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Seroprevalence of Bovine Viral Diarrhea Virus and Factors Associated with the Serological Status in Dairy Cattle in Western Region of Thailand

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ABSTRACT

Background: Bovine viral diarrhea virus (BVDV) causes productive losses and reproductive failure in dairy farms. This study defined the seroprevalence and determined the factors associated with BVDV infection in dairy cows in the western region of Thailand.

Methods: Blood samples were collected from 732 cows in 30 randomly selected dairy herds. The BVDV antibody was detected using a commercial indirect ELISA kit (IDEXX®BVDV Total Ab, IDEXX Laboratories Inc., Maine, USA). A questionnaire about the herd management was used to collect data via interviews with farm owners. The variables identified by Fisher's Exact Test ($p<0.20$) were then analyzed using multivariate logistic regression.

Result: Individual prevalence of BVDV infection was 36.89% (270/732), while herd prevalence was 93.33% (28/30). Significant risk factors of BVDV based on the univariate analysis were identified as area, herd size, feeding type, history of abortion, pen of calving, biosecurity and pet on farm ($p<0.05$). Biosecurity using no disinfectant ($p=0.02$) (OR, 1.32; CI 95%, 1.038-1.669), pet on farm ($p=0.03$) (OR, 4.72; CI 95%, 1.142-19.501) and history of abortion ($p=0.00$) (OR, 0.02; CI 95%, 0.004-0.140) were significant, based on multivariate regression analysis. Pen of calving ($p=0.01$) (OR, 0.24; CI 95%, 0.084-0.667) was a protective factor for BVDV infection. BVDV infection in dairy cattle herds was distributed throughout the western region of Thailand.

Key words: BVD, Risk factor, Seroprevalence.

INTRODUCTION

Bovine viral diarrhea (BVD) is an infectious disease in cattle worldwide causing significant economic losses. BVD virus (BVDV) is a single stranded RNA virus of the genus *Pestivirus* in the family *Flaviviridae*. This BVDV has been detected and causes diseases in a wide variety of livestock and wildlife species (Hause *et al.*, 2021). BVDV can be classified into two groups as BVDV-1 and BVDV-2 which may be differentiated from other Pestiviruses by monoclonal antibody reactions with E2 protein (Khodakaram-Tafti and Farjanikish, 2017). An atypical member of *Pestivirus* or Hobi-like viruses also referred to as BVDV-3 which is genetically and antigenically related with BVDV-1 and 2 (Uzal *et al.*, 2016). Hobi-like virus (BVDV-3) was identified in fetal bovine serum imported from Brazil, Europe, Southeast Asia including Thailand (Kampa *et al.*, 2004; Schirrmeier *et al.*, 2004). BVDV can be classified into 2 biotypes which are cytopathic (CP) and non-cytopathic (NCP) on the basis of their pathologic effects in cultured cells without significant serological differences. (Deregt and Loewen, 1995). Both biotypes of BVDV usually establish subclinical infection. Non-cytopathic BVDV is capable of causing persistent infection allowing this biotype to maintain its circulation in herds. As a consequence, NCP virus has been detected more regularly in cattle.

Evidence of published epidemiological studies of BVDV infection around South East Asia are inadequate. The BVDV prevalence was reported in Cambodia with only 6.4% seropositivity from 471 cattle serum samples and 3.4% from 29 buffalo serum samples (Olmo *et al.*, 2021). Also, frozen serum samples were screened for BVDV antibodies in Laos with 4.9% and 10% serological prevalence from buffalo and cattle samples respectively (Olmo *et al.*, 2018).

An epidemiological study of BVD in Thailand was conducted in the northeastern and northern region during 2001-2004. In 2001, 73% of serological prevalence was reported while the results in 2002 showed an increase in herd exposure of up to 92%. A moderate level of 72% prevalence in bulk milk tank was reported by Kampa *et al.* (2004). Moreover, there was the first incidence of atypical 'HoBi-like' Pestivirus from an infected calf in 2007 in Thailand (Ståhl *et al.*, 2007). A study by Nilnont *et al.* (2016) showed

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that in North-eastern Thailand has a high seroprevalence (62.5%) of BVD infection whereas the latest study by Amonongart *et al*. (2020) demonstrates overall prevalence of 28.44% with results of 43.51% and 25.89% prevalence in Kanchanaburi and Nakhon Pathom respectively.

