East African Medical Journal Vol. 92 No. 11 November 2015
COMPARATIVE PERFORMANCE OF ANIGEN © FMD NSP AB ELISA (KOREA), NSP PRIOCHECK® KIT, LIQUID PHASE BLOCKING ELISA KITS AND VIRUS NEUTRALISATION TESTS IN THE DETECTION OF FOOT AND MOUTH DISEASE VIRUS ANTIBODIES IN ANIMALS SERA FROM KILIFI COUNTY, KENYA
N. K. Ruuri, Institute of Tropical Medicine and Infectious Diseases (ITROMID), P.O. Box 62000-00200, Nairobi, A. W. T. Muigai, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, R. A. Lwembe, Institute of Tropical Medicine and Infectious Diseases (ITROMID), P.O. Box 62000-00200, Nairobi and Kenya Medical Research Institute, Center for Virus Research, P.O. Box 54628-00200, Nairobi and A. K. Sangula, Institute of Tropical Medicine and Infectious Diseases (ITROMID), P.O. Box 62000-00200, Nairobi, FMD National reference lab, Ministry of Agriculture, Livestock and Fisheries, P.O. Box 18021-00500, Industrial Area, Nairobi

Request for reprints to: N. K. Ruuri, Institute of Tropical Medicine and Infectious Diseases (ITROMID), P.O. Box 62000-00200, Nairobi, Kenya, Email: kiriinyanicholas@gmail.com

COMPARATIVE PERFORMANCE OF ANIGEN © FMD NSP AB ELISA (KOREA), NSP PRIOCHECK® KIT, LIQUID PHASE BLOCKING ELISA KITS AND VIRUS NEUTRALISATION TESTS IN THE DETECTION OF FOOT AND MOUTH DISEASE VIRUS ANTIBODIES IN ANIMALS SERA FROM KILIFI COUNTY, KENYA

N. K. RUURI, A. W. T. MUIGAI, R. A. LWEMBE and A. K. SANGULA

ABSTRACT

***Background*: Foot and mouth disease (FMD) is a highly contagious viral infection of hoof cloven animals. Performance of serological tests used for FMD is faced with challenges like low test sensitivity, low tests agreement levels, in concise test cut-offs, cost of the tests and numerous FMD serotypes which may lead to misdiagnosis.**
***Objective*: To determine the sensitivity of assays Anigen © FMD NSP Ab ELISA –Korea, NSP Priocheck® kits with Liquid Phase Blocking Enzyme Linked Immunosorbent Assay compared with Virus neutralisation test (the gold standard).**
***Design*: A cross-sectional study design.**
***Setting*: Kilifi County.**
***Subjects*: Four hundred and twenty one cattle.**
***Results*: The results showed that both Anigen © FMD NSP Ab ELISA (Korea), NSP Priocheck® Netherlands assays had low sensitivity but high specificity. Liquid Phase Blocking ELI SA had the highest sensitivity 100%. LPBE had a Positive Predictive Value (PPV) =100%, Anigen © FMD NSP Ab ELISA–Korea 94.44% and NSP Priocheck® Netherlands had the lowest 44.74% compared with the VNT 100%. The Cohen's kappa coefficient showed a perfect agreement level of 90%, 93%, 100 and 100% respectively.**
***Conclusion*: This study showed no significant difference in sensitivity and both structural and non-structural proteins tests should always be used concurrently.**

INTRODUCTION

Foot and mouth disease (FMD) virus remains a major livestock burden worldwide. FMD outbreaks has lead to a cost of 5 billion to almost 10 million US dollars in Uruguay, Japan, UK, Taiwan, Rep and Korea in the year 2011 and 2012, where there were ten reported outbreaks in FMD free areas (1). It was estimated that 32 million livestock units (LSU) are affected by FMD in a year, although the figure could be between 28 and 79 million (2,3). Foot and mouth disease sero-prevalence in cattle in Kenya was reported in 2013 to be 52.5% (14). Prevalence of foot and mouth disease virus (FMDV) across the world keeps on changing in different countries leading to difficulties in the diagnosis, management and control of FMD. Prevalence rates of FMDV are dropping in some countries in the world due to more awareness of infected animal products, vaccination campaigns with a vaccine which contains O, A, SAT1 and SAT2 serotypes and more frequent surveillance programmes. Several research studies have also shown more evidence supporting cattle movements, contacts of blood and animal products and export of infected meat and meat products as the leading risk factors for the spread of the virus. Most studies on FMDV prevalence use meat and its products to report the frequency of FMD. It is important to note, however, that using animal products screening information as the prevalence source may underestimate the real prevalence of the disease and the animals are generally a healthier population stratum (4). Diagnosis of FMD is difficult due to low test sensitivity, low tests agreement levels, in concise test cut-offs, cost of the

