

Münstermann S.⁽ⁱ⁾, Mokopasetso M.⁽ⁱ⁾, Monyame K.⁽ⁱⁱ⁾, Hyera J.⁽ⁱⁱ⁾, Moagabo K.⁽ⁱⁱ⁾, Baipoledi K.⁽ⁱⁱ⁾, Letshwenyo M.⁽ⁱⁱⁱ⁾

Summary

In this study the performance of two commercial tests (Chekit® and Ceditest FMDV-NS®) for the detection of antibodies to non-structural proteins (NSP) of the foot and mouth disease virus (FMDV) was tested on cattle sera, collected during an outbreak of FMDV SAT2 in the vaccinated zone of Botswana in 2007. The study was extended to three other distinct disease control zones during an outbreak in a FMD free zone in 2008. The Ceditest® provided positive results only in sera from infected herds, whereas the Chekit® gave positive results also for sera from uninfected herds. The Kappa statistic showed fair to substantial agreement (Kappa = 0.562; CL: 0.347 – 0.784) between the two tests for locations with clinical FMD and poor agreement (Kappa = 0) for locations with no clinical signs of FMD. The results indicate a higher sensitivity and specificity for the Ceditest® under field outbreak situations in vaccinated and in non-vaccinated animals.

Introduction

Botswana manages FMD through a systematic approach based on zoning into disease-free (with or without vaccination) zones and livestock movement control. In order to maintain the disease-free status and export beef from the non-vaccinated "green" zones, regular vaccination programmes are implemented in the "red" zones where contact with buffalo is possible. Routine sero-surveillance using the liquid phase blocking ELISA (LPBE) is carried out to monitor vaccine performance. In situations of FMD infection the use of serological tests (3ABC-ELISA) that detect antibodies against FMD viral NSPs is useful in discriminating infected from non-infected animals. NSPs are exclusively produced as a result of infection and therefore the presence of anti-NSP antibodies in animal sera indicates past or present infection (Robiolo *et al.*, 1997). However, it has been reported that commercial NSP tests may not be as suitable for SAT-type viruses as they are for the Eurasian virus types.

Objective

To assess the performance of two commercial NSP test kits for detecting evidence of SAT-type infection.

Material and Methods

Study Area

In October 2007 samples were collected from infected cattle crushes at Habu in the Maun district and three other locations in the same district in crushes of different infection status (Table 1). All locations were situated in the "red" zone where all animals are vaccinated regularly. In October 2008 samples were collected from Newlook farm in Ghanzi district, located in the "green" disease-free, non-vaccinated zone. Additional samples were taken from another district in the "green" zone (Mahalapye, November 2008) and from the "red" zone which had never experienced an outbreak (Nata, April-May 2009) as shown in Table 2. The different locations are shown in the Map.

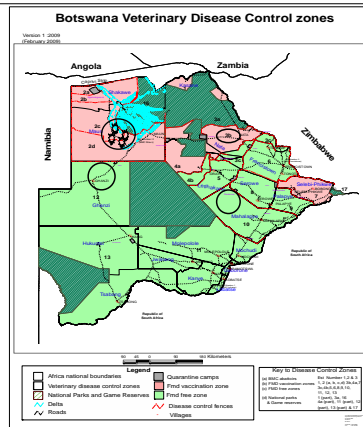


Table 1: Samples obtained during the Habu outbreak (October 2007)

Period	Clinical signs		Crush status		Vaccinated		Category	No samples
	Yes	No	Infected	Non-infected	Yes	No		
22 – 30 October 2007	Yes	No	Infected	Non-infected	Yes	No	IAIC	52
	No	No	Infected	Non-infected	Yes	No	NAIC	80
	No	No	Non-infected	Non-infected	Yes	No	NANC	61
	No	No	Surveillance	Non-infected	Yes	No	NASC	55
Total								248

NB: IAIC: Infected Animal within Infected Crush; NAIC: Non-Infected Animal within Infected Crush; NANC: Non-infected Animal within Non-Infected Crush; NASC: Non-Infected Animal within Surveillance Crush (surveillance crushes here refers to crushes within a specific demarcated area for outbreak control purposes)

Table 2: Samples obtained in Ghanzi, Nata and Mahalapye districts

Period	Clinical signs	Crush status	Vaccinated	Category	No of samples
27 – 28 Oct 08	Yes	Infected	No	Ghanzi	61
24 Nov 08	No	Non-Infected	No	Mahalapye	100
11 Apr – 10 May 09	No	Non-infected	Yes	Nata	168
Total					336

Sample testing

Two commercial NSP tests, Ceditest FMDV-NS® and Chekit® (Bommeli), were used for the detection of antibodies against NSP of FMD virus. Both kits are blocking ELISAs using FMD 3ABC viral antigen.

