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Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

Prevalence of Blood Parasites Infestation among Buffaloes with Abortion in Nakhon Phanom Province, Thailand

Prakit Srisai[1,*], Manvika Pholpark[2], Pichet Tongpan[2], Netchanok Jiwaganon[2], Parinya Punnurit[2], Supranee Dermpun[2], Haruthai Rungruang[3], Noppadol Panlinfa[3]

1 Field Epidemiology Training Program, Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand

2 Veterinary Research and Development Center (Upper Northeastern Region), Khon Kaen Province, Thailand

3 Provincial Livestock Office, Nakhon Phanom Province, Thailand

*Corresponding author, email address: prakitsai@hotmail.com

Abstract

From 2007 to 2011, the buffalo population in Nakhon Phanom Province decreased 34% with approximately 150-300 aborted buffaloes each year. However, the etiology was not systematically examined. Our objectives were to determine prevalence of blood parasites in buffaloes with abortion, and identify causes and possible factors related to abortion. We conducted a case control study in Nakhon Phanom from April 2011 to March 2012. Cases were buffaloes that reported abortion and controls were female buffaloes that previously calved without history of abortion in the same sub-district with the case. Farmers were interviewed, and placenta, aborted fetus, blood, serum, and fecal specimens were collected from both cases and controls. The specimens were tested for blood parasites, brucellosis, and gastrointestinal parasites and hematocrit using Giemsa staining of blood smear and hematocrit centrifugation technique by Woo's method, and identified for blood parasites by microscopy. *Trypanosoma* spp. was the only blood parasite found with the true prevalence of 16% (95% CI =2.6-30.0). We identified risk factors for abortion using logistic regression. Significant risk factors for abortion were high density of tabanus (adjusted OR=12.9, 95% CI=1.2-135.7) and blood parasite (adjusted OR=13.1, 95% CI=1.2-142.9). Reducing the density of the vector such as tabanus might reduce the risk of abortion in buffaloes. In addition, a surveillance system on abortion etiology should be established.

Keywords: blood parasites, buffalo, abortion, Nakhon Phanom

Introduction

Nakhon Phanom is one of the provinces with the highest number of buffaloes in Thailand apart from Ubon Ratchathani, Surin, Buriram, Sisaket and Sakon Nakhon Provinces. From 2007 to 2011, the number of buffalo decreased from 95,331 to 32,529 heads while the overall buffalo population across the country reduced from 1,577,798 to 1,234,179 during the same period.[1] This posed a crucial problem for Thailand where livestock is one of the most important farming commodities.

Buffalo productivity in Thailand is limited due to low pregnancy rate. Estrus detection in Thai buffalo is difficult to observe and thus, effective insemination remains challenging. Furthermore, farmers' preference for female buffaloes resulted in low number of male buffalo. Other factors such as inbreeding, low pregnancy and production rates of calves, including weak or small calves, contribute to the low buffalo production.[2] In addition, abortion is one of the major factors for production loss, leading to low birth rate and less productivity.[3] Economic loss could arise from diagnosis and treatment expense of sick animals, replacing animals, and low milk and meat productivity from animal loss.[4] Due to the facts mentioned above, factors contributing to abortion in buffaloes should be assessed and the etiology should be investigated systematically, especially when the abortion rate was higher than 3% or more animals in a herd aborted when compared with that of the same period from the previous year.[5]

Blood parasites, such as *Trypanosoma evansi*, are one of the factors causing abortion among cattle and buffaloes in Thailand.[6,7] Other causes include bacteria, virus, fungus, chemicals, protozoa such as *Neospora caninum*, *Babesia* spp., *Anaplasma* spp., *Theileria* spp., microfilaria, *Tritrichomonas* spp., and *Toxoplasma* spp.[5,8,9] Other risk factors associated with abortion in cattle and buffaloes were season, parity, farm size, age at pregnancy, malnutrition and parasitic infection during pregnancy.[6,10-12]

Abortion in buffaloes has been a common problem in Nakhon Phanom Province. There were a total of 249 buffaloes with abortion in 2008 while 258 and 149 buffaloes aborted in 2009 and 2010 respectively. Nevertheless, etiology of buffalo abortion was never systematically studied before in Nakhon Phanom. This study aimed to determine the prevalence of blood parasites in buffaloes with a history of abortion, and identify causes and possible factors related to abortion in Nakhon Phanom Province during April 2011 to March 2012.