tests and present but unknown numerous sero types in some counties in Kenya leading to FMD misdiagnosis. This complicates surveillance purposes using serology technique. Anigen © FMD NSP Ab ELISA – Korea, NSP Priocheck® Netherlands kits and Liquid Phase Blocking Enzyme Linked Immunosorbent Assay may give different sensitivities (7). When false negative results are produced, it may lead to loss of livestock due to misdiagnosis. The tests may produce unspecified of range values which makes interpretation of the tests difficult hence mismatched agreements level. The variation of sensitivity and specificity of the tests may lead to wrong diagnosis of the disease. In order to develop and have confidence in vaccination and testing strategies, it is essential that NSPs, LPBE, and VNT should be validated using samples from Kenya as it may be different elsewhere. Commercially available genotyping assays are too expensive especially for developing countries, which are yet to fully embrace genotyping in management of FMD. These genotyping assays are considered expensive compared to current available platforms developed. This justifies reliability of serology assays and test kits for FMD control and management. The inability to detect occurring serotypes may results in more animal infection and failure to produce the right effective vaccine for specific serotypes.

Previously some of the authors compared two FMD 3ABC ELISAs using the VNT as the "gold-standard" (5), i.e. it was assumed that the Se and Sp of the VNT were both 100%. However, there might be reasons to question the justification of using the VNT as a "gold-standard". It appears, that reliable estimates of the duration of the different antibody responses in cattle are lacking. Thus, there might be justification in disregarding the VNT and compare the NSP tests to each other without assuming that the true status of the animal is known (i.e. the disease status is latent). The latent class approach does not assume any of the tests is a "gold standard". This paper follows on from previous evaluation of two NSP and one SP tests using a conventional approach with the VNT as a "gold-standard". The objective of the study was to determine the sensitivity and specificity of assays Anigen © FMD NSP Ab ELISA –Korea, NSP Priocheck® Netherlands kits with Liquid Phase Blocking Enzyme Linked Immunosorbent Assay compared with Virus neutralization test as the gold standard. The test properties were estimated for each of the three tests in a cattle population from kilifi county Kenya. The analysis used Cohen's kappa formulation for test evaluation in the presence of a "gold-standard" (6).

MATERIALS AND METHODS

Blood Specimens for this study were collected from cattle Kilifi County at the coastal region of Kenya where outbreaks of FMD frequently occur. Blood samples were collected from Bahari, Kaloleni, Ganze, Malindi and Magarini constituencies between March and September 2012. The sample size was calculated from the previous prevalence rate of 52.5% (14). Kilifi County is located on an estuary of River Rare that provides water for domestic and agricultural purposes. During this feeding time transmission may occur by direct or indirect contact (droplets). Farmers practice dairy farming which accounts to a significant quantity of milk consumed in Kilifi town and other towns such as Mombasa and Lamu hence animate vectors (humans, live animals & products etc.) are possible suggested method of FMDV transmission. Roads passing all the areas where livestock are kept are passed by inanimate vectors like vehicles when transporting goods and people to their destinations; hence they may carry the FMDV.

This was a cross-sectional study. The blood samples were collected in non EDTA Vacucontainer tubes to allow for serum collection. Blood sample collection bottles were labeled and stored in in a cool box where the samples were triple packaged. The samples were transported to the laboratory in Nairobi and stored in a deep freezer at –200C.In the FMD laboratory, four hudriend and twenty one blood samples were selected from One thousand six hundred and twenty four collected from Bahari, Kaloleni, Ganze, Malindi and Magarini constituencies using multistage process.

