

Epidemic Characteristics of Foot and Mouth Disease on the Buffaloes and Cows in Lang Son Province, Vietnam

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To cite this article:

Dang Xuan Binh, Dam Thi Phuong Mai. Epidemic Characteristics of Foot and Mouth Disease on the Buffaloes and Cows in Lang Son Province, Vietnam. *Animal and Veterinary Sciences*. Vol. 4, No. 4, 2016, pp. 62-71. doi: 10.11648/j.avs.20160404.13

Received: July 14, 2016; **Accepted:** August 23, 2016; **Published:** August 25, 2016

Abstract: From 2011 to 2015, in Lang Son province, Vietnam, foot and mouth disease (FMD) occurred in 11 districts and town. The virus causing FMD on the cattle in Lang Son belonged to type O and type A. While type Asia 1 virus had occurred in some other provinces in Vietnam, it was not found in Lang Son. FMD epidemic in Lang Son province had no regularity. In one district, the epidemic occurred continuously in two or three years (e.g. in Binh Gia and Van Quan districts) while in some others, it occurred only once (e.g. Cao Loc district and Lang Son city). On average, 1.26% of the buffaloes and cows were infected with FMD per year. Precisely, 1.39% of the buffaloes and 0.75% of the cows were infected. FMD on the buffaloes and cows in Lang Son was under the influence of (i) the weather and climate conditions (especially the years with extremely cold winter), (ii) the seasonal agricultural practice in the locality (during the time of the year when the cattle were used for ploughing and pulling carts for the crops, their health was relatively declined), and (iii) the results of vaccination in early Autumn – Winter season (the smaller the number of cattle vaccinated, the higher the number of cattle infected). These influences were clearly observed in 2011 with the highest number of cattle infected (8,929 infected cattle, accounting for 5.3% of the herds, whereas the average rate of infection in the whole five studied years was 1.26%). The sources of FMD were identified as mainly came from the infected cattle which had been medically treated and clear of the symptoms, yet still carried and shed the virus to the environment. On average, the rate of natural virus infection on the cattle in Lang Son was 33.23%. Of which, the rate was highest in the following districts: Bac Son (66.66%), Van Quan (50%), Trang Dinh (32.88%) and Binh Gia (28.57%). After being injected with FMD vaccine (Aftovax Bivalent, with 2 types of O and A), 100% of the cattle had immune response. 30 days after vaccination, the rate of protection varied from 86.6% to 100%. Six months after the first vaccination shot, a second one must be done for the vaccination to be effective.

Keywords: FMD, Type A, Type O, Disease, Buffaloes, Cows

1. Introduction

Foot and mouth disease is an acute communicable disease which spreads quickly and causes serious damages to the cattle such as the buffaloes and cows and pigs. It is listed in list A of the dangerous communicable diseases by Office International Epizooties (OIE) [1, 2, 11, and 17].

There are seven types of FMD virus: A, O, C, Asia1, SAT1, SAT2, and SAT3. In South East Asia, the most common ones are types O, A, and Asia1. In Vietnam, the ones which have caused diseases on the buffaloes and cows and pigs are types

O, A and Asia1 [3, 4, 6, 18, 19, 20 and 21].

Lang Son is a border mountainous province in the Northeast of Vietnam, with national roads running through the province, and the north of the province borders Chongzuo (Quangxi, China), with a border line of 253 kilometres, two international bordergates, two national bordergates, seven border markets, and local markets, which make the province an important center for exchanging and trading between Vietnam and China, to Central Asian and European countries.

During the five years, from 2011 to 2015, there were 187 FMD outbreaks in Lang Son. On average, 3.4 outbreaks occurred in each district or town per year. The number of

outbreaks which becomes epidemics varied among the districts. During the five years, there were seven districts with 20 outbreaks or more, including Trang Dinh (21 outbreaks), Van Lang (26 outbreaks), Chi Lang (23 outbreaks), Huu Lung (27 outbreaks), Bac Son (23 outbreaks), Binh Gia (24 outbreaks) and Van Quan (29 outbreaks); there were only four districts with less than 10 outbreaks, including Cao Loc (2 outbreaks), Loc Binh (2 outbreaks), Dinh Lap (4 outbreaks) and Lang Son City (6 outbreaks).

The complex situation of FMD epidemic in Lang Son province and in the whole country in the past few years requires studies on the distribution and occurrence of FMD virus, to serve as a scientific ground for selection of suitable vaccines. This would contribute to improve the effectiveness of epidemic prevention and fighting, to ensure that the cattle are safe. These are the reasons why the study entitled “*Epidemic Characteristics of Foot and Mouth Disease on the Buffaloes and Cows in Lang Son Province, Vietnam*”, was carried out from 2011 to 2015.