Methods

A case-control study was conducted in Nakhon Phanom Province from 1 Apr 2011 through 31 Mar 2012. Among 13,341 farmers who raised 64,751 buffaloes in Nakhon Phanom during 2012, 69% were female buffaloes with 30% had history of calving (more than first parity).[1]

A case was a buffalo that reported abortion while a control was a buffalo from other farms in the same village with normal calving and within two months before the matching case abortion date. Controls were selected by simple random sampling. If such buffaloes were not found, buffaloes in an adjacent village in the same sub-district with similar criteria were selected by simple random sampling.

Sample size was calculated by Epi Info version 3.5.1. The case-to-control ratio was 1:2, 312 samples (104 cases and 208 controls) is required to achieve 95% confidence interval (CI) and 80% power. Expected prevalence of blood parasites infection among controls and the studied population was set at 15.5% and 30.0%.[13]

Owners that reported buffalo abortion were interviewed using a questionnaire, including information of farmers, characteristics of farms and potential risk factors for abortion in buffaloes such as rearing and production context of buffaloes, parasitic control, source of feed/pasture and water, history of vaccination, disease outbreak in the farm and history of buffalo abortion in the village.

Age of aborted fetus was estimated through farmer interviewed including size measurement of the fetus[14]. We collected and submitted the fetus and placenta for laboratory testing. Blood, blood smear slide, serum and fecal specimens from both cases and controls were collected, and submitted to the Veterinary Research and Development Center VRDC (Upper Northeastern Region) in Khon Kaen Province for testing blood parasites, brucellosis, gastrointestinal (GI) parasites and hematocrit (Hct). Blood parasites were tested by two methods which included Giemsa staining of blood smear slide and hematocrit centrifugation technique by Woo's method[15]. Blood parasites (*Anaplasma* spp., *Theileria* spp., *Babesia* spp. and *Trypanosoma* spp.) were identified by direct microscopic observation. If any of the methods tested positive, it was considered to have that infection. Rose Bengal test was examined for brucellosis diagnosis while floatation technique and simple sedimentation were carried for GI parasites infestation.

A descriptive analysis was performed using frequency, percent, mean and apparent prevalence. Abortion rate (number of aborted buffaloes/number of pregnant buffaloes) and proportion of each aborted fetus age (number of aborted in each fetus age/total of aborted buffaloes) were calculated. Apparent prevalence was produced from method of the survey data analysis (cluster analysis), accounting for clustering effect of some buffaloes being from the same village. True prevalence was calculated using the following formula to adjust for errors from imperfect sensitivity and specificity of the diagnostic tests.[16]

$$TP = \frac{(AP + Sp - 1)}{(Se + Sp - 1)}$$

$$CI. = TP \pm Z_{\alpha/2} \frac{1}{(Se + Sp - 1)} \sqrt{\frac{AP(1 - AP)}{n}}$$

where TP = true prevalence, AP = apparent prevalence, Se = sensitivity, Sp = specificity, CI = confidence interval (Direct microscopic examination: Se = 50.0[17], Sp = 100[18,19]). For CI wider than 0-1, 0 or 1 was applied as the prevalence will not go beyond 0-1.

Due to hierarchical nature of the data, i.e. buffaloes in the same farm possibly shared several characteristics and therefore, not independent to each other, potential risk factors for abortion were examined using multiple logistic regression. Farm and animal-level factors included as random effects and model intercepts were included. The model also accounted for the matching effect from the way cases and controls were selected. All factors from the univariate analysis with p-value equal to or less than 0.2 with complete information were included in

multivariate analysis. The results of Hct were also compared by t-test.

Results

Epidemiological Characteristics of Buffaloes

Twenty two out of 1,123 villages from nine sub-districts (99 sub-districts in total) in seven districts (12 districts in total) reported buffalo abortion. Phi Man Sub-district in Na Kae District reported the highest abortion rate of 8.4% compared with the number of buffalo that had the first parity and above (Figure 1).