A total of four hundred and twenty one sera samples was selected for the study out of one thousand six hundred collected. The stored sera were removed from the freezer and was put on the bench to thaw. A multi stage sampling method was applied. At the first Stage, five Constituencies in the Kilifi County were selected to form clusters. At the second stage, samples were sorted according to the constituency of origin. At the third stage, serum was sampled from each cluster to represent the animals using systematic random sampling technique. At the fourth stage, the table of random numbers was used to select the first and then every Kth serum preservation bottle in the serial list until the sample size was realised.

Data analysis was done with SPSS version 16.0. Criteria for sensitivity, specificity and predictive values Reference / gold standard tests were used. Exact 95% confidence limits for binomial proportions were calculated from F-distribution. Kappa statistics (coefficient) and Chi-square test were employed for parameters analysis and calculating this extent for purposes of Comparing sensitivity agreement levels of the tests. Any value of 0.75 and above was established as excellent agreement, between 0.40 and 0.75 intermediate agreement and below 0.40 poor agreements. The samples were expressed as either negative or positive. The data was presented in frequency data tables.

Criteria for determining sensitivity, specificity and predictive values (Reference / gold standard test)
Sensitivity (SE) = True Positive / (True Positive +False Positive) x 100
Specificity (SP) = True Negative / (False Negative + True N) x 100
Positive predicative value (PPV) = True Positive / (True Positive +False Positive) x 100
Negative predicative value (NPV) = True Negative /(False Negative + True Negative) x 100

Cohen's kappa
Cohen's kappa was used to measure the agreement levels between AniGen © FMD NSP Ab ELISA – Korea, NSP Priocheck Netherlands, LPBE and VNT which each classify N items into C mutually exclusive categories (6).
The equation for K is:

$$k = \frac{Pr\,(a) - Pr\,(e)}{1 - Pr\,(e)}$$

Where Pr (a) is the relative observed agreement among tests assays, and Pr (e) is the hypothetical probability of chance agreement, using the observed data to calculate the probabilities of each test assay randomly saying each category. If the assay are in complete agreement then K = 1; where (K) is the agreement level. If there is no agreement among the test assays other than what would be expected by chance (as defined by Pr (e)), $\kappa = 0$.

Interpretation of the values
Test Sensitivity: The sensitivity of a test was considered valid if the test indicated that antibodies are present in serum. A sensitive test was considered positive when there was a color change in which case the virus antigens were considered present in serum and the disease as present. The AniGen© FMD NSP Ab ELISA - Korea and NSP priocheck Netherlands kits were considered efficient and they could pick serum with the antibody of FMD virus whenever the serotype specific test LPBE and VNT (gold standard) picked the antibody in the serum.

Test specificity: The specificity of the test was considered valid if the test indicates that antibodies are absent in serum. A sensitive test was considered negative when there was no color change and the virus was considered absent in serum and the disease as absent. The AniGen FMD NSP Ab ELISA - Korea and NSP priocheck Netherlands kits was considered efficient and they can peak serum with no antibody of FMD virus and if the specific test LPBE and VNT (gold standard) did not pick the antibody in the serum.

Positive predicative value: Where a "true positive" the event made the test makes a positive prediction, and the subject had a positive result under the gold standard, and a "false positive" is the event that the test will made a positive prediction, and the subject will had a negative result under the gold standard.

Negative predicative value: For a "true negative" the event took the test made a negative prediction, and the subject had a negative result under the gold standard, and a "false negative" is the event that the test made as a negative prediction, and the subject has a positive result under the gold standard.

Ethical considerations: Scientific and Ethical approvals were obtained from Kenya Medical Research Institute Scientific steering committee and ethical review committees. Authorisation was obtained from the officer in charge FMD national reference laboratory, Ministry of Agriculture, Livestock and Fisheries to access the serum samples. All procedures were carried out in accordance to FMD Bio safety guidelines and waste disposal.

RESULTS

The sensitivity of the Anigen © FMD NSP Ab ELISA – Korea, NSP priocheck© Netherlands, LPBE and virus neutralisation assays were identified. Four Serological tests were used. Anigen © FMD NSP Ab ELISA -Korea and NSP priocheck© Netherlands kits were used to detect viral nonstructural proteins (NSPs) antibodies while LPBE and virus neutralisation test (VNT) was used to detect serotype specific structures as Reference / gold standard test.