Results

As shown in Figure 1 for the groups of vaccinated cattle, both tests were able to detect antibodies to NSP in infected crushes (IAIC and NAIC), but the Ceditest® test showed a higher specificity, as it did not detect NSP antibodies in non-infected crushes (NANC and NASC) whereas the Chekit® did.

The same pattern was found in non-vaccinated animals in Ghanzi and Mahalapye (non-infected) as shown in Figure 2, where the Ceditest® detected NSP antibodies in infected animals only while the Chekit® detected animals as positive in all three groups, including the group of vaccinated, non-infected animals at Nata.

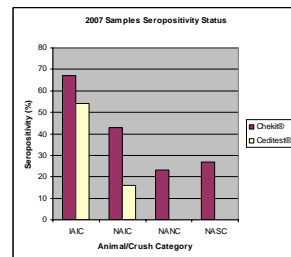


Figure 1: test results in vaccinated animals

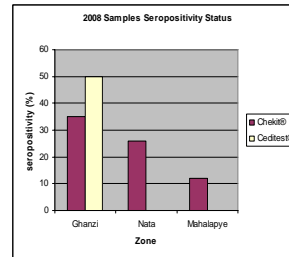


Figure 2: test results in non-vaccinated and vaccinated (never infected) animals

The Kappa statistic showed fair to substantial agreement (Kappa = 0.562; CL: 0.347 – 0.784) between the two tests for locations with clinical FMD and poor agreement (Kappa = 0) for locations with no clinical signs of FMD as shown in Table 3 and 4.

Conclusion

These results reaffirm the apparent low sensitivity and specificity of commercially available kits designed to detect antibodies to NSPs following infection with SAT-serotype FMD viruses.

Results reported here originated from field testing during two outbreak situations and are in line with the laboratory-based comparisons between the Chekit® and Ceditest® for SAT 2 viruses of southern African origin which found the Ceditest® to have higher specificity and to detect antibodies earlier and for longer time periods than Chekit®.

The results add further weight to the need to develop NSP testing systems specific for the SAT-serotypes. They also highlight the necessity for the production of purified SAT vaccines to enable application of the DIVA principle.

Discussion

In a recent literature review on the use of currently available serological tests for differentiating antibodies produced following SAT virus infection versus SAT vaccine induced antibodies (Roeder, 2007) the author concluded that the 3ABC-based NSP tests were not as sensitive in detecting SAT-type infection as they were for the Eurasian-origin viruses A,O,C and Asia 1. Vosloo *et al.*, 2007, also tested this hypothesis using 4 commercially-available NSP tests to detect SAT 1-3 virus-induced antibodies in sera from experimentally infected cattle. The authors concluded that different topotypes of SAT viruses influence the sensitivity of all tests used in the comparison. Both papers contain the conclusion that more research is needed on the characterisation of SAT NSPs and that the development of SAT-specific NSP tests is required. However, these SAT specific tests are not yet available, and therefore an assessment of performance of currently available NSP tests under field outbreak situations was used to assess their application for differentiating infection from vaccination antibodies (DIVA). The application of the DIVA principle in Southern Africa is also hampered by the fact that the vaccine currently in use is not purified and hence could potentially induce antibodies to NSP proteins. This factor needs to be taken into account particularly in areas where successive vaccination campaigns have been conducted over many years such as the Maun District of Botswana.

The results indicate that the Ceditest® is more specific in this situation, as it detected NSP antibodies only in animals that showed either clinical signs of FMD (IAIC and Ghanzi) or were in-contact animals (NAIC). These findings covering two outbreaks and for a total of 584 samples are in line with those of Vosloo *et al.*, 2007 whose results were based on a laboratory study. Our results also support the Vosloo *et al.*, 2007 finding that the Chekit® detects NSP antibodies in SAT type virus outbreaks only over a short time interval.

References

- Robiolo B. *et al.* (2006): Analysis of immune response to FMDV structural and non-structural proteins in cattle in Argentina by the combined use of liquid phase and 3 ABC-ELISA tests. Vaccine 24 (2006): 997-1008.
- Roeder, P. (2007): The use of currently available serological tests for differentiating antibodies developed as a result of infection with SAT-types of foot-and-mouth disease virus and those which result from the use of vaccines against SAT-type viruses in control actions. SADC report (2007).
- Vosloo, W., Maree, F., Botha, B. (2007): Preliminary report on a comparison between four different tests to detect antibodies to the non-structural proteins of the SAT type foot and mouth disease virus

Acknowledgement

This study was published with the permission of the Director of Veterinary Services of Botswana. Funding for the test kits was provided by the EU funded SADC FMD project.