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Original Article

Seroprevalence of Foot and Mouth Disease Virus (FMDV) and associated risk factors in unvaccinated sheep and goats in Pyawbwe and Meikhtila townships of Myanmar

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ABSTRACT

Objective: In this study, a serological survey was conducted in unvaccinated sheep and goat populations at Pyawbwe and Meikhtila townships of Mandalay region in Myanmar to determine the seroprevalence and associated risk factors of foot and mouth disease (FMD).

Materials and methods: A total of 110 sheep and 107 goat sera samples were randomly collected from Pyawbwe. Similarly, 108 sheep and 109 goat sera were collected from Meikhtila. All samples were tested for the presence of non-structural protein (NSP) specific antibodies to FMD virus (FMDV) by Ceditest FMDV-NSP Enzyme-lined Immunosorbent Assay (ELISA), and were confirmed by Liquid Phase Blocking ELISA (LPB ELISA) .

Results: Overall seroprevalence was 42.4%(n=184/434) by Ceditest-NSP ELISA, and 46.8%(n=203/434) by LPB ELISA against FMDV serotype O. The presence of antibodies against FMDV serotype O was higher ($P<0.01$) as compared to those of serotype A and Asia-1. The seroprevalence in Meikhtila (49.77%) was higher ($P<0.01$) than that of Pyawbwe (35.2%). The seropositivity in sheep and goats that were in-contact (77.19%) with infected cattle and pigs was higher ($P<0.01$) as compared to those in-contact with non-infected animals (37.14%). Similarly, the seropositivity in sheep and goats from high animal trade areas (49.4%) was higher ($P<0.05$) than that of those from low animal trade areas (37.97%).

Conclusion: Rearing of sheep and goats in-contact with FMDV-infected cattle and pigs, and high animal trading areas are the major associated risk factors for FMDV infection for sheep and goats in the study areas in Myanmar.

KEYWORDS

Ceditest ELISA; ELISA; FMD; FMDV; Risk factor; Seroprevalence

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INTRODUCTION

Foot and mouth disease (FMD) is one of the most economically important diseases of cloven foot animals. FMD is caused by FMD virus (FMDV) belonging to the genus Aphthovirus, which has seven serotypes namely FMD serotype A, O, C, SAT 1, SAT 2, SAT 3 and Asia 1 (Rweyemamu et al., 2008). As of 2002, the OIE reported FMD to be endemic in 7 out of the 10 South East Asian countries including Myanmar (Leforban and Gerbier, 2002). Serotype O was reported to be the most predominant type associated with outbreaks in all the endemic countries (Olabode et al., 2014; Alam et al., 2015; Chowdhury et al., 2015; El-Khabaz and Al-Hosary, 2017). FMD serotypes O and Asia 1 were the most reported in Myanmar (Gleeson, 2002). FMDV rapidly replicates and spreads from infected animals to in-contact susceptible animals through aerosol. There are many examples of FMDV outbreaks resulting from importation of the disease into previously disease-free countries through movement of infected sheep and goats. In 1983, FMD was spreaded from Spain to Morocco by infected sheep. In 1989, FMD was introduced into Tunisia by infected sheep. Similarly, sheep served as carriers of the virus from Turkey to Greece and Bulgaria in 1994 and 1993, respectively (Kitching and Hughes, 2002). Recently, Japan and South Korea, which have been free from FMD without vaccination, reported cases of FMD outbreaks associated with serotypes O and A, which were believed to be imported from South East Asian countries (Knowles et al., 2012).

Small ruminants such as sheep and goats play an important role in transmission and epidemiology of FMD as they rarely show typical clinical signs of the disease; thus, these animals are assumed to act as carriers. Infected herds or nomadic farmers can spread the infection to other herds. Shipping or trading of live sheep and goats, a common phenomenon worldwide, is another way of spreading the virus to non-endemic regions (Ganter et al., 2001; Di Nardo et al., 2011).

The animals sufferging from sub-clinical infections may disseminate the disease when come in contact with susceptible livestock. The risk of introduction of sub-clinical FMD into FMD-free countries may significantly increase the disease incidence in susceptible large ruminants (Sutmoller and Casas, 2002). The Malaysia, Thailand, Myanmar campaign for FMD freedom was initiated in 2003 with the goal of eradicating the virus from the respective countries. However, up to date, the virus is still endemic in these countries primarily due to its persistence in Myanmar, which has the highest population of cattle in the region (Smith, 2012).