Table 1
Sensitivities and Specificity of NSP Ab ELISA - Korea against virus neutralization test

TEST		Positives	Negatives		Predictive values	Kappa stat
Screening test: NSP Ab ELISA –Korea	Positives	17(TP)	1(FP)	18	TP / (TP + FP) 94.44%	
	Negatives	15 (FN)	388 (TN)	403	TN / (FN + TN) 96.28%	90%
	TOTAL	Sensitivity: TP / (TP + FN) = 53.13%	Specificity: TN / (FP+TN)=99.74%	421		

T- True
P -positive
F-False
N-negative
Sensitivity= TP / (TP+FP) x 100
Specificity= TN / (FN+TN) x 100
Positive predicative value = TP / (TP+FP) x 100
Negative predicative value = TN / (FN+TN) x 100
Table 1 indicates sensitivity of NSP Ab ELISA Korea used as screening test. NSP Ab ELISA – Korea identified 17 serum samples to contain FMDV antibodies and 1 serum sample as negative. VNT indicated that 15 serum samples contained FMDV antibodies while in 388 antibodies were absent. However both assays agreed that 17(53.13%) serum were true positive and 388(99.74%) true negatives (specificity). 15(46.88%) were false negatives and 1 (0.028%) false positive. Analysis was done by screening test and confirmed by the gold standard. 94.44% was calculated as positive predictive value and 96.28% as negative predictive value. The large positive predictive value (PPV = 94.44%) and a negative predictive value of 96.28% indicates that NSP Ab ELISA Korea is highly sensitive assay. Then Cohen's kappa coefficient was used to measure the agreement level between NSP Ab ELISA Korea assay and VNT and the agreement level was 90%. This was a perfect agreement. The results show that NSP Ab ELISA Korea assay is a highly sensitive assay.

Table 2
Sensitivity and Specificity of NSP priocheck© Netherlands against virus neutralisation test

	VNT (gold standard test)				
Screening test: NSP Ab ELISA –Korea	Positives	Negatives		Predictive values	Kappa statistics
Positives	17(TP)	21(FP)	18	TP / (TP + FP) 44.74%	
Negatives	21 (FN)	380 (TN)	403	TN / (FN + TN) 94.76%	93%
TOTAL	Sensitivity: TP/ (TP+FN)=44.74%	Specificity: TN / (FP+TN) = 94.76%	421		

Table 2 show sensitivity of NSP priocheck© used as screening test. NSP Priocheck© – Netherlands identified 17 serum samples to contain FMDV antibodies and 21 as negative while VNT indicated that 21 serum samples contained FMDV antibodies and 380 not to have FMDV antibodies. The two tests identified on agreement that 17(44.74%) of serum were true positive and 388(96.28%) as true negatives. 4 (19.05%) were false negatives and 20 (54.05%) false positive. The samples were first subjected to screening test and confirmed by the VNT gold standard test.
17(44.74%) was calculated as positive Predictive values and 44.74% as negatives Predictive values.

The positive predictive value (PPV = 44.74%) indicates that NSP priocheck Netherlands is a medium sensitive assay but a negative predictive value of 94.76% indicate that it is a high specificity assay. Cohen's kappa coefficient was used to measure the agreement level between NSP Ab ELISA Korea assay and VNT and the agreement was level 93%. This was rated as excellent agreement. The results show that NSP priocheck© Netherlands assay is a low sensitive assay but with high specificity.

Table 3
Sensitivity of LPBE against virus neutralisation test

	VNT (gold standard test)				
TEST	Positives	Negatives		Predictive values	Kappa stat
Screening test: LPBE Positives	23(TP)	0(FP)	18	TP / (TP+FP)100%	
Negatives	0 (FN)	398 (TN)	403	TN / (FN + TN) 100%	100%
TOTAL	Sensitivity: TP/(TP + FN)=100%	Specificity: TN/ (FP+TN)=100%	421		

Table 3 show the sensitivity of LPBE as used in screening tests in reference to VNT the gold standard. LPBE identified 23 serum samples to contain FMDV antibodies and none as negative and VNT confirmed indicated that 23 serum samples contained FMDV antibodies and 398 did not have FMDV antibodies. However 23(100%) were identified as true positive and 398(100%) as true negatives. The large positive predictive value 100% and negative 100% indicates that LPBE is highly sensitive assay and is serotype specific. The high result indicates the accuracy of LPBE statistic. Cohen's kappa coefficient was used to measure the agreement level between NSP Ab ELISA Korea assay and VNT and the agreement was 100%. This was rated as perfect agreement. The findings show that LPBE assay is a high sensitive assay and high specificity.