In Thailand, the economic losses in dairy farms due to BVDV are not fully established. The infection has negative effects on reproductive performances such as abortion, mummification and infertility (Kampa *et al*., 2011) and is also associated with immunosuppression which predisposes infected animals to increased morbidity and mortality when co-infection with other pathogens occurs.

Many studies have documented the possible risk factors of BVDV transmission in dairy herds. Studies have shown that herd structure, herd size and density impact the spread of BVDV within herds (Ezanno *et al*., 2008). The introduction of new cattle into herds was the cause of BVD outbreaks in Iran and Europe (Karimi *et al*., 2022; Van Roon *et al*., 2020). Moreover, Karimi *et al*. (2022) reported that traditional housing systems also induced high seroprevalence of BVD infection in Iran. However, there are a limited studies concerning the BVD status in dairy cattle in the western part of Thailand. Therefore, this study determined BVD disease prevalence and factors associated with dairy farming patterns in the western region of Thailand.

MATERIALS AND METHODS

Ethical approval

The research was approved by the Animal Care and Use for Scientific Research Committee, Kasetsart University, Bangkok, Thailand (ACKU63-VET-018). Farmers were willing to provide information about cattle and to permit blood sampling from their animals.

Populations and sample size

The study was performed in the western region of Thailand which consists of five provinces: Ratchaburi, Kanchanaburi, Nakhon Pathom, Phetchaburi and Prachuap Khiri Khan (Fig 1). The Epi Info^{TM} 7.1.5.0 software (Windows version) was used to calculate the sample size at 43.51% of the prevalence (Amonongart *et al*., 2020) with 5% allowable error and 95% confidence interval. The estimation of total population of dairy cows in Western Thailand was 106,164 (DLD, 2019). Under this condition, the total number of sampled animals was 376 cows.

Sample and data collection

Blood samples were collected at the coccygeal vein from dairy cattle age over 1 year. A questionnaire was used for information about possible risk factors of BVD including herd size, housing system, feeding type, history of abortion and aborted fetus condition, history of illness (diarrhea and respiratory signs), brucellosis testing, biosecurity, new cattle introduction, pen of calving and the existence of pets and mice on their farms.

Herd size was defined as small (less than 20 cows), medium (20 to 50 cows) and large (more than 50 cows). Different housing systems in Thailand were tie stall (tied all the time), free stall (free all the time) and mixed housing (being tied at milking and feeding time). Feeding types were separated into four groups as separate feeding (concentrate and roughage), total mixed ration (TMR), same container and separate container. A biosecurity system was evaluated presence and absence of disinfectant.

Serological evaluation

All serum samples were tested to detect BVDV antibodies with an ELISA test kit (IDEXX®BVDV Total Ab, IDEXX