2. Materials and Methods

2.1. Contents

- Survey on epidemic situation of FMD on the buffaloes and cows in Lang Son province from 2011 to 2015.
- Serological assesment of the positive rate with the strains of FMD virus which have been found in Vietnam (O, A, Asia 1) on the buffaloes and cows in Lang Son.
- Identification of the type of virus which cause FMD on the buffaloes and cows in Lang Son province

2.2. Animal Testing

- Buffaloes and cows of different age groups

2.3. Samples

- Samples were the epithelium tissues and vesicles on the infected buffaloes and cows collected from outbreaks in Lang Son province.
- Blood samples of healthy buffaloes and cows in Lang Son for examining the presence of FMD virus.
- FMD virus.
- Antisera to FMD virus collected from buffaloes and cows blood samples in Lang Son province.
- Other necessary equipments, materials, and environment chemicals.

2.4. Time Duration

- Data from epidemic survey from 2011 to 2015
- Study the presence of FMD virus on the buffaloes and cows in the two years of 2014 and 2015.

2.5. Study Area

- The samples were collected by the Animal Health Department of Lang Son province.

- The tests and examinations of the samples were conducted at the National Centre for Veterinary Diagnosis and the Regional Animal Health Office No. II.
- The epidemic characteristics of FMD were studied on the buffaloes and cows in Lang Son province from 2011 to 2015
- Serological assessment of positive rate with the main virus strains (O, A, Asia 1), and assessment of the virus infection rate on the buffaloes and cows were conducted at the laboratories of the National Centre for Veterinary Diagnosis and the Regional Animal Health Office No. II.
- Identification of the types of virus which cause FMD on the buffaloes and cows were done in Lang Son province

2.6. Methodology

2.6.1. ELISA

ELISA technique was used to identify the antigen and examine the serotypes of FMD virus. This is a quick dianogsis method, with high specifity and sensitivity. The tests based on the principles of serology to FMD virus included two types: (i) tests in which antibodies to structural proteins (SPs) of virus were identified, and (ii) tests in which antibodies to nonstructural proteins (NSTs) of virus were identified.

The blood type specific SP tests identifying the antibodies were developed by vaccination and infection.

2.6.2. 3ABC - ELISA

ELISA methodology allows for identification of antibodies (types and density of antibodies), and of the types of FMD virus causing the disease. However, it is not possible to use ELISA technique to determine whether the existence of the antibodies relates to natural infection or it is due to vaccination. In order to clear the confusion, 3ABC-ELISA method was used to determine the types of antibodies against non-structural proteins 3ABC antigen of FMD virus.

In fact, only cattles which are natural infected with FMD virus are possitive with 3ABC-ELISA reactions.

2.6.3. Dianogsis with Real-Time RT-PCR Technique

According to [17, 19, 20], primers FM1F-FM1R can be used to multiply the non-coding gene area 5'UTR from RNA templates of the strains O, A, C, and Asia 1, and the product was cDNA with the lengths of 323- 328 nucleotide, and the lengths vary according to the types.

Primer sequence 1 (FM1F): 5'-GCCTG-GTCTT-TCCAG-GTCT-3' (positive strand)

Primer sequence 2 (FM1R): 5'-CCAGT-CCCCT-TCTCA-GATC-3' (negative strand)

Cycle parameter: 94°C in five minutes (1 cycle); 94°C in one minute, 55°C in one minute, 72°C in two minutes (30 cycles); 72°C in seven minutse (1 cycle).

Designed primers which can magnify the encoding gene sequences 1D (VP1) of FMD virus related to the strains of O, A, C, and Asia. The sense primer of the virus was designed

to have the same sequences as the 1C (VP3) of the virus. The antisense primer NK61 was designed based on the gene sequence 2B of the strains O, A, C, and SAT2. The sequence

of primer NK72 used for identifying FMD virus was used in all the tests on RNA sequence [5, 9, 13, and 15].

Table 1. Primers used for identification of the prevalence of FMD virus type O, A and Asia1.