Table 4
Controls of NSP Ab ELISA - Korea

	Mean OD negative control	OD sample	Sample optical density	Calculated cut off	Interpretation
Negative control	1.251	0.868=	1-(0.868/1.251)*100 = 30.6	>50	negative
Ab positive				<50	positive

Table 4 shows the negative and positive controls. Calculated mean negative control using the absorbance values obtained and the calculated PI (percentage inhibition) value of positive and each test sample using [1-(OD sample / mean OD negative)]*100 as the formulae. The value of PI will lead to interpretation of the test.

Table 5
Controls of NSP Priocheck ©

	Mean OD negative control	OD sample	Sample optical density	Calculated cut off	Interpretation
Negative control	1.000	0.868=	100-[*100=30.6	>50	negative
Ab positive	<70			<70	positive
weak positive control				<50	weak positive

Table 6
Sensitivity and Specificity of Virus Neutralisation Test (VNT) (Reference/gold standard test)

TEST	+ve	-ve	TOTAL	Predictive values
Positives	22 (TP)	0 (FP)	22	= PPV = TP/(TP + FP) 100%
Negatives	0 (FN)	399 (TN)	398	= NPV = TN/(FN + TN) 100%
Total	22 Sensitivity: TP/(TP + FN) = 100%	399 Specificity: TN/ (FP+TN) = 100%	421	

Table 6 shows sensitivity of VNT (gold standard). 22(100%) were identified as true positive and 399 (100%) as true negatives. Positive and negative Predictive value of 100% was obtained and this indicated that the gold standard (VNT) is a high sensitive assay and is serotype specific. This is because it uses cell culture technique where live viruses are cultured. This means if the virus is present it will grow and if it is not it will not grow. A negative predictive value of 100% means that 100% of animals with a negative test are in fact FMD free. In conclusion, animals with a negative test have a 100% chance of being FMD free. The high sensitive value and positive predictive indicates the accuracy of VNT statistically.

Controls of LPBE: Control of LPBE can be explained by upper control limit and lower control limit. Control (Optical Density) has an upper control limit of 1.9 and lower control limit of 0.8.

Ca (PI) percent inhibition had upper control limit 25 and lower control limit of -25, C++(PI) had upper control limit 100 and lower control limit of 85, C+(PI) had upper control limit of 85 and lower control limit of 50. Finally C-(PI) had upper control limit of 49 and lower control limit of 0.

Generally, Optical density Control readings should be between 1.9 and 0.8, control percent inhibition, strong percent inhibition 100-85, weak percent inhibition 85-50 and negative 0-49. The control performed well.

Titter Controls of VNT: Calculating of titter of < 1/23 was interpreted as Protective. >1/23 was interpreted as Exposed but not protective and <8/8 was interpreted as Unexposed. Their interpretation can be in this sense; A titter of greater than 1/23 indicated the animal had antibodies to FMDV and is protected. 8 to 16 show that the animal is exposed but not protected and titters of less than 8/8 indicates that the animal is un exposed.