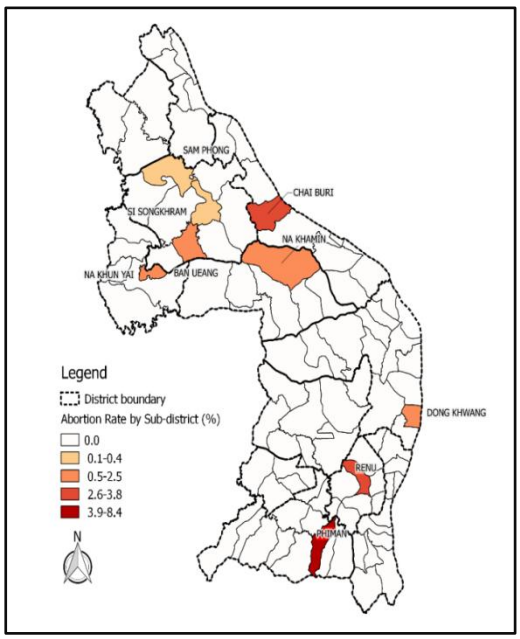

Figure 1. Distribution of buffaloes with abortion reported in sub-districts of Nakhon Phanom Province, Thailand, April 2011 to March 2012

Of total 13,341 farms in Nakhon Phanom Province, 123 farms participated in this study, there were 42 farms with the cases and 81 farms with the controls (Figure 2). Of 42 case farmers, 78.0% were male. Most of them raised the buffalo in a stall outside the house (75.6%) and did not clean the stall regularly (63.4%). In general, farmers did not examine buffaloes for pregnancy and noticed pregnancy by visual observation (Table 1). If a buffalo did not show heat behavior after breeding and was observed with gravid abdomen, it was considered to be pregnant.

A total of 758 buffaloes were raised in the studied farms with the median of four buffaloes per household (range 1-14). Out of 567 (74.8%) female buffaloes, 229 (40.4%) had history of pregnancy. Among them, 88 buffaloes with recent abortion were reported. However, we were able to collect samples from 62 buffalo cases. Median age of the 62 cases and the 118 controls were six years old (range 2-15 years). Ages of buffaloes that bred for the first time were three years (range 2-6 years) for the cases and four years (range 2-5 years) for the controls. Pregnancy rate of the cases and the controls was 33% for first-calf heifers. The majority of female buffaloes (96.7%) were bred naturally. The buffaloes were raised mostly for tracking in the field (58.1%) or were free-range (37.1%) (Table 2).

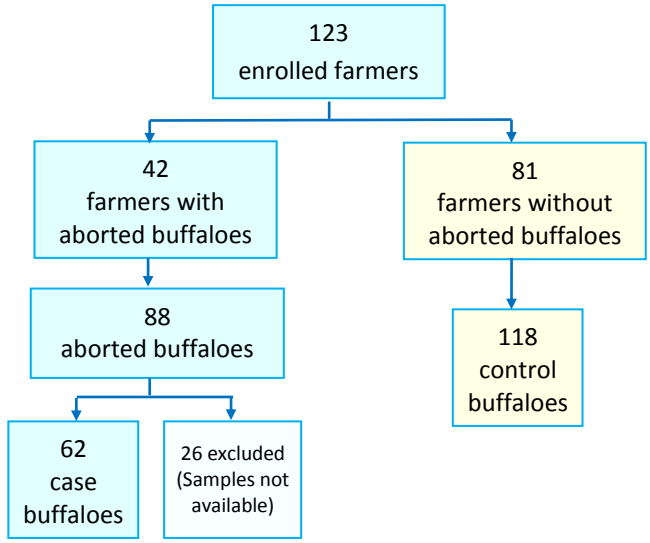

Figure 2. Number of samples collected in this study

Table 1. Characteristics of farmers and buffalo management in Nakhon Phanom Province, Thailand, April 2011 to March 2012 (n=42)

Characteristic	Number of farmer	Percent
Male	32	78.0
Primary school	28	68.3
Raising type		
Tying in the stall	1	2.4
Tying under the long-legged house	2	4.9
Tracking in the field	21	51.2
Free-range	17	41.5
Stall		
None	2	4.9
Outside the house	31	75.6
Under the long-legged house	8	19.5
Clean stall	15	36.6
Food for buffaloes		
Grass	41	100.0
Thatch	38	92.7
Rice bran	2	4.9
Other crops	3	7.3
Supplement foods	0	0
Mixed food	0	0
Deworming in buffalo herd	7	17.1
Vaccination in buffalo herd	15	36.6
Breeding		
Natural	40	97.6
Insemination	1	2.4
Pregnancy examination	1	2.4

Abortion rate of buffalo population in this study was 38.4% (88/229). About 93.5% (58/62) of them were not reported to have other symptoms beyond the observed abortion. Estimated age of the aborted fetus (the maternal gestational age) ranged from 3-10 months. Proportions of abortion in each age group (3-10 months) were 6.9%, 13.8%, 15.5%, 31.1%, 20.7%, 5.2%, 5.2% and 1.7%. Abortion was reported every month, except March 2012, with the highest number of abortion in July 2011 (Figure 3). No samples were collected from September to December as the livestock personnel were occupied by an emergency mission.