Primer	Nucleotide sequence (5'-3')	Size (bp)
Positive sense		
O-1C124 (ARS4)	ACCAACCTCCTTGATGTGGCT	1301
O-1C564	AATTACACATGGCAAGGCCGACGTG	861
O-1C609 (Ovp3)	TAGTGCTGGTAAAGACTTTGAGCT	816
A-1C562	TACCAAATTACACACGGGAA	863-866
A-1C612	TAGCGCCGGCAAAGACTTTGA	813-816
As1-1C505	TACACTGCTTCTGACGTGGC	908-914
As1-1C616	GGCAAGGACTTTGAGTTTCGC	797-803
Negative sense		
FMD-2B58 (NK61)	GACATGTCCTCCTGCATCTG	
FMD-2A34 (NK72)	GAAGGGCCCCAGGGTTGGACTC	

2.6.4. Blood Sample Collection

- 3 ml of blood was sucked from the neck vein of each buffaloes and cows by syringes
- Blood serum was separated, stored in Eppendorf tubes at the temperature from 2 - 4°C, and sent to the laboratory.
- The blood serum was stored at 4°C if examined within one week or at -30°C if examined later.

2.6.5. Epithelium Tissue Sample Collection

- Clinical examination and selection of the cattles with FMD at early stage. The samples were collected from the new wounds, and not from the ones which had formed scars or had been sterised and treated.
- Epithelium tissue samples were collected from the cattles which showed symptoms of FMD according to the guidelines of the Agency of Animal Health of Vietnam and the principles of biosafety of OIE for examining and identifying the types of virus.
- Epithelium tissue samples include those from the tongue, gum, hoof gaps, the peeling skin surrounding the hooves formed when the vesicles broke, and the fluid substances in the vesicles.
- The samples were stored at 4 - 8°C in the mixture of PBS 0.04 M and Glycerin (Rate 1: 1) pH = 7.2 - 7.6 during transportation to the laboratory.
- The samples which were not examined immediately were stored at -80°C for later laboratory performances.

3. Results and Discussion

3.1. Epidemic Characteristics of the FMD on Buffaloes and Cows in Lang Son Province

In order to evaluate the occurrence of FMD in Lang Son province in recent years, a retrospective study was carried out, in which, data on the FMD outbreaks on buffaloes and cows in Lang Son province in five consecutive years from 2011 to 2015 was collected. The results are presented in table 2.

Table 2. FMD on buffaloes and cows in Lang Son province.

TT	FMD areas (districts)	Number of outbreaks					Total
		2011	2012	2013	2014	2015	
1	Trang Dinh	20		1			21
2	Van Lang	20	5		1		26
3	Cao Loc	2					2
4	Loc Binh	1			1		2
5	Dinh Lap	2			2		4
6	Lang Son City	4	2				6
7	Chi Lang	19	1	1	2		23
8	Huu Lung	26	1				27
9	Bac Son	17		5		1	23
10	Binh Gia	19	3	2			24
11	Van Quan	22	3	3		1	29
Total		152	15	12	6	2	187

Table 2 shows that in the five years from 2011 to 2015, in Lang Son province, there were 187 FMD outbreaks. On average, there were 3.4 outbreaks/year in each district/town (187 outbreaks/11 districts/five years = 3.4).

The number of FMD outbreaks depended on the locality. In the five years, there were seven districts with 20 outbreaks or more, including Trang Dinh (21 outbreaks), Van Lang (26 outbreaks), Chi Lang (23 outbreaks), Huu Lung (27 outbreaks), Bac Son (23 outbreaks), Binh Gia (24 outbreaks) and Van Quan (29 outbreaks); four districts with less than ten outbreaks, including Cao Loc (2 outbreaks), Loc Binh (2 outbreaks), Dinh Lap (4 outbreaks) and Lang Son City (6 outbreaks). FMD occurred in Lang Son in all the five years. The number of FMD outbreaks among the years varied. On average, there were 37.4 outbreaks/year, with the highest rate of 152 outbreaks in 2011. The FMD in Lang Son occurred irregularly. In one district, there were outbreaks every year in two or three consecutive years (e.g. Binh Gia and Van Quan districts), while in some others the outbreaks occurred and did not happen again several years after that (Cao Loc, Lang Son City). This finding was similar to that of [9, 10].

3.1.1. Statistics of the Buffaloes and Cows Infected with FMD

Statistics on the buffaloes and cows infected with FMD in

Lang Son province in five years from 2011 to 2015 were collected. The results are presented in tables 3a and 3b.

Table 3a. Buffaloes and cows infected with FMD.

Year	Buffaloes			Cows		
	Total number	Infected	Rate (%)	Total number	Infected	Rate (%)
2011	133,850	8,253	6.16	34,414	676	1.96
2012	128,531	63	0.05	31,168	3	0.009
2013	121,375	349	0.28	31,006	266	0.86
2014	121,309	73	0.06	32,109	242	0.75
2015	122,060	31	0.02	32,783	35	0.11
Total	627,125	8,769	1.39	161,480	1,222	0.75

Table 3b. Buffaloes and cows with FMD.