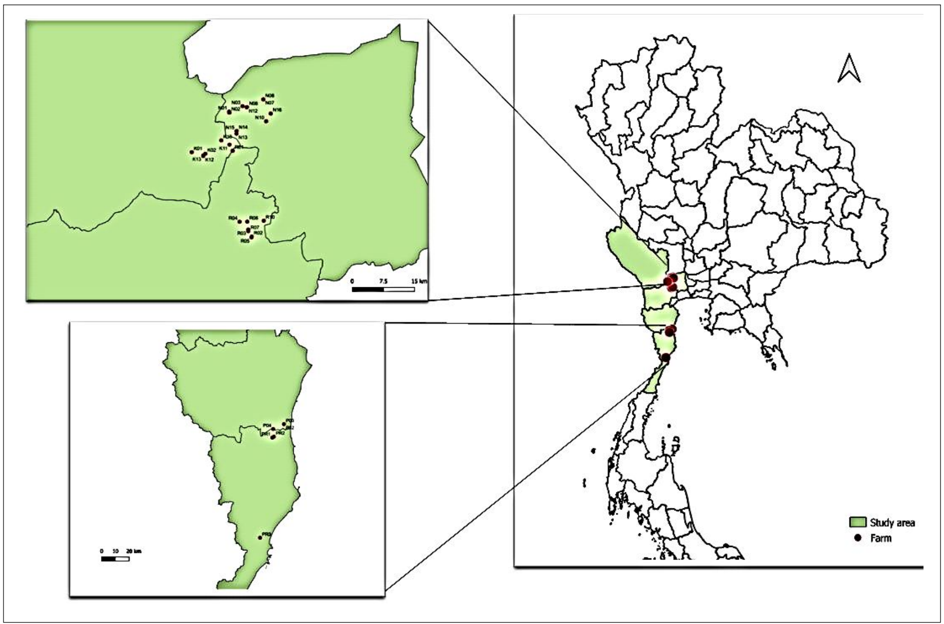

Fig 1: Geographic location of Western, Thailand.

Laboratories Inc., Maine, USA) following the manufacturer's instructions. For results to be valid, mean negative optical densities absorbance should read below 0.25 and the difference between mean negative control and mean positive control should be greater than 0.15, calculated based on the sample to positive ratio (S/P). Samples with S/P ratio 0.3 or greater were considered as positive, samples with S/P ratio less than 0.2 were considered negative and those with S/P ratio between 0.2 and 0.3 were considered suspected.

Statistical analysis

All analyses were conducted using the statistical software package STATA (version 17, Stata Corp., College Station, TX) to estimate herd and individual prevalence of BVDV infection. Correlation and multivariate logistic regression models were used to determine the relationship between the factors and BVDV seroprevalence. Fisher's exact test for univariate analysis was applied to assess the association between BVDV antibody level and each variable. Relationships between variables (BVDV antibody level, province, herd size, feeding type, history of abortion, pen of calving, biosecurity and the existence of pets and mice on farm) were also tested. Possible risk factors identified from univariate analysis with p-value < 0.20 were then evaluated by multivariate logistic regression analysis. Variables with p-value > 0.5 were removed by the backward stepwise method.

RESULTS AND DISCUSSION

This cross-sectional study was carried out between May 2019 and July 2021. Seven hundred and thirty-two blood samples were obtained from 30 dairy herds in the western region of Thailand. These animals did not receive any BVDV vaccine. The results showed that herd prevalence was

Table 1: Prevalence of bovine viral diarrhea virus infection within herd.

Province	Farm code	No. of sample	No. of positive	No. of negative	Prevalence (%)
Ratchaburi	1	18	5	13	27.78
	2	12	11	1	91.67
	3	27	8	19	29.63
	4	26	22	4	84.62
	5	20	19	1	95.00
	6	14	6	8	42.86
	7	18	2	16	11.11
	8	30	7	23	23.33
	9	32	3	29	9.38
	10	15	3	12	20.00
		212	86	126	40.57
Kanchanaburi	11	52	8	44	15.38
	12	33	15	18	45.45
	25	56	9	47	16.07
	26	20	3	17	15.00
		161	35	126	21.74
Phetchaburi	13	38	29	9	76.32
	14	28	28	0	100.00
	15	29	28	1	96.55
	16	30	2	28	6.67
		125	87	38	69.6
Nakhon Pathom	17	19	17	2	89.47
	18	19	3	16	15.79
	19	20	13	7	65.00
	20	5	0	5	0.00
	21	11	0	11	0.00
	27	16	9	7	56.25
	28	5	1	4	20.00
	29	31	2	29	6.45
	30	21	2	19	9.52
		147	47	100	31.97
Prachuap Khiri Khan	22	26	8	18	30.77
	23	29	4	25	13.79
	24	32	3	29	9.38
		87	15	72	17.24
Overall	30	732	270	462	36.89

93.33% (28/30) while 2 herds had 100% seronegative samples. Individual prevalence was 36.89% (270/732) and prevalence within the herd ranged from 0 to 100%. Individual prevalence of seropositivity status in each location was shown in Table 1.