Laboratory Results

Blood and fecal specimens were collected from 62 out

Table 2. Characteristics of buffaloes with abortion (cases) and normal delivery (controls) included in this study

Characteristic	Case			Control		
	Median	Range	Percent	Median	Range	Percent
Age in year (n=180)	6	2-15	-	6	2-15	-
Age of the first breeding in year (n=173)	3	2-6	-	4	2-5	-
Chest length in cm (n=62)	186	160-213	-	183.5	173-210	-
Herd size (number of animal)	4	1-14	-	5	2-36	-
Parity	2	1-11	-	2	1-11	-
Gestational age of aborted buffalo in month (n=59)	6	3-10	-	-	-	-
First parity (n=173)	-	-	33.3	-	-	33.9
Age ≥ 10years (n=172)	-	-	11.7	-	-	9.8
Abortion history (n=180)						
Individual buffalo	-	-	6.5	-	-	11.0
Herd	-	-	0.0	-	-	16.1
Adjacent household	-	-	12.9	-	-	10.2
Village	-	-	43.5	-	-	73.7
Natural breeding (n=178)	-	-	96.7	-	-	93.2
Deworming history (n=178)	-	-	5.0	-	-	58.5
Vaccination history (n=176)	-	-	35.0	-	-	81.0
Raising type (n=180)						
Tying in the stall	-	-	1.6	-	-	1.7
Free-range	-	-	37.1	-	-	50.0
Tracking in the field	-	-	58.1	-	-	43.2
Tying under the long-legged house	-	-	3.2	-	-	5.1
Stall						
None (n=179)	-	-	3.2	-	-	0.9
Outside the house (n=179)	-	-	80.6	-	-	82.1
Under the long-legged house (n=177)	-	-	33.9	-	-	49.6

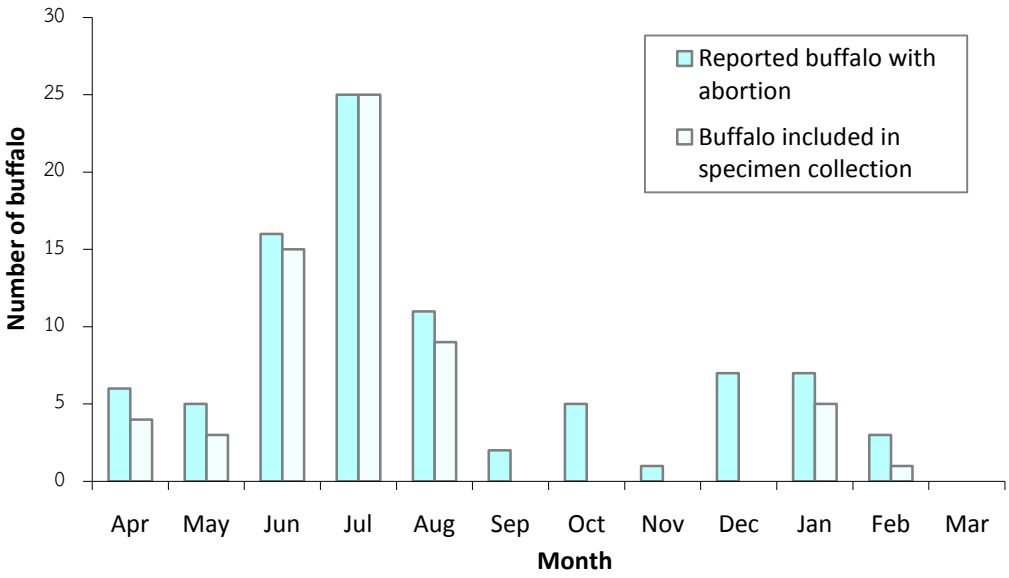

Figure 3. Number of buffaloes reported and included in this study, Nakhon Phanom Province, Thailand, April 2011 to March 2012 (n=175)

of 88 cases and all 118 control buffaloes. Blood parasites were identified in seven out of total 180 specimens tested, with 8% (5/62) from the cases and 2% (2/118) from the controls. No brucellosis and other pathogenic bacteria were detected. Fecal examination revealed GI parasite coccidia and rumen flukes (Table 3). Thirteen specimens of fetus and placenta were collected, and laboratory results were all negative.