Year	Total number of buffaloes and cows	Infected	Rate (%)
2011	168,264	8,929	5.30
2012	159,699	66	0.04
2013	152,381	615	0.40
2014	153,418	315	0.20
2015	154,843	66	0.04
Total	788,605	9,991	1.26

Tables 3a and 3b show that the buffaloes and cows with FMD account for an average of 1.26%/year; with 1.39%/year for buffaloes and 0.75%/year for cows. The disease occurred every year from 2011 to 2015. The number of buffaloes and cows infected varied among the months due to the influence of the weather and vaccination. For example in 2011 when it was extremely cold in the winter, the Rate of buffaloes and cows with FMD was highest with 8,929 infected (or 5.3%, compared to the five year's average of 1.26%). This finding was similar to that of [9, 10, and 14].

With the above results, epidemically speaking, it could be confirmed that the source of the disease still existed. The main source was the buffaloes and cows with FMD which had been treated with the disease and shown no symptom, yet still carried the viruses and release them to the surrounding environment. The new buffaloes and cows which had no immunity or no longer been protected would be sensitive to the viruses and infected with the disease. This result was similar to that of [10, 15, 20, and 21].

3.1.2. FMD on the Buffaloes and Cows in Lang Son Province with by Seasons

In order to determine the influence of climatic and weather conditions to the outbreaks of FMD, statistics on cases of FMD on buffaloes and cows (Totally and separately)

occurring in each of the the four seasons in Lang Son province in five years from 2011 to 2015. The results are presented in tables 4a, 4b, and 4c.

Table 4a. FMD on the buffaloes and cows in Lang Son province by seasons (in the five years from 2011 to 2015).

Areas	Number of buffaloes and cows with FMD by seasons				
	Spring	Summer	Autumn	Winter	Total
Trang Dinh	368	0	23	514	905
Van Lang	526	77	121	651	1,375
Cao Loc	0	0	0	56	56
Loc Binh	0	0	0	175	175
Dinh Lap	15	0	0	56	71
Lang Son City	347	0	0	446	793
Chi Lang	825	30	181	1,763	2,799
Huu Lung	479	0	65	682	1,226
Bac Son	282	13	20	14	329
Binh Gia	298	27	0	445	770
Van Quan	631	0	0	861	1,492
Total	3,771	147	410	5,663	9,991

Table 4a shows that from 2011 to 2015, the number of buffaloes and cows with FMD was 3,771 in Spring; in Summer; 410 in Autumn; and 5,663 in Winter. The total number of buffaloes and cows infected with the disease in Lang Son was 9,991 in the five years.

Table 4a shows that FMD in Lang Son was critical. Besides, it is very difficult to control the sources of the disease. It is because once infected with the disease and being treated, although there might be no sign of the symptom, the cattle still carry and release FMD viruses to the environment for a long time after that (one or two years), which causes great challenges to the prevention of the disease's outbreaks.

Table 4b. FMD on Buffaloes by seasons.

Year	Total number of Buffaloes	Infected	Seasons							
			Spring		Summer		Autumn		Winter	
			Infected	Rate (%)	Infected	Rate (%)	Infected	Rate (%)	Infected	Rate (%)
2011	133,850	8,253	3,402	41.22	98	1.19	341	4.13	4,412	53.46
2012	128,531	63	63	100	0	0	0	0	0	0
2013	121,375	349	0	0	40	11.46	23	6.59	286	81.95
2014	121,309	73	0	0	0	0	0	0	73	0
2015	122,060	31	0	0	0	0	6	19.35	25	80.65
Total	627,125	8,769	3,465	39.41	138	1.57	370	4.22	4,796	54.70

Table 4b shows the situation of FMD on Buffaloes by seasons in 11 districts and towns in Lang Son from 2011 to 2015. Out of the 627,125 buffaloes, 8,769 were found with FMD. Of which, the number of buffaloes infected with the disease was 3,456 in Spring, accounting for 39.41%; 138 in Summer, accounting for 1.57%; 370 in Autumn, accounting for 4.22%; and 4,796 in winter, accounting for 54.70%.

The results correspond with those in Table 4a. It can be

easily seen that there were most cases in winter (accounting for 54.80% of the total buffaloes infected with the disease); and the number of cases was lowest in Summer (accounting for 1.57%). Thus, it can be seen that the climatic and weather conditions affect seasonal agriculture and husbandry practices in Lang Son and other provinces of similar conditions in Northern mountainous areas in Vietnam. This finding was similar to that of [11, 13].

Table 4c. FMD on cows by seasons.