Associations between seropositive dairy cattle and the risk factors found in the univariate analysis are shown in Table 2. It shows that out of ten variables, seven variables were identified as possible risk factors (p<0.2): area, herd size, feeding type, history of abortion, pen of calving, biosecurity and the existence of pet on farm. Table 3 presents factors impacted BVDV seropositivity (p<0.05). The existence of pets on farms and the absence of disinfectant were factors that increased BVDV infection in herd, while pen of calving was a protective factor. As the result of this study, we found that seroprevalence of BVDV was widespread throughout the western region of Thailand. Previous observations reported variations in the seroprevalence of BVDV at the individual level in different areas of the country (Kampa *et al*., 2004; Nilnont *et al*., 2016; Virakul *et al*., 1997).

In Eastern China, serological investigations found that 77.8% of herds were BVDV antibody positive. Average positive ratios of calves, heifers and lactating cows were 15.94%, 40.16% and 41.7%, respectively (Hou *et al*., 2019). The study in Korea reported 91.5% that were determined to be positive BVDV from bulk-milk tank (Park *et al*., 2016). In

Table 2: Prevalence of bovine viral diarrhea virus infection categorized by each variable.

Variable	Positive number	Negativenumber	Prevalence (%)	p-value
Area*				
Ratchaburi (R)	86	126	40.57	0.08
Kanchanaburi (K)	35	126	21.74	
Phetchaburi (P)	87	38	69.60	
Nakhon Pathom (N)	47	100	31.97	
Prachuap Khiri Khan (PK)	15	72	17.24	
Housing system				
Tie stall	63	99	38.89	0.58
Free stall	185	273	40.39	
Mixed	22	90	19.64	
Herd size*				
Small (<20 cows)	32	65	32.99	0.10
Medium (20-50 cows)	177	180	49.58	
Large (>50 cows)	61	217	21.94	
Feeding type*				
Separate feeding	20	82	19.61	0.17
Total mixed ration; TMR	0	0	0.00	
Same container	250	374	40.10	
Separate container	0	5	0.00	
History of abortion*				
Aborted	197	459	30.03	0.00
Non-aborted	73	3	96.05	
Calving pen*				
Yes	30	217	12.15	0.00
No	240	245	49.48	
New cattle introduction to herd				
Yes	90	170	34.62	0.76
No	180	292	38.14	
Biosecurity*				
Disinfectant	81	223	26.64	0.17
No disinfectant	189	239	44.16	
Mice on farm				
Yes	217	403	35.00	0.41
No	53	54	49.53	
Pets on farm*				
Yes	243	375	38.32	0.09
No	10	87	10.20	

*Variable having p-value <0.2 by univariate analysis.

Table 3: Factors associated with bovine viral diarrhea virus serological status of dairy cattle from Western Thailand.

Variable	OR	p-value	95% CI
History of abortion			
Non - aborted	-	-	-
Aborted	0.022	0.000*	0.004-0.140
Biosecurity			
Disinfectant	-	-	-
No disinfectant	1.316	0.023*	1.038-1.669
Pen of calving			
No	-	-	-
Yes	0.237	0.006*	0.084-0.667
Pets on farm			
No	-	-	-
Yes	4.719	0.032*	1.142-19.501

*Variable having p-value ≤0.05 by multivariate regression analysis.

Jordan, true prevalence of antibodies against BVDV at the individual and herd levels were 31.6% and 80.7%, respectively (Talafha *et al.*, 2009). Our results are in conformity with these reported in Jordan, with less individual seroprevalence but high herd level. In Turkey, prevalence of BVDV positive were 89.58% from blood serum and the presence positive BVDV RNA was 66.66% from internal organ of aborted calves (Yilmaz, 2016; Yilmaz *et al.*, 2016). Moreover, there reported small-sized ruminant farms and the results confirmed that BVDV was still in circulation (Tamer *et al.*, 2018). While overall seroprevalence of BVDV was 51.1% in Bangladesh (Uddin *et al.*, 2017). An India survey during 1999-2004 was 30% (Sood *et al.*, 2007). Seroprevalence in our study was lower than those reported in middle east and similar to India.