It is a known fact that FMD is endemic in Myanmar (Smith, 2012). However, there are few studies on the role of unvaccinated in-contact farm animals in propagation of FMDV and its associated risk exposure. This study was thus designed to determine the seroprevalence and associated risk factors of FMDV in unvaccinated small ruminants in two Myanmar townships; Pyawbwe and Meikhtila.

MATERIALS AND METHODS

Pyawbwe (20.5833°N, 96.0667°E; https://goo.gl/maps/km361H4GXhH2) and Meikhtila (20.8833°N, 95.8833°E; https://goo.gl/maps/Wrmr7njG9QG2) townships located at Mandalay Region in Myanmar were selected for this study. These two townships were selected on the basis of their geographical location, proximity to livestock market and socio-economic status. From each township, 8 villages were randomly selected as a herd, while small ruminants (sheep and goats) were randomly selected to be sampled. Accordingly, 16 herds comprising of 218 sheep and 216 goats were included in this study. All the animals used in this study were unvaccinated. Sampling was done from November to April representing the cold and dry seasons.

Ethical consideration: The approval for the study was granted by the Ministry of Livestock and Fisheries, Livestock Breeding and Veterinary Department and University of Veterinary Science, Yezin, Myanmar (No: UVS-12-0457). Blood samples were collected from the animals with prior consent from the owners.

Study design: A cross-sectional survey was conducted for determination of FMDV seroprevalence in sheep and goats. In order to estimate the sample size to evaluate the presence of FMDV in these townships, the following formula, as mentioned by Thrusfield (1995a), was used. The animals of one village were considered as one herd. An expected prevalence of 15% with a confidence level of 95% was used in this unit (**Table 1**).

$$n = \{1- (1- \alpha)^{1/d}\} \{N - d/2\} + 1$$

Where,

'N' is the population size

'd' is the number of affected animals in the population

'n' is the required sample size

'α' is the desired confidence level (probability of finding at least one case in the sample)

Serum sample collection: Five to seven milliliters of blood per animal was collected from the jugular vein aseptically. The blood samples were kept to clot in cold boxes with ice and 1.5-2 mL of serum was decanted into sterile cryovials, and was transported to FMD Section,

Table 1: Calculated sample size for each flock

No.	Townships	Villages	Sheep	Goats	Sheep samples	Goat samples
1.	Pyawbwe	Kintar	352	62	14	12
		Yelekway	620	759	14	14
		Phwathinn	300	681	14	14
		Htantawgyi	890	1460	14	14
		Yonkone	580	114	14	13
		Kontkokhahla	100	720	13	14
		Yonekone	668	170	14	13
		Twinywa	167	200	13	13
2.	Meikhtila	Montai	790	2650	14	14
		Monpin	63	1970	12	14
		Kyaukphyukone	59	61	12	12
		Laitaw	860	2960	14	14
		Nyaungpintha	620	1350	14	14
		Htahattann	610	920	14	14
		Thayatpin	1450	730	14	14
		Tapyaw	1513	200	14	13
		Total	9642	15007	218	216

LBVD, Myanmar for serological diagnosis. Sera were centrifuged at 2500 revolutions per minute (rpm) for 20 min. The clear sera were stored at -20°C until serological tests were carried out. The serum samples were then tested by using Ceditest FMDV-NSP ELISA, and were confirmed by using LPB ELISA at the Regional Reference Laboratory for FMD in Southeast Asia, Pakchong, Thailand.

Detection of infected cattle and pigs: In-contact animals infected with FMD were diagnosed based on classical FMD lesions which included sores and ulcers in the mouth and foot.

Laboratory analysis of FMDV: The Ceditest FMDV-NSP ELISA detects antibodies directed against the non-structural 3ABC protein of FMDV. This ELISA kit detects FMDV infected animals independent of the serotype that causes the infection and independent of the fact that the animal is vaccinated or not. However, since there is no history of vaccination in the animals sampled, a negative test was considered as an absence of FMDV infection. All the ELISA were carried out according to manufacturer's instruction.

Equation 1: Formula for calculation of Percentage Inhibition (PI)

$$PI = 100 - \left[\frac{OD_{450}\ \text{test sample}}{OD_{450}\ \text{max}}\right] x\ 100$$

Interpretation of the percentage inhibition

PI = <50% means negative, or no antibodies against the NS protein of FMDV.
PI = ≥50% means positive or presence of antibodies against the NS protein of FMDV was detected.