Year	Total number of cows	Infected	By seasons trong Year							
			Spring		Summer		Autumn		Winter	
			Infected	Rate (%)	Infected	Rate (%)	Infected	Rate (%)	Infected	Rate (%)
2011	34,414	676	321	47.48	0	1.33	26	3.85	320	47.33
2012	31,168	3	3	100	0	0	0	0	0	0
2013	31,006	266	0	0	0	0	0	0	266	0
2014	32,109	242	24	9.92	0	0	0	0	218	90.08
2015	32,783	35	0	0	0	0	14	40	21	60
Total	161,480	1,222	306	25.04	9	0.74	40	3.27	867	70.95

Table 4c shows FMD on cows in 11 districts and towns in Lang Son by seasons. Out of the total 161,480 cows, 1,222 were found with FMD. The number of cows with FMD was 306 in Spring, accounting for 25.04%; 9 in Summer, accounting for 0.74%; 40 in Autumn, accounting for 3.27%; and 867 in Winter, accounting for 70.95%. The results show that the situation of FMD on cows was relatively different from that on buffaloes. The number of cows with FMD was lower than that of buffaloes in Spring, Summer, and Autumn, but higher in Winter (70.95% and 54.70%).

3.1.3. FMD on Buffaloes and Cows by Age

In fact, besides being species dependent, the sensitivity of the buffaloes and cows towards FMD also depends on age and other factors relating to active and passive immunity. In order to determine the relationship between age factor and FMD, a retrospective study of the statistics of the buffaloes and cows with FMD by age from 2011 to 2015 was carried out. The cattle were divided into three age groups: (i) less than two years of age, (ii) from two to five years of age, and (iii) over five years of age. The results are presented in tables 5a, 5b and 5c.

Table 5a. Buffaloes with FMD by age.

Year	No. of Buffaloes with FMD	Age of Buffaloes (Year)					
		<2		2 – 5		>5	
		No. infected	%	No. infected	%	No. infected	%
2011	8,253	453	5.49	851	10.31	6,949	84.21
2012	63	7	11.11	14	22.22	42	66.67
2013	349	29	8.31	53	15.18	267	76.50
2014	73	6	8.22	11	15.06	56	76.71
2015	31	3	9.68	4	12.90	24	77.42

Table 5b. Cows with FMD by age.

Year	Total Cows with FMD	Age of cows					
		<2		2 – 5		>5	
		No. infected	%	No. infected	%	No. infected	%
2011	676	26	3.84	67	9.91	583	86.25
2012	3	0	0	1	33.33	2	66.67
2013	266	11	4.13	46	17.29	209	78.57
2014	242	15	6.20	58	23.69	169	69.84
2015	35	2	5.71	12	34.29	21	60

Table 5c. Buffaloes and Cows with FMD by age.

Year	Total No. of buffaloes and cows with FMD	Age of buffaloes and cows (Year)					
		<2		2 – 5		>5	
		No. infected	%	No. infected	%	No. infected	%
2011	8,929	479	5.36	918	10.28	7,532	84.35
2012	66	7	10.60	15	22.72	44	66.67
2013	615	40	6.50	99	16.09	476	77.39
2014	315	21	6.67	69	21.90	225	71.42
2015	66	5	7.57	16	24.24	45	68.18

Tables 5a, 5b and 5c show that FMD occurs on buffaloes and cows of all the age groups; (i) less than two years of age, (ii) from two to five years of age, and (iii) over five years of age. Of which, the highest rates of FMD infection occurred on Buffaloes and cows over five years of age, with 66.67% to 84.21% of the buffaloes, 60% to 86.25% of the cows; and 68.18% to 84.35% of the total buffaloes và cows with FMD. This result corresponds to previous reports on FMD on buffaloes and cows in Bac Kan, Cao Bang, and several other Northern mountainous provinces in Vietnam.

3.1.4. Buffaloes and Cows Died of FMD

The number of buffaloes and cows died of FMD in 11 districts and towns in Lang Son province from 2011 to 2015 was studied using veterinary epidemiology criteria. The results are presented in Table 6.

Table 6. Buffaloes and cows died of FMD.

Epidemiological Criteria	No. infected	Dead toll	Rate (%)
The buffaloes and cows died of FMD by age			
<2	552	34	6.15
2 – 5	1,117	87	7.78
> 5	8,322	168	2.02
The buffaloes and cows died of FMD by seasons			
Spring	3,771	109	2.89
Summer	147	2	1.36
Autumn	410	14	3.41
Winter	5,663	184	3.25
The buffaloes and cows died of FMD by year			
2011	8,929	285	3.19
2012	66	0	0
2013	615	9	1.46
2014	315	14	4.44
2015	66	1	1.51

3.1.5. Number of Buffaloes and Cows Naturally Infected with FMD Viruses (Those Without FMD Vaccination)

Blood samples from healthy buffaloes and cows (those

with no clinical symptoms or clinical signs of FMD) were collected to assess natural FMD viruses infection (The buffaloes and cows had not been injected with FMD vaccines). The results are presented in Table 7.

