Thin slide preparation

The blood from the EDTA tube was mixed by inverting 5–10 times ensuring the sample did not contain a partial clot. Using an autopipette or capillary tube took blood from the tube and drop 20–30 µl onto a slide. Then ploughed blood into a bullet shape by the spreader and let it dry.

Giemsa's staining

Giemsa working solution for staining were prepared by mixing Giemsa dye and buffer pH 7.4 at a ratio of 1:9. One slide used 3 mL of working Giemsa solution. The slide was fixed by immersing in absolute methanol for 2 min and let it dry completely. Put the dried slide onto the staining rack, poured on the Giemsa working solution, wait for 2 min and then washed with water. After the slide completely dried, take it to be examined under a microscope.

Microscopic diagnosis

Put the thin slide on the stage of the microscope then screened the blood parasite with the low-magnification objective (10X) then turned to objective lens 40X. The species of blood parasite were identified with high magnification objective (100X) using oil emulsion and reported the presence of blood parasites.

Micro-haematocrit centrifugation technique (Woo's method)

The blood from the EDTA tube was mixed by inverting 5–10 times ensuring the sample did not contain a partial clot. Insert the capillary tube into the EDTA tube, gently tilted the tube downward to let the blood flow into the capillary tube, filling about 3/4 of the tube. Covered one end of the capillary tube with plasticine or sigillum wax to prevent blood leakage from the tube as it is centrifuged at 12,000 rpm for 5 min. Cleaned and put the capillary tube on the stage of the microscope. Start observed with the low-magnification objective (10X) at the boundary between the buffy coat and the plasma. When the image is clearly seen, converted to objective 40X. Reported the presence of parasites.

Statistical analysis

Raw data were analyzed as a percentage by using Microsoft Excel (Microsoft, Washington DC, USA). The percentage of positive samples were calculated by divided number of total positive with total sample, then multiplied by one hundred in order to convert it to a percent. The comparisons of percentage of positive samples were based on descriptive statistics. The inferential statistics is unable to conduct due to an obvious unequal number of samples among provinces.

RESULTS

A total of 1906 blood samples were collected from bullfighting cattle that showed the clinical signs of gasping, pale mucous membrane, haematuria, anorexia, sneezing and weakness. Overall, 42.50% (810/1906) of samples were identified as carrying vector-borne disease infections. The most prevalent was of the vector-borne protozoa *Theileria* spp. (38.20%, 728/1906), followed by microfilaria of *Setaria* spp. (2.68%, 51/1906), *Trypanosoma* spp. (1.21%, 23/1906), *Babesia* spp. (0.94%, 18/1906) and *Anaplasma* spp. (0.10%, 2/1906; Figure 1, Table 1). Out of 810 positive samples, there were co-infections between *Theileria* spp. and *Babesia* spp. (0.99%, 8/810), *Theileria* spp. and *Setaria* spp. (1.36%, 11/810) and *Theileria* spp., *Trypanosoma* spp. and *Setaria* spp. (0.37%, 3/810). Total percentage of positive sample of vector-borne protozoa in eight breeding areas ranged from 39.71 to 75%, but there was no significant difference between them. However, the results showed that *Theileria* spp. was the most prevalent in all breeding areas, ranging from 34.32 to 75% (Table 2). The provinces which have fighting arenas and regularly hold bullfights, such as Songkhla, Pattalung, Satun and Nakhon Si Thammarat, showed high percentage of positive sample of *Theileria* spp., of 38.32, 34.80, 37.50 and 32.35%, respectively. The results from bullfighting cattle from Nakhon Si Thammarat showed a percentage of positive sample of *Babesia* spp. of 2.94% and of *Trypanosoma* spp. of 5.88%, while other provinces showed very low percentage of positive sample of both parasites. In Yala province, the presence of microfilaria of *Setaria* spp. (6.25%) was the highest of all provinces.

Table 1 The percentage of positive sample of vector-borne protozoa identified in bullfighting cattle (n = 1906)

Blood parasite	Bullfighting cattle examined (1906)	
	No. of positives	Percentage of positive sample (%)
***Babesia* spp.**	18	0.94
***Theileria* spp.**	716	37.53
***Anaplasma* spp.**	2	0.10
***Trypanosoma* spp.**	23	1.26
***Setaria* spp.**	51	2.62
Total positives	810	42.45

Table 2 Comparison of the percentage of positive sample of vector-borne protozoa in the provinces of southern Thailand

Province	No. of cattle	Positive sample (%)					Total positive sample (%)
		Theileria spp.	*Babesia* spp.	*Anaplasma* spp.	*Trypanosoma* spp.	*Setaria* spp.	
Songkhla	1511	38.32	1.13	0.13	0.99	2.51	43.08
Pattalung	204	34.80	0	0	1.96	2.94	39.71
Satun	104	37.50	0	0	0.96	3.85	42.31
Nakhon Si Thammarat	34	32.35	2.94	0	5.88	5.88	47.06
Pattani	24	50.00	0	0	4.17	0	54.17
Yala	16	43.75	0	0	0	6.25	50.00
Trang	9	66.67	0	0	0	0	66.67
Narathiwat	4	75.00	0	0	0	0	75.00