Liquid phase blocking ELISA (LPB-ELISA): The results were read by a spectrophotometer at wavelength of 450 nm and PI was calculated. The PI for control and test samples were calculated according to equation 2 and 3, respectively. The cut-off PI for each serotype was ≥40.

Equation 2: Formula for calculation of PI on the control and quality assurance acceptance

$$PI = \frac{100 - (\text{Replicate OD of Control antigen} \times 100)}{\text{Median OD of Ca}}$$

Ca = control antigen

Equation 3: Formula for calculation of PI on the test sera and quality assurance acceptance

$$PI = \frac{100 - (\text{Replicate OD of test serum} \times 100)}{\text{Median OD of Ca}}$$

Ca = control antigen

Data Analysis: The questionnaires and laboratory results were analyzed using SPSS (ver. 17.0). The level of agreement between ELISA tests were analyzed by *kappa* statistic (Thrusfield, 1995b). Finally, logistic regression (SPSS) was used to determine risk factors associated with seropositivity of the disease in the study area.

Risk factor analysis: For each individual flock, pre-tested questionnaire surveys were conducted for assessment of the associated risk factors through calculation of the relative risk (RR), as described by Thrusfield (1995c).

Relative risk (RR)
= Risk in exposed / Risk in non-exposed
= (a / a + b) / (c / c + d)

Interpretation:
<1 indicates positive or protection
=1 is no association between outcomes
>1 indicates negative or increased risk

RESULTS

Seroprevalence: In this experiment, a total of 434 unvaccinated sheep and goats sera samples from two Myanmar townships were collected and tested for FMDV; 110 sheep and 107 goats from Pyawbwe and 108 sheep and 109 goats from Meikhtila, respectively. The overall FMDV seroprevalence was 42.4%(n=184/434) by Ceditest-NSP ELISA, and 46.8% (n=203/434) by LPB ELISA against FMDV serotype O. Anti-FMDV antibody positive sera detected by Ceditest-NSP ELISA was 38.2%(42) in sheep and 31.8%(34) in goats in Pyawbwe, and 45.4%(49) in sheep and 54.1%(59) in goats in Meikhtila. The antibody titers of anti-FMDV were significantly higher for serotype O than serotype A and Asia-1 in all positive sera by LPB ELISA. Seroprevalence of sheep and goats in Meikhtila was significantly higher (P<0.01) than that of Pyawbwe (**Table 2**).

Risk analysis: Seropositive sheep and goat in-contact with infected cattle and pig and those in-contact with non-infected cattle and pig detected by Ceditest FMDV-NSP ELISA are shown in **Table 3**. Seropositivity of sheep and goat in-contact with infected cattle and pig were significantly higher (P<0.01) than those in-contact with non-infected cattle and pig. Rearing of sheep and goats together with cattle and pig also increased the risk of FMD because the values of relative risk were more than 1 in all tests.

Relative risk (RR)
= Risk in exposed / Risk in non-exposed
= (a / a + b) / (c / c + d)
= (44/ 57) / (140/ 377)
= 2.1

Seropositive sheep and goats in the high animal trade area and those in low animal trade areas detected by Ceditest FMDV-NSP ELISA are shown in **Table 4**. The seropositivity of sheep and goats in high animal trade area was significantly higher (P<0.05) than those in low animal trade areas detected by Ceditest FMDV-NSP. High animal trade in the villages was also an associated risk factor because the value of RR was more than 1 with Ceditest FMDV-NSP.

Relative risk (RR)
= Risk in exposed / Risk in non-exposed
= (a / a + b) / (c / c + d)
= (83/ 168) / (101/ 266)
= 1.3

Logistic regression: Logistic regression was used to confirm the associated risk factors for FMDV infection. Rearing of small ruminants in-contact with FMD-infected cattle and pig in the mixed system and high animal trade areas were identified as associated risk factors for FMD infection in sheep and goats in the study areas (**Table 5**). The agreement between the ELISA systems used in this experiment was determined by analysis of *Kappa* value (Thrusfield, 1995c). There was a substantial agreement between Ceditest FMDV-NSP and LPB ELISAs (*Kappa* value = 0.753).