Table 7. Buffaloes and cows which are positive with 3ABC antigen of FMD viruses in the nature.

NO.	Areas	No. of samples examined	No. of positive samples	Rate (%)
1	Trang Dinh	298	98	32.88
2	Binh Gia	7	2	28.57
3	Bac Son	3	2	66.66
4	Van Quan	2	1	50
Total		310	103	33.23

Table 7 shows that the blood samples of the buffaloes and cows in the four districts of Trang Dinh, Binh Gia, Bac Son and Van Quan found positive with 3ABC antigen of FMD viruses accounted for an average of 33.23%. The rate was highest in Bac Son (66.66%), Van Quan (50%), Trang Dinh (32.88%) and Binh Gia (28.57%).

The result shows the relatively high rate of buffaloes and cows carrying FMD virus (accounting for 33.23%), which means for every three cattles, there is one carrying FMD viruses. This is a source of the disease. The buffaloes and cows or other sensitive cattles such as pigs and goats which have not been immuned against FMD could easily catch the disease. The result also shows that there is a relation between the number of buffaloes and cows carrying the virus and the number of buffaloes and cows suffered from FMD from 2011 to 2015. This result was similar to that of [7, 9].

3.1.6. Buffaloes and Cows Infected with FMD Virus in Natural Conditions After Being Injected with FMD Vaccine

Blood samples from healthy buffaloes and cows which showed no symptom or clinical signs of FMD to assess the natural infection of FMD in natural conditions after being injected with FMD vaccine in April 2015. The results are presented in Table 8.

Table 8. Vaccinated buffaloes and cows found positive with 3ABC antigen of FMD virus existing in natural environment in Bac Son district.

NO.	Sample taking time (month/year)	No. of samples examined	No. of samples positive	Rate (%)
1	5/2015 (1 month after vaccination)	10	7	70
2	6/2015 (2 months after vaccination)	10	6	60
3	8/2015 (3 months after vaccination)	10	6	60

Of the 30 blood samples of the buffaloes and cows taken one, two, and three months after vaccination, 60% to 70% were found positive with 3ABC antigen of the FMD virus existing in the environment.

The results correspond with the local reality. In Bac Son district, FMD occurred in many consecutive years. Some buffaloes and cows were treated and showed no symptom of the disease, yet still carried the viruses and release them to the environment, which infected the cattles, both vaccinated and non-vaccinated ones, and cause the outbreaks of the disease every year. Similar to the results discussed in tại Table 6, the buffaloes and cows here were also the source of the disease in the environment. If other buffaloes, cows or

other sensitive cattles such as pigs and goats are not vaccinated, they could easily develop the disease. This result corresponds to that of [7, 9].

3.2. Types of FMD Viruses on the Buffaloes and Cows in Lang Son Province

3.2.1. Types of FMD Viruses in the Blood Samples Taken from the Buffaloes and Cows

Blood samples collected from the vaccinated and non-vaccinated buffaloes and cows in four districts which represented the different areas in Lang Son province. The results are presented in Table 9, and 10.

Table 9. Types of FMD viruses in the blood samples taken from the buffaloes and cows.

Areas (districts)	No. of samples collected	Result					
		Type O	Rate (%)	Type A	Rate (%)	Type Asia 1	Rate (%)
Chi Lang	2	0	0	1	50	0	0
Binh Gia	7	0	0	1	14.28	0	0
Van Quan	3	2	66.6	0	0	0	0
Bac Son	2	1	50	0	0	0	0
Dinh Lap	3	2	66.6	0	0	0	0
Loc Binh	2	0	0	1	50	0	0
Van Lang	2	1	50	0	0	0	0
Total	21	6	28.5	3	14.2	0	0

Table 9 shows that out of the 21 blood samples from the buffaloes and cows with FMD collected in the districts of Chi Lang, Binh Gia, Bac Son, Dinh Lap, Loc Binh, Van Lang and Van Quan, 6 belongs to type O virus (Van Quan, Dinh Lap, Van Lang and Bac Son); 3 belong to FMD virus type A (Chi Lang, Loc Binh and Binh Gia); virus type Asia 1 was not found. Thus, the viruses found in the seven districts belong to two types: type O (accounting for 28.5%) and type A (accounting for 14.2%). The result correspond to the reports of the Department of Animal Health on the occurrence and types of FMD viruses on the buffaloes and cows in Vietnam

in the two years of 2014, 2015, and OIE's update on the types of FMD viruses on buffaloes and cows in the world (2016).