Only 155 out of 180 specimens could be tested for Hct as the other specimens were clotted. Cases average Hct was 30.6 (SD = 5.5) and control was 30.9 (SD = 6.0). The difference was not statistically significant. There was no significant difference between four cases and nine controls with low Hct (<24, p-value = 0.8).

Prevalence of Blood Parasites

Two types of blood parasites, *Trypanosoma evansi* and *Trypanosoma theileria*, were identified in seven buffaloes (3.9%, 95% CI = 1.9-7.8). The case apparent prevalence was 8.1% (5/62, 95% CI = 1.1-15.0) and the control was 1.7% (2/118, 95% CI = 0.7-4.1). True prevalence for the cases and the controls were 16.2% (95% CI = 2.0-30.4) and 3.4% (95% CI = 0.0-8.1) respectively.

Risk Factors Related to Abortion of Buffaloes

Out of 10 risk factors considered in the analysis, infection with blood parasites, free-range raising, and prevalence of tabanus and stable flies were included in the multivariate analysis. The risk factors that were likely to be associated with abortion in buffalo included prevalence of tabanus (adjusted OR=12.9, 95% CI=1.2-135.7) and infection with blood parasites (adjusted OR=13.1, 95% CI=1.2-142.9) (Table 4).

Discussion

This study confirmed *T. evansi* infection among buffaloes in Nakhon Phanom Province as it was detected in buffaloes with and without history of abortion. *T. evansi* infection could be detected in many areas of Thailand. The report of VRDC (Upper Northeastern Region) revealed that the infection was identified in every province of the upper northeastern region during 2008-2012, with the highest rate in Nakhon Phanom Province. Beef cattle were infected most, followed by buffalo, dairy cattle and domestic pigs. In Nakhon Phanom, the most common type of infected animal was buffalo.[17] In 2010, the VRDC (Northern Region) reported that 3.9% of buffaloes in the northern Thailand were infected with *T. evansi*.[20] In the previous studies, the seroprevalence of *T. evansi* in beef cattle in Phayao Province was as high as 82.7% and that of dairy cattle in the northern region was higher than 15%.[21] In2007, the prevalence among dairy cattle in the central region was 8.1% while the seroprevalence of *T. evansi* was 15% or more[22]. The report from the southern region in 2003-2005, 0.3% of *T. evansi* infection was found in Nakhon Sri Thammarat Province.[23] These different results might be affected by various laboratory methods used in each study.

Blood parasites infection can cause less efficient animal production, and blood parasites could affect the reproductive system by causing ovarian cyst, ovarian inactivity, delayed puberty and uterine inflammation. In addition, it can interfere the level of progesterone, iron, zinc and selenium. These could be main causes for less reproductive activity[24] as well as reduced fertility rate in buffaloes[25].

Moreover, *T. evansi* infection could suppress the immune system in lymph nodes, spleen and bone marrow,[26] and also have an effect on immune response after vaccination against hemorrhagic septicemia[27,28] and foot and mouth disease[29]. All these facts stated above can reduce the efficiency of production.

Table 3. Laboratory results and prevalence of buffaloes with abortion (cases) and normal delivery (controls) in Nakhon Phanom Province, Thailand, April 2011 to March 2012

Type of infection	Case (n=62)			Control (n=118)		
	Number positive	Apparent prevalence (95% CI)	True prevalence (95% CI)	Number positive	Apparent prevalence (95% CI)	True prevalence (95% CI)
Blood parasites	5	8.1 (1.1-15.0)	16.2 (2.0-30.4)	2	1.7 (0.7-4.1)	3.4 (0-8.1)
T. evansi	4	6.5 (0.2-12.7)	13.0 (0.3-25.7)	2	1.7 (0.7-4.1)	3.4 (0-8.1)
T. theileria	1	1.6 (-1.6-4.8)	3.2 (0-9.5)	0	0	0
Gastrointestional nematodes	1	1.6 (-1.6-4.8)	-	0	0	-
Rumen Fluke	34	54.8 (42.1-67.6)	-	29	24.6 (13.9-35.2)	-
Coccidia	0	0	-	1	0.9 (0.2-4.6)	-

Remark: All the specimens were tested negative for *Fasiola hepatica* and brucellosis.