DISCUSSION

In sheep and goats, FMD is generally mild and can be difficult to distinguish from other common health conditions (Donaldson and Sellers, 2000). After clinical recovery from the disease, some sheep and goats carry

Table 2: Seropositive sheep and goats in Pyawbwe and Meikhtila tested by Ceditest FMDV-NS ELISA

Species	Ceditest-NSP results		Total	Seropositivity (%)	*Chi*-square test	
	Positive	Negative			Calculated Value	Significance level
Sheep and goats (Pyawbwe)	76	141	217	35.02	9.661	P<0.01
Sheep and goats (Meikhtila)	108	109	217	49.77		
Total	184	250	434			

Table 3: Seropositive sheep and goats in-contact with infected cattle and pig and those in-contact with non-infected cattle and pig tested by Ceditest FMDV-NS ELISA

Status of sheep and goats	Ceditest-NSP results		Total	Seropositivity (%)	Chi-square test	
	Positive	Negative			Calculated value	Significance level
In-contact with infected animals	44	13	57	77.19	32.533	P<0.01
In-contact with non-infected animals	140	237	377	37.14		
Total	184	250	434			

Table 4: Seropositive sheep and goat in high animal trade area and those in low animal trade area tested by Ceditest FMDV-NS ELISA

Animal trade	Ceditest-NSP results		Total	Seropositivity (%)	*Chi*-square test	
	Positive	Negative			Calculated value	Significance level
High	83	85	168	49.40	5.513	$P<0.05$
Low	101	165	266	37.97		
Total	184	250	434			

Table 5: Level of risk factors that influence FMD infection in sheep and goat in Pyawbwe and Meikhtila townships

Variables	Score	df	Sig.
Outbreak in farm	2.049	1	.152
Mixed farming	1.091	1	.296
Infected in mixed animal	34.912	1	.000
Animal origin (own and buy)	.231	1	.630
Animal movement	1.616	1	.204
Animal trade	5.732	1	.017
Distance from live market	.548	1	.459
Usage of disinfectant	.169	1	.681
Overall statistics	51.536	8	.000

the virus for as long as 9 months and 4 months, respectively in the mucous membrane of the esophagus, pharynx and tonsils (Jensen and Swift, 1982; Leforban, 1999). Thus, animals which are positive from the ELISA tests are considered to be naturally infected animals that have sub-clinical infection of FMDV. In this study, a Ceditest FMDV-NSP ELISA test kit was used for detection of specific antibody to NSP of FMDV in sheep and goats. The detection of antibody against the 3ABC (Non-structural protein) of FMDV is a useful indicator of FMD virus infection with any of the seven serotypes of the FMDV (Mackay et al., 1998).

Ceditest FMDV-NSP has a higher sensitivity and specificity (more than 98%) than other NSP ELISA kits such as CHEKIT FMD-3ABC and 3ABC-ELISA (Niedbalski, 2004). The LPB ELISA detects and quantifies FMDV antibodies for serotyping in serum of both infected and vaccinated animals (Hamblin et al., 1986). The antibodies detected by LPB ELISA in this experiment was due to natural infection because there was no history of vaccination in sheep and goats in the study areas.

Sheep and goats are more populated in the central part of Myanmar such as Mandalay, Magwe and Sagaing divisions as compared to other parts of the country. The selected two townships (Pyawbwe and Meikhtila) are among those with the highest population of sheep and goats in the Mandalay division. In this study, the seroprevalence of FMDV in sheep and goats in Meikhtila was found significantly higher ($P<0.01$) than that of Pyawbwe. This might be attributed to the high animal population density in Meikhtila as compared to Pyawbwe. Meikhtila also has more animal population and a very famous live ruminant market, which allow the mixing of different species of animal and disease transmission. Thus, it can be speculated that there are more chances of animals being infected with FMDV in Meikhtila township as compared to Pyawbwe. According to these geographical and economic conditions, it can be assumed that Meikhtila is more FMD risk prone area than Pyawbwe, and this fact might increase the occurrence of FMD within this area.

Based on the questionnaire survey, all sheep and goats sampled in Pyawbwe and Meikhtila townships were not vaccinated before, and were grazed on common pasture together with cattle. This seemed to be the most important risk factor for FMDV seropositivity in sheep and goats from these areas. It has been indicated that transmission of FMDV most commonly occurs by close contact between acutely infected and susceptible animals, often following the movement of infected animals (Donaldson and Alexandersen, 2001). Potential contamination of fomites and feedstuffs, including concentrates, hay and straw, by saliva, feces and urine, are considered as responsible for a certain amount of its spread (Parker, 1971).