3.2.2. Types of FMD Viruses from the Clinical Samples of Epithelium Tissue of Infected Buffaloes and Cows

In order to identify the types of FMD viruses in the area, 35 clinical samples of epithelium tissue, mouth, tongue, and the hoof gaps of buffaloes and cows with FMD in six district in Lang Son was collected in 2014 and 2015, using RT-PCR. The results are presented in Table 10.

Table 10. Types of FMD viruses from the clinical samples of epithelium tissue of infected buffaloes and cows.

No.	Areas (districts)	Result					
		No. of Samples collected	No. of positive samples	Rate (%)	Type O	Type A	Type Asia1
1	Dinh Lap	2	2	100	1	1	0
2	Van Lang	1	1	100	0	1	0
3	Chi Lang	2	2	100	1	1	0
4	Loc Binh	1	1	100	1	0	0
5	Bac Son	1	1	100	1	0	0
6	Van Quan	1	1	100	0	1	0
Total		8	8	100	4	4	0

The results show that all the eight epithelium tissue samples collected from the infected buffaloes and cows in the six districts with FMD outbreaks in the two years of 2014 and 2015 were found positive. In other words, the positive rate was 100%. Of which, half were positive with FMD virus type O, and the other half were positive with type A. None was found positive with type Asia1 virus. The result corresponds to that of Table 8 which identifies the types of FMD virus. This result was similar to that of [2, 3, 4, 12, 17, and 18].

3.3. Vaccines for the Buffaloes and Cows in Lang Son Province

3.3.1. Types of FMD Vaccines for the Buffaloes and Cows

Based on the types of FMD virus found above, and the capacity of vaccine provision in Vietnam, recommendation on the suitable types of FMD vaccines was made to the Animal Health Sub-Department of the province so as they could vaccinate their cattles accordingly. The results are presented in table 11.

Table 11. Vaccination of the buffaloes and cows in Lang Son.

Districts	Types of virus found			Type of vaccines recommended
	Type O	Type A	Type Asia 1	
Van Lang	1	1	0	Bivalent vaccine types O, A
Chi Lang	6	3	0	Bivalent vaccine types O, A
Bac Son	1	1	0	Bivalent vaccine types O, A
Binh Gia	1	1	0	Bivalent vaccine types O, A
Van Quan	8	2	0	Bivalent vaccine types O, A

Table 11 shows that the local Animal Health Sub-Department selected Aftovax Bivalent vaccine types O and A for the buffaloes and cows in five districts of Van Lang, Chi Lang, Bac Son, Binh Gia and Van Quan. The Bivalent vaccine types O và A meet the required scientific criteria, suitable to the condition of the local areas and the instruction of the Department of Animal Health of Vietnam. This finding was similar to that of [17, 18].

3.3.2. FMD Vaccination for the Buffaloes and Cows

A retrospective study on FMD vaccination for the buffaloes and cows in Lang Son province in the years 2011, 2012, 2013, 2014 and 2015. The results are presented in table 12.

Table 12. FMD vaccination for the buffaloes and cows in Lang Son province from 2011 to 2015.

Year	No. planned for vaccination	No. vaccinated	Rate of implementation vs planning (%)
2011	202,700	78,018	38.49
2012	204,000	164,079	80.43
2013	165,458	162,789	98.34
2014	164,200	134,685	82.02
2015	165,800	161,657	97.50

Table 12 shows that the vaccination of the buffaloes and cows in Lang Son met the technological requirements on the proportion of the herds being vaccinated i.e. from 80.43% to 97.50%, except for the year 2011 when the rate of vaccinated cattle was low (38.49%).

3.4. Immune Response and Duration of the Buffaloes and Cows in Lang Son Province

In order to assess the level of immune response and duration of the buffaloes and cows after vaccination (Aftovax Bivalent, 2 types O and A produced by Merial - France), blood samples of the buffaloes and cows were collected at days 30, 60, 120 and 180 after being vaccinated in three areas previously experienced FMD outbreaks with the following variations: (i) one shot for the cattle living in the areas previously affected by FMD outbreaks and for those which had been vaccinated six months before; and (ii) two shots for the ones which had never been vaccinated before. The samples were then examined in the laboratory for antibody titer assessment using ELISA method. The results are shown in Table 13.

Table 13. Protection for the buffaloes and cows injected with FMD vaccines.