In Cameroon, estimates of herd level and within herd seroprevalences adjusted for test imperfections were 92% and 30%, respectively with 16.5% of herds classed as having a PI calf (Handel *et al.*, 2011). Studies in Africa that evaluated antigen BVDV and investigated PI calves showed varied results. Individual and herds with positive BVDV antibodies were reported at 36% and 69% in Colombia (Ortega *et al.*, 2020). In Ecuador, individual and herd BVDV seroprevalences were 36.2% and 74%, respectively (Saa *et al.*, 2012) with BVDV seroprevalence recorded at 47.8% in Mexico (Segura-Correa *et al.*, 2016). Study results in South America concurred with BVDV seroprevalence in the western region of Thailand. These studies indicated that BVDV is globally distributed.

It could be seen from Table 3 that the history of abortion (0.022) was significant. One more report confirms that aborted cows were significantly more seropositive than non-aborted cows (Uddin *et al.*, 2017).

Generally, farmers clean their barns using ground water. Sometimes stools remain on pillars and at floor corners. Biosecurity by 'No disinfectant' was associated with BVDV infection. BVDV can be transmitted to susceptible cows and between herds from secretion and mucus, workers' clothing, contaminated equipment, contaminated biologicals, injectables contaminated with small amounts of nasal secretions from PI animals and biting flies (Lindberg and Houe, 2005). Strict biosecurity is essential to prevent high seropositivity of BVDV within the herd.

Dogs on farms have direct access to cows. They eat placental fluid and lick the noses of cattle. In Kenya, farm dogs with access to bovine aborted fetuses and dogs whelping on the farm were identified as risk factors for BVDV infection. Factors associated with co-infection included direct contact between dairy cattle, dogs and goats (VanLeeuwen *et al.*, 2021).

The calving pen was also identified as a major factor that decreased opportunities for BVDV infection in our study. Absence of calving pens showed 88% seroprevalence (Talafha *et al.*, 2009). Fetal fluid from the calving of PI animals can infect other animals in the herd for 24 to 48 hours after calving, while tools and equipment used on PI animals carry transmittable numbers of live virus (Lindberg *et al.*, 2004).

There was no association between province, herd size, feeding type and BVDV status in the final model. Dairy cattle herds in each province varied in size, but quite similar in geographical area. The low dairy cattle population in each province is another possibility. Farms located in high-density areas have more and closer neighboring farms (Saa *et al.*, 2012). Feeding type was not a significant risk factor for within-farm transmission. Four feeding patterns are popular in western areas of Thailand as separate feeding, TMR, same container and separate container. In separate feeding, concentrate and roughage are separated. Some farms fed cows from the same container while others used separate containers.

Housing system, new cattle introductions and mice on farm were not a significant risk factor. Most farmers replaced only 1-2 heifer cows per year that were sourced from nearby areas. Study results in Spain suggested that BVDV infection could be controlled by unvaccinated livestock-trade control (Mainar-Jaime *et al.*, 2001). Seropositive animals were not the main risk of BVDV infection, with PI (seronegative) animals identified as the major risk factor (Segura-Correa *et al.*, 2016). Mice on farm were not a significant risk factor for within-farm transmission. Farmers usually place concentrate feed bags near the barn. This feed storage behavior induces mice infestation and increases the opportunity of contact between vermin and dairy cows. However, dogs and cats on the farm generally control vermin populations.

CONCLUSION

Results demonstrated that BVDV infection is ubiquitous in dairy cattle herds in the western region of Thailand. Individual seroprevalence of BVDV was 36.89%, with herd seroprevalence 93.33%. There were 28 seropositive herds. Factors associated with BVDV infection included history of abortion, biosecurity, pets on farm and pen of calving.

Biosecurity of no disinfectant was a significant protective factor using multivariate regression analysis. Pet on farm was associated with increasing BVDV infection within the herd. History of abortion was an associated factor for monitoring the seropositive status of the disease, while pen of calving showed decreased BVDV infection. This study provided additional information to understand the factors associated with BVDV and design control strategies for dairy farmers. Good management practices can control BVD infection. Outbreak investigation and development of local vaccine strains should be considered in future studies.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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