DISCUSSION

Many developing countries are reluctant to intensively screen animals, animal products and employ specific control strategy for FMD. This is mainly due to high cost, time and high technical skills required with serology technique based tests. This study was aimed to compare performance of serological tests for the detection of FMDV antibodies in Kilifi County, Kenya. NSP Ab ELISA (Korea) assay is a new kit which was designed to detect FMDV NSP abs like the priocheck© kit. The results indicated that though different assays are designed to detect antibodies or antigens, they produce varying sensitivity results. NSP Ab ELISA Korea assay showed a sensitivity of 17(53.13%) compared to NSP priocheck© Netherlands (44.74%) having a variation of 8.96%. The large positive predictive value (PPV = NSP Ab ELISA Korea (94.44%), LPBE (100%) and VNT (100%) and a negative predictive value of 96.28%, 94.76%, and 100% respectively indicates that NSP Ab ELISA Korea is highly sensitive assay. The results point out that both NSP Ab ELISA (Korea) assay and NSP priocheck© Netherlands gave different results despite they are nonstructural proteins (NSPs) tests. Comparing the scenario with all three tests to the C-ELISA verses the I-ELISA there were no relevant differences, but when either of these two tests are evaluated alone against the CHEKIT ELISA the estimates seem to shift towards something that indicates a changed perception of what constitutes a "diseased" case (7). However, it was the author's experience that this shift might also be explained by the combination of the poor sensitivity of the CHEKIT ELISA and the relatively small sample size. Studies done in Cameroon (7) showed that nonstructural protein tests are non-specific tests because they only tell if the blood sample has antibodies to FMDV or not. The study done in 2007 on comparison of sensitivity and specificity in three commercial foot-and-mouth disease virus non-structural protein ELISA kits with swine sera in Taiwan concurred with results obtained from this study in that: that non-structural protein ELISA kits may give varying results (8). The weakness of this study was the sensitivity was not compared based on age of the animals since exposure and antibodies level would vary.

The sensitivity of LPBE was used as screening tests in reference to VNT the gold standard. Their

results realised comparable findings and both recorded similar sensitivity of 100% with positive predictive values of 100% and negative predictive value of 100% for two assays respectively. LPBE identified 23 serum samples to contain FMDV antibodies and none as negative and VNT confirmed indicated that 23 serum samples contained FMDV antibodies and 398 did not have FMDV antibodies. However 23(100%) were identified as true positive and 398(100%) as true negatives. The large positive predictive value 100% and negative 100% indicates that LPBE is highly sensitive assay and is serotype specific. The high result indicates the accuracy of LPBE statistic. These results concurred with studies done in eastern and central Africa (9).

The study established that NSP Ab ELISA (Korea) assay Specificity 99.74%, NSP priocheck© Netherlands 94.76% (Table 3) LPBE 100% gave a high specificity results. The study pointed the need of incorporating more than one assay when demonstrating test's specificity. The results showed that when numerous assays are incorporated in a test, a researcher can comfortably declare specificity tests results. This was demonstrated by the very narrow range difference among the assays in comparison. In studies done in Taiwan their specificity results differed with these results in that their specific results was low while those of Kilifi were high but in sensitivity all results were highly correlated (7,10). The specificity results suggest that the NSP Ab ELISA (Korea), NSP priocheck© (Netherlands) and LPBE assys are useful tools for screening cattle for infection with FMDV.

In all the assys; NSP Ab ELISA Korea, NSP priocheck© Netherlands, LPBE and VNTN recorded agreement level. Cohen's kappa coefficient was used to measure the agreement level between NSP Ab ELISA Korea assay 90%, NSP priocheck© Netherlands (93%) and LPBE and VNT 100%. This was rated as perfect agreement. Cohen's kappa coefficient was used to measure the agreement level between NSP Ab ELISA Korea assay and VNT and the agreement was level 93%. This was rated as excellent agreement. Statisticians have used Cohen's kappa coefficient as a statistical measure of inter-rater agreement or inter-annotator agreement for qualitative (categorical) items. It is generally thought to be a more robust measure than simple percent agreement calculation since κ takes into account the agreement occurring by chance. In studies done in Cameroon, the results showed that the accuracy of assays done in FMD-endemic area were comparable to those obtained from serologic testing (11). These results concurred with the findings in this study. These results showed excellent agreement level of 95.75%.

Finally the study incorporated controls tests. The controls performed well. Both negative and positive controls acted as guide during results interpretation.

In conclusion, the study concluded that both Anigen © FMD NSP Ab ELISA –Korea and NSP priocheck© kits had a low sensitivity and a high specificity in determining antibodies to FMD. Anigen © FMD NSP Ab ELISA –Korea has a higher sensitivity than NSP priocheck© kit and do not add further benefit to detect FMDV infections by enhancing sensitivity due to potential contingency to trace viral capsid antigens, a fact that needs further evaluation. On the other hand LPBE and VNT are effective serological tools among FMD serotyping.