Areas	Samples taken at No. of days after vaccination	No. of samples examined	Sample collection date	No. of samples (+)	Positive rate (%)	No. of samples protected	Rate of protection (%)
Bac Son	30	29	29 May 2015	29	100	28	96.5
Van Lang	30	30	01 Jun 2015	30	100	30	100
Lang Son City	30	30	02 Jun 2015	30	100	26	86.6
Bac Son	60	29	30 Jun 2015	29	100	25	86.2
Van Lang	60	30	02 Jul 2015	30	100	26	86.6
Lang Son City	60	30	03 Jul 2015	30	100	25	83.3
Bac Son	120	29	30 Aug 2015	29	100	22	75.8
Van Lang	120	30	05 Sept 2015	30	100	22	73.3
Lang Son City	120	30	06 Sept 2015	30	100	21	70.0
Bac Son	180	29	30 Nov 2015	18	62.0	8	44.4
Van Lang	180	30	02 Dec 2015	21	70.0	9	42.8
Lang Son City	180	30	03 Dec 2015	20	66.6	6	30.0

According to the regulations of the Department of Animal Health of Vietnam, it is required that vaccination of FMD vaccines be done in accordance with the instruction document No. 752/TY-DT issued by the Department on 16 June 2006, and the instruction of the vaccine providers. Thus, for the buffaloes and cows which had never been vaccinated before, it was required that after 28 days after the first shot, the second shot be done with the same type of vaccine. After that, it is necessary to repeat the shots every six months to ensure immunity. The number of buffaloes and cows vaccinated had to be at least 80% of the total herd.

Table 13 shows that 100% of the vaccinated buffaloes and cows had immunity response. The rate of protection was from 86.6% to 100% 30 days after vaccination, from 83.3% to 86.6% 60 days after vaccination; 120 days after vaccination, the rate of protection seemed to be decrease, with protection rate accounted for 70% to 75.8%; 180 days after vaccination, the rate of positive samples (with antibody titer against the types O, A of FMD viruses) only 62% to 70% of the herd are protected. The rate of protection decreased dramatically, with only 30% to 44.4%. The results were similar to the technical criteria issued by the

Department of Animal Health of Vietnam. In order to ensure protection for the cattle, six months after the first shot, the cattle had to be injected with the second shot of FMD vaccines. This result was similar to that of [8, 16].

4. Conclusion

FMD on the buffaloes and cows in Lang Son occurred repeatedly in 11 districts and towns in Lang Son province from 2011 to 2015. With the total of 187 outbreaks, on average, there were 3.4 outbreaks per year in each district or town.

FMD in Lang Son occurred irregularly. In some districts, there might be FMD outbreaks in two or three consecutive years (E.g. Binh Gia, Van Quan) while in some others, it occurred only once (e.g. Cao Loc district and Lang Son city).

In total, the buffaloes and cows with FMD accounted for 1.26%/year, with buffaloes accounted for 1.39%/year, and cows 0.75%/year. FMD on the buffaloes and cows in Lang Son was under the influence of (i) the weather and climate conditions (especially the years with extremely cold winter), (ii) the seasonal agricultural practice in the locality (during the time of the year when the cattle were used for ploughing and pulling carts for the crops, their health was relatively declined), and (iii) the results of vaccination in early Autumn – Winter season (the smaller the number of cattle vaccinated, the higher the number of cattle infected). These influences were clearly observed in 2011 with the highest number of cattle infected (8,929 infected cattle, accounting for 5.3% of the herds, whereas the average rate of infection in the whole five studied years was 1.26%).

In Lang Son, the sources of FMD were identified as mainly came from the infected cattle which had been medically treated and clear of the symptoms, yet still carried and shed the virus to the environment.

On average, the rate of natural virus infection on the cattle in Lang Son was 33.23%. Of which, the rate was highest in the following districts: Bac Son (66.66%), Van Quan (50%), Trang Dinh (32.88%) and Binh Gia (28.57%).

The virus causing FMD on the cattle in Lang Son belong to type O and type A. While type Asia 1 virus had occurred in some other provinces in Vietnam, it was not found in Lang Son. Thus, the suitable vaccine for the cattle in Lang Son would be Aftovax Bivalent, with 2 types of O and A.

After being injected with FMD vaccine, 100% of the cattle had immune response. 30 days after vaccination, the rate of protection varied from 86.6% to 100%. Six months after the first vaccination shot, a second one must be done for the vaccination to be effective.

Acknowledgments

We would like to send out special thanks to OIE and OIE Bangkok for their financial and technical supports, and the help of the Department of Animal Health of Vietnam, Sub-Department of Animal Health in Lang Son, and colleagues who helped to make this study possible.

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