According to the risk factor analysis by *Chi*-square method, the seropositivity of sheep and goats in-contact with infected cattle and pigs was significantly higher ($P<0.01$) than that of those in-contact with non-infected cattle and pigs. This indicates that rearing of sheep and goats together with FMDV infected cattle is a major associated risk factor for getting an FMD infection in these animals. Similarly, the high seropositivity of sheep and goats from a high animal trade area than that of those from low animal trade areas suggests transportation and trading of FMDV infected animals among villages to be a risk factor for infection and spread of FMDV. In essence, sheep and goats become carriers and present as reservoirs for the spread of FMDV, thus becoming a major risk factor in the trade of live sheep and goats to disease-free countries (Barnett and Cox, 1999). Uppal (2009) discussed the relevance of small ruminants in the

control of FMD in endemic areas. Nevertheless, most of the current control strategies of FMDV in endemic countries do not include small ruminants.

Since the antibody against NSP only exists in field infections, the antibodies detected in sheep and goats in this study was due to natural infection with FMDV in the study area. In both Pyawbwe and Meikhtila townships, according to the questionnaire survey, there was no record of clinical manifestation resembling FMD in sheep and goats. Therefore, the results of this study indicated the presence of sub-clinical FMD infection in sheep and goats in Pyawbwe and Meikhtila townships.

CONCLUSION

FMDV is endemic in sheep and goats populations in Pyawbwe and Meikhtila townships**,** Myanmar, but its prevalence and associated risk factors have been under-reported. This study shows that presence of animal trade markets and raring of small ruminants in-contact with cattle and pigs are strong associated rick factors for higher seropositivity against FMDV in goats and sheep.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTION

All authors contributed equally.

REFERENCES

1. Alam MA, Rahman M, Hossen ML, Ahmed S, Parvej MS, Khan MFR, Rahman MB (2015). Reverse transcription polymerase chain reaction (RT-PCR) based detection and serotyping of FMD Virus from field samples of Gazipur, Bangladesh, and adaptation of the virus in BHK-21 cell. Journal of Advanced Veterinary and Animal Research, 2(3): 291-295. https://dx.doi.org/10.5455/javar.2015.b88
2. Barnett PV, Cox SJ (1999). The role of small ruminants in the epidemiology and transmission of foot-and-mouth disease. Veterinary Journal, 158(1): 6-13. https://doi.org/10.1053/tvjl.1998.0338
3. Chowdhury MMR, Hossen ML, Amin KB, Nazir KHMNH, Ahmed S, Rahman M, Khan MFR, Rahman MT, Rahman MB (2015). Assessment of immune response in cattle against experimentally prepared trivalent (O, A, and Asia-1) FMD vaccine in Bangladesh. Journal of Advanced Veterinary and Animal Research, 2(4): 475-480. https://dx.doi.org/10.5455/javar.2015.b121
4. Di Nardo A, Knowles N, & Paton DJ (2011). Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. Revue scientifique et technique, 30(1): 63-85. https://doi.org/10.20506/rst.30.1.2022
5. Donaldson AI, Alexandersen S (2001). Relative resistance of pigs to infection by natural aerosols of FMD virus. Veterinary Record, 148: 600-602. https://doi.org/10.1136/vr.148.19.600
6. Donaldson AI, Sellers RF (2000). Foot-and-mouth disease. In: Diseases of sheep. (Eds. Martin W. B. and I. D. Aitken). Blackwell Science, Oxford, United Kingdom; pp 254-258.
7. El-Khabaz KAS, Al-Hosary AAT (2017). Detection and identification of Foot and Mouth disease virus serotypes in Assiut governorate, Egypt. Journal of Advanced Veterinary and Animal Research, 4(1): 32-38. https://doi.org/10.5455/javar.2017.d186
8. Ganter M, Graunke WD, Steng G and Worbes H (2001). Foot and mouth disease in sheep and goats. Deutsche Tierärztliche Wochenschrift, 108(12): 499-503.
9. Gleeson LJ (2002). A review of the status of foot and mouth disease in South-East Asia and approaches to control and eradication. Scientific and Technical Review of the Office International des Epizooties, 21(3): 465-472. https://doi.org/10.20506/rst.21.3.1346
10. Hamblin C, Barnett I and Crowther JR (1986). A new enzyme linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouth disease virus. Journal of Immunological Methods, 93: 123-129. https://doi.org/10.1016/0022-1759(86)90442-4
11. Jensen R, Swift BL (1982). Foot-and-Mouth Disease. In: Diseases of Sheep, 2nd Edn. (Edn. Jensen R and