It also suggests that the NSP priocheck© kit, Anigen © FMD NSP Ab ELISA –Korea and LPBE assay are highly sensitive tests for use in cattle populations with multiple exposures to different FMD virus serotypes. However, these NSP priocheck© tests lack sensitivity and this may create difficulties in designing sero-surveillance strategies as the number of herds with false positives may overwhelm the available resources to deal with them unless a confirmatory test such as the VNT is also used.

RECOMMENDATIONS

1. The study recommends further evaluation of LPBE and VNT would be instrumental in FMD diagnosis and management in Kenya.
2. The non- structural proteins tests should always be used in accompaniment with structural proteins tests. Therefore running the nonspecific assay with specific assay is vital and this will provide better validation of the assays and provide better base for results declaration.

ACKNOWLEDGEMENTS

I give my gratitude to the National reference lab, Ministry of Agriculture, Livestock and Fisheries, for financial(funding), moral support and venue acceptance. Your input is very much appreciated.

REFERENCES

1. OIE, 2013. World Animal Health Information Database (WAHID) http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home> (accessed 20.03.13).
2. Wint G.R.W. & Robinson T.P., 2007. Gridded livestock of the world. Food and Agriculture Organization of the United Nations, Rome. <ftp://ftp.fao.org/docrep/fao/010/a1259e/a1259e01.pdf> (accessed 01.07.11).
3. Sumption K., Rweyemamu M., Wint W. Incidence and distribution of foot-and-mouth disease in Asia, Africa and South America; combining expert opinion, official disease information and livestock populations to assist risk assessment. Transbound. *Emerg. Dis.* 2008;**55**:5–13.
4. Vosloo, W Bastos, ADS, Sangare O, Hargreaves, SK, and Thomson, G.R. Review of the status and control of foot and mouth disease in sub-Saharan Africa. *Rev.*

SciTech. Off. Int. Epiz. 2002; **21**: 437-449.
5. Hui SL, Walter SD: Estimating the error rates of diagnostic tests. *Biometrics* 1980, **36**:167-171.
6. Cohen, Jacob. "A coefficient of agreement for nominal scales". Educational and Psychological Measurement 1960; **20**: 37–46.
7. Barend M deC Bronsvoort, Nils Toft, Ingrid E Bergmann, Karl-Johan Sørensen, John Anderson, Viviane Malirat, Vincent N Tanya and Kenton L Morgan. Evaluation of three 3ABC ELISAs for foot-and-mouth disease non-structural antibodies using latent class analysis in Cameroon *BMC Veterinary Research* 2006, **2**:30.
8. Chen SP, Ellis TM, Lee MC, Cheng IC, Yang PC, Lin YL, Jong MH, Robertson ID, Edwards JR. Comparison of sensitivity and specificity in three commercial foot-and-mouth disease virus non-structural protein ELISA kits with swine sera in Taiwan. *Vet Microbial.* 2007;**119**:164-172. Epub 2006 Nov 16.
9. Bronsvoort BM, Parida S, Handel I, McFarland S, Fleming L, Hamblin P, Kock R. Serological survey for foot-and-mouth disease virus in wildlife in eastern Africa and estimation of test parameters of a nonstructural protein enzyme-linked immunosorbent assay for buffalo. *Clin Vaccine Immunol*. 2008;**15**:1003-1011.
10. Kenton L. Morgan, Ian G. Handel, Vincent N. Tanya, Saidou M. Hamman, Charles Nfon, Ingrid E. Bergman, Viviana Malirat, Karl J. Sorensen, and Barend M. de C. Bronsvoort Accuracy of Herdsmen Reporting versus Serologic Testing for Estimating Foot-and-Mouths Disease Prevalence. *CDC journal* Volume 20, Number 12—December 2014
11. Bronsvoort BMC, Sørensen KJ, Anderson J, Corteyn A, Tanya VN, Kitching RP, Morgan KL: A comparison of two 3ABC ELISA's in a cattle population with endemic, multiple serotype foot-and-mouth disease. *Journal of Clinical Microbiology* 2004, **42**:2108-2114.
14. Kibore, B. Gitao, C.G, Sangula A. and Kitala. Foot and mouth disease sero-prevalence in cattle in Kenya Journal of Veterinary Medicine and Animal Health. Vol.5 (9), pp.262268, September, 2013 DOI 10.5897/JVMAH2013.0220© 2013 Academic Journalshttp://www.academicjournals.org/JVMAH.