DISCUSSION

The results from this research indicate that bullfighting cattle are infected with significant tick-borne haemoparasite, such as *Theileria* spp., *Babesia* spp. and *Anaplasma* spp., which affect the health and productivity of cattle in Thailand. The results from this study are similar to those of a previous study in beef cattle and water buffalo of Thailand, which showed high percentage of positive sample of *Theileria* spp. infection in beef cattle and water buffalo, of 48.1% and 25.9%, respectively. However, the percentage of positive sample of *Anaplasma* spp. in water buffalo was higher than this: about 42.8% (Nguyen and Kaewthamasorn, 2019). In the northern and northeastern regions of Thailand, molecular detection revealed that T. orientalis was the most prevalent (30.1%) followed by *B. bigemina* (13.1%) and *B. bovis* (5.5%) (Jirapattharasate et al., 2016), while in western region, the prevalences of *B. bovis*, *B. bigemina*, *T. orientalis* and *A. marginale* were 11.1, 12.5, 7.8 and 39.1%, respectively (Jirapattharasate et al., 2017). Studies of other animals by using a genus-specific 18S-rRNA PCR determined that 103 ticks removed from mammals in Thailand were identified with hepatozoa and breeding area *Theileria* spp. of 8% and 18%, respectively. (Sumrandee et al., 2015). The study by PCR amplification of a fragment of the 18S-rRNA gene of *Babesia* and *Theileria* spp., and the 16S rRNA gene of bacteria in the family Anaplasmataceae in ticks' pools were 57.0% (239/419) with the highest infection rate for *Anaplasma* spp. at 55.6% (233/419) (Wattanamethanont et al., 2018). In neighboring countries, there was the first report of an outbreak of *T. orientalis* in dairy cattle imported to Vietnam from Australia 72.3% (Gebrekidan et al., 2017). The result of study in Zambia was similar to that from Thailand: the most prevalent vector-borne disease in cattle were from *Theileria* spp. such as *T. mutans* (54.5%) and *T. velifera* (51.5%) (Tembo et al., 2018). However, this report contrasted with the study by Ybañez et al. (2013) in the Philippines, which indicated that they found *Anaplasma* spp. (54.7%), *B. bigemina* (15.4%), *B. bovis* (10.0%) and *Trypanosoma* theileri (12.0%) but did not find *Theileria* spp. Protozoa identification in cattle and sheep in Bangladesh determined the prevalence of *Anaplasma* spp. 43%, *Babesia* spp. 19%, *Theileria* spp. 4% coinfection of *Anaplasma* spp. with *Babesia* spp. 33%, and *Anaplasma* spp. with *Babesia* spp. and *Theileria* spp. 1% (Hassan and Hassan, 2018). Identification of *Trypanosoma* spp. in this study was very low (1.21%) similar to the previous study by Kamyingkird (2020) which could not detect DNA in the blood sample of fighting bull cattle.

In this study, bullfighting cattle were infected mostly with *Theileria* spp., which cause theileriosis and are mostly transmitted by ixodid ticks of the genera Rhipicephalus, Amblyomma, Hyalomma and Haemaphysalis (Sumrandee et al., 2015; Scoles and Ueti, 2013). Those genera of ticks are commonly found in Thailand; therefore, the pathogen can circulate for a long time. Theileriosis knowns as bovine anaemia; young calves (2–3 months of age) are at most risk of infection. The clinical signs of theileriosis are those associated with severe anemia (pale mucous membrane) and include lethargy, lack of appetite and exercise intolerance. If forced to run, the animals may stagger and gasp for breath and some may collapse and die. Pregnant cows may abort, and stillbirths are common. In dairy cows a drop in milk production will occur. Death rates are highest in heavily pregnant cows (thecattlesite.com). Livestock aged more than 2 years of age (52%) are found to be more susceptible to blood protozoan diseases than animals aged 1–2 years of age (33.97%) (Mohanta et al., 2013).

Of greater interest for this research, the microfilaria of *Setaria* spp. were identified in 2.68% of cases. Seteria is a genus of filarial roundworms that infect domesticated mammals such as pigs, camels, cattle and horses (Cheng, 1986). Three species: *S. digitata*, *S. cervi* and *S. labiatopapillosa* were identified in cattle (Sundar and D'Souza, 2015). Adult Setaria worms in the peritoneal cavity of livestock are considered as non-pathogenic and do not cause any significant economic damage. However, erratic larvae may reach the central nervous system and migrate along nerves and cause substantial damage. The diseases can appear overnight and feature muscular weakness and uncoordinated movements of all four limbs or only the hind limbs. This can lead to paralysis and death in a few days. The study by Mohanty et al. (2000) found that about 12.5% of cattle were found to harbor both adult worms in the peritoneum and microfilariae in circulation. In Thailand, the worms were collected from the cattle in slaughterhouse showed morphologically as *S. digitata* (Subhachalat et al., 1999).

CONCLUSION

Bullfighting cattle raised in the south may play a role as reservoirs of Theileria spp. and other haemoparasites to domestic cattle. The parasites may also be reinforced in the bulls and domestic cattle because they are genetically close. The eradication of vectors by using acaricide is the best way to control those pathogen transmissions. However, it has been found that the eradication of disease vectors is difficult in tropical and subtropical areas because the environment is suitable for the multiplication and growth of vectors, and acaricide is expensive. Therefore, disease control and prevention in both bullfighting and domestic cattle must be considered along with vector control. This study indicates that investigation into species of infection should be carried out, together with clarify the correlation of vector genera and protozoan transmission in order to establish specific vector borne control program for bullfighting cattle in Southern of Thailand.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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