Swift BL). Lea and Febiger, Philadelphia, USA; pp 182-185.
12. Kitching RP, Hughes GJ (2002). Clinical variation in foot and mouth disease: sheep and goats. Scientific and Technical Review of the Office International des Epizooties. 21(3): 505-512. https://doi.org/10.20506/rst.21.3.1342
13. Knowles NJ, He J, Shang Y, Wadsworth J, Valdazo-González B, Onosato H, Kim SM (2012). Southeast Asian foot-and-mouth disease viruses in Eastern Asia. Emerging Infectious Diseases, 18(3): 499-501. https://doi.org/10.3201/eid1803.110908
14. Leforban Y (1999). Prevention measures against foot-and-mouth disease in Europe in recent years. Vaccine, 17: 1755-1759. https://doi.org/10.1016/S0264-410X(98)00445-9
15. Leforban Y, Gerbier G (2002). Review of the status of foot and mouth disease and approach to control/eradication in Europe and Central Asia. Scientific and Technical Review of the Office International des Epizooties, 21(3): 477-489. https://doi.org/10.20506/rst.21.3.1345
16. Mackay DK, Forsyth MA, Davies PR, Berlinzani A, Belsham GJ, Flint M and Ryan MD (1998). Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA. Vaccine, 16: 446-459. https://doi.org/10.1016/S0264-410X(97)00227-2
17. Niedbalski W (2004). Comparison of three ELISA kits for the detection of antibodies against foot-and-mouth disease virus non-structural proteins. Bulletin of Veterinary Institute of Pulawy, 49: 147-151.
18. Olabode HO, Kazeem HM, Raji MA, Ibrahim ND, Nafarnda WD (2014). Geo-spatial distribution of serologically detected bovine Foot and Mouth Disease (FMD) serotype outbreaks in Ilesha Baruba, Kwara. Journal of Advanced Veterinary and Animal Research, 1(3): 94-99. https://dx.doi.org/10.5455/javar.2014.a20
19. Parker J (1971). Presence and inactivation of foot-and-mouth disease virus in animal feces. Veterinary Record, 88: 659-662. https://doi.org/10.1136/vr.88.25.659
20. Rweyemamu M, Roeder P, Mackay D, Sumption K, Brownlie J, Leforban Y, Saraiva V (2008). Epidemiological Patterns of Foot-and-Mouth Disease Worldwide. Transboundary and Emerging Diseases, *55*(1): 57-72. https://doi.org/10.1111/j.1865-1682.2007.01013.x
21. Smith P (2012). Epidemiological and risk-based approaches to accelerating achievement of foot and mouth disease free-zone in the Malaysia-Thailand-Myanmar peninsula (Doctoral Dissertation, Murdoch University).
22. Sutmoller P, Casas OR (2002). Unapparent foot and mouth disease infection (sub-clinical infections and carriers): implications for control. Scientific and Technical Review of the Office International des Epizooties, 21(3): 519-529. https://doi.org/10.20506/rst.21.3.1366
23. Thrusfield M (1995a). Estimation of disease prevalence. Chapter 13. Surveys. In: Veterinary Epidemiology. 2nd Edn. (Edn. Thrusfield M). Blackwell Science; pp 178-198.
24. Thrusfield M (1995b). Agreement between tests: the kappa statistic. Chapter 17. Diagnostic testing. In: Veterinary Epidemiology. 2nd Edn. (Edn. Thrusfield M). Blackwell Science; pp 266-285.
25. Thrusfield M (1995c). Measures of association. Chapter 15. Observational studies. In: Veterinary Epidemiology. 2nd Edn. (Edn. Thrusfield M). Blackwell Science; pp 220-255.
26. Uppal PK (2009). Foot-and-Mouth disease in small ruminants–an issue of concern. Food and Agricultural Organization, Appendix, 26: 192-196.

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