Table 4. Multi-level logistic regression on possible risk factors for abortion in buffaloes, Nakhon Phanom Province, Thailand, April 2011 to March 2012 (n=175)

Factor	Case (n=59)		Control (n=116)		Adjusted odds ratio		
	Yes	No	Yes	No	Point	95% CI	P-value
Blood parasites	5	54	2	114	13.1	(1.2-142.9)	0.04
Free-range	23	36	59	57	0.4	(0.05-3.04)	0.37
High *Stomoxys* density	35	24	57	59	0.3	(0.03-2.28)	0.24
High tabanus density	44	15	63	53	12.9	(1.2-135.7)	0.03

The causes of abortion in buffaloes apart from brucellosis are blood and GI parasites, including *Toxoplasma* spp., *Neospora* spp. and bovine viral diarrhea virus[30-32]. Detection of blood parasite was possibly a co-infestation. The actual cause of infection could be confirmed by testing the implicated specimens such as the aborted bovine fetus or placenta.[7] Since this study collected mainly blood and serum, the results may not reflect other causes of abortion. Nevertheless, if there was no infectious agent identified, other non-infectious causes and predisposing risk factors for abortion in buffaloes should be explored.

This study revealed that having vectors such as tabanus or multiple stable flies and parasitic infection in the GI tract could increase the risk of abortion in buffaloes[33]. Furthermore, animals with malnutrition and low immunity could be prone to infections. The gestational complications, not only due to parasitic infection in the GI tract, but also by increasing stress-related hormones in the blood stream.[34] We hypothesized that Hct could be used as an indicator in preliminary screening of animals for the surveillance system. However, different Hct between the cases and the controls in this study was not statistically significant. Moreover, due to the nature of livestock farming and subject selection in this study, we calculated the blood parasites prevalence by survey data analysis (cluster analysis) and used multilevel analysis method to identify risk factors of the abortion. Although we could not conclude that abortion among buffaloes in the study area was caused by blood parasites, the buffaloes with blood parasites had higher risk of abortion than those without blood parasites (13.5 times).

Most specimens from aborted fetus, placenta or blood of the fetus were not available due to delayed reporting from the farmers. The blood specimens with ethylenediaminetetraacetic acid (EDTA) did not reach the laboratory within 24 hours after collection which might detect less parasites in some specimens with low parasite count as storage of specimens in high temperature could increase glucose utilization by parasites. A study suggested that preparing blood smear slides just after blood collection could provide higher yield for parasite detection.[17]

Specimen collection was not able to conduct from September to December 2012 due to limited human resources even though the abortion in buffaloes was reported during that period. This might decrease the sample size and affect the analysis in some aspects.

The other limitation of this study was that diagnosis of blood parasites was carried out by microscopy. Though the method is easy, convenient and inexpensive, it takes some time to conduct the testing which can affect the sensitivity of the testing method. As a result, the studied prevalence might be lower than the reality. Despite that, when occurrence of abortion in buffaloes is higher than usual or if there are sick animals suspected of infection by blood parasites, the method could be used as a preliminary testing, along with calculating true prevalence, to overcome the limitation of the testing and assess the actual situation in the affected area. This could foster better planning on finance, resource, equipment and medical supplies for effective prevention and control. In case of aiming to study the true prevalence, the testing should be carried out together with other methods with high sensitivity such as *T. evansi* antibody and antigen detection by enzyme-linked immunosorbent assay (ELISA)[35], indirect hemagglutination test, complement fixation test, indirect fluorescent antibody test and polymerase chain reaction[36-38].

Recommendations

Findings from this study along with the results from other researches indicated that blood parasitic infection in buffaloes from Nakhon Phanom Province was higher than other provinces in the upper northeastern region. Hence, surveillance on blood parasites among buffaloes must be established. Surveillance and reporting systems on abortion in buffaloes and other animals such as cattle and domestic pigs should be improved to be able to determine the situation rapidly and collect the

appropriate specimens in time for better diagnosis. The testing of blood parasites in buffaloes with abortion should be performed by laboratory methods with higher sensitivity and specificity than the parasitological methods.

In order to reduce abortion risk among buffaloes, the sick buffalo and others in the herd and the nearby herd should be treated with diminazene aceturate (berenil)[17] or quinapyramine group (anticide R) once it is reported[39]. Normally, the drug could be administered for single dose as 3.5-5mg/kg body weight by deep intramuscular injection in neck or hip to reduce the swelling. However, the drug could get rid of all parasites completely in buffaloes that required 5-7 mg/kg body weight. Since the infection in most animals is mild, the animals recover rapidly after single dose treatment.

Furthermore, preventive measures for vectors control such as tabanus and stable flies should be recommended to farmers, and deworming of parasites in the GI tract should be carried out consistently.

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