DIRECTORATE ANIMAL HEALTH, DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES
SOUTH AFRICA

Report on the prevalence survey for Foot and mouth disease in the KwaZulu-Natal protection zone that was declared in June 2011

31 January 2012 to 6 March 2012

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Contents

Tables:

Figures:

1. Introduction and background

The World Animal Health Organization (OIE) had recognized South Africa as having a zone free from Foot and mouth disease (FMD) without vaccination until 2011 as can be seen in Figure 1.

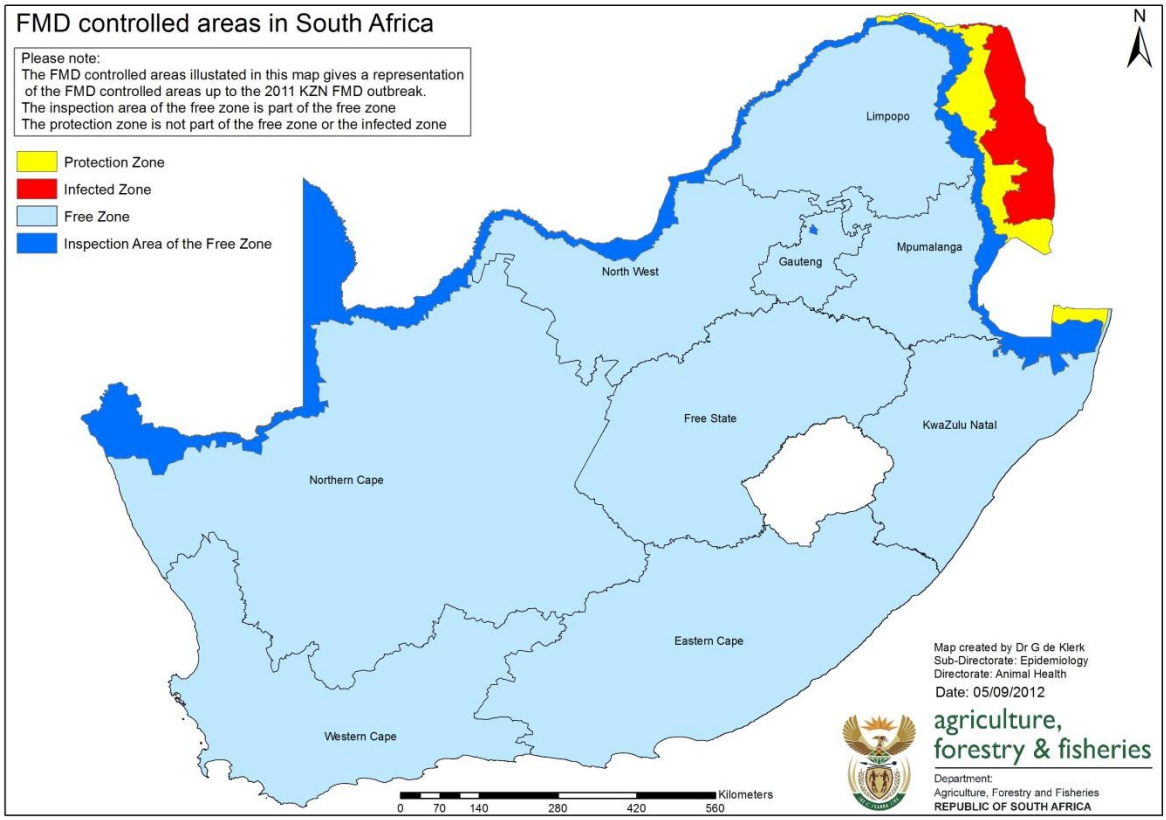

Figure 1: FMD controlled areas up to the 2011 KwaZulu-Natal outbreak

Prior to the 2011 FMD outbreak, the majority of South Africa was considered free from Foot and mouth disease (FMD) without vaccination. The Kruger National Park and adjacent areas were defined as an infected zone (where FMD carrier buffalo are present) and an adjacent buffer area called the protection zone; both of these were excluded from the FMD free zone. The FMD controlled areas and the legally prescribed FMD control measures are described in the Animal Diseases Act, 1984 (Act No 35 of 1984), the accompanying Animal Diseases Regulations (as amended) and the FMD protocol, that has recently been updated into the FMD Veterinary Procedural Notice (VPN).

An outbreak FMD in the FMD free zone was detected in February 2011, after positive FMD serology results in cattle were obtained, following routine sampling of cattle, at diptanks in the northern part of KwaZulu-Natal (KZN). No conclusive clinical signs of FMD were ever observed during the investigation of the outbreak. The outbreak was confirmed on the 11th of February 2011 and reported to the OIE on the 25th of February. Quarantine and movement control were implemented in the area and cattle in the infection zone north of the N2 highway were vaccinated. An initial protection zone, depicted by a the yellow area in Figure

2 was proposed in KZN and included almost 50% of the province, while the initial infected zone, indicated by the red area in Figure 2, included the north-eastern part of KZN. These areas were discussed and decided on during a joint meeting between the KZN Provincial Veterinary Service and the Department of Agriculture, Forestry and Fisheries (DAFF) in the first week of March 2011, soon after the outbreak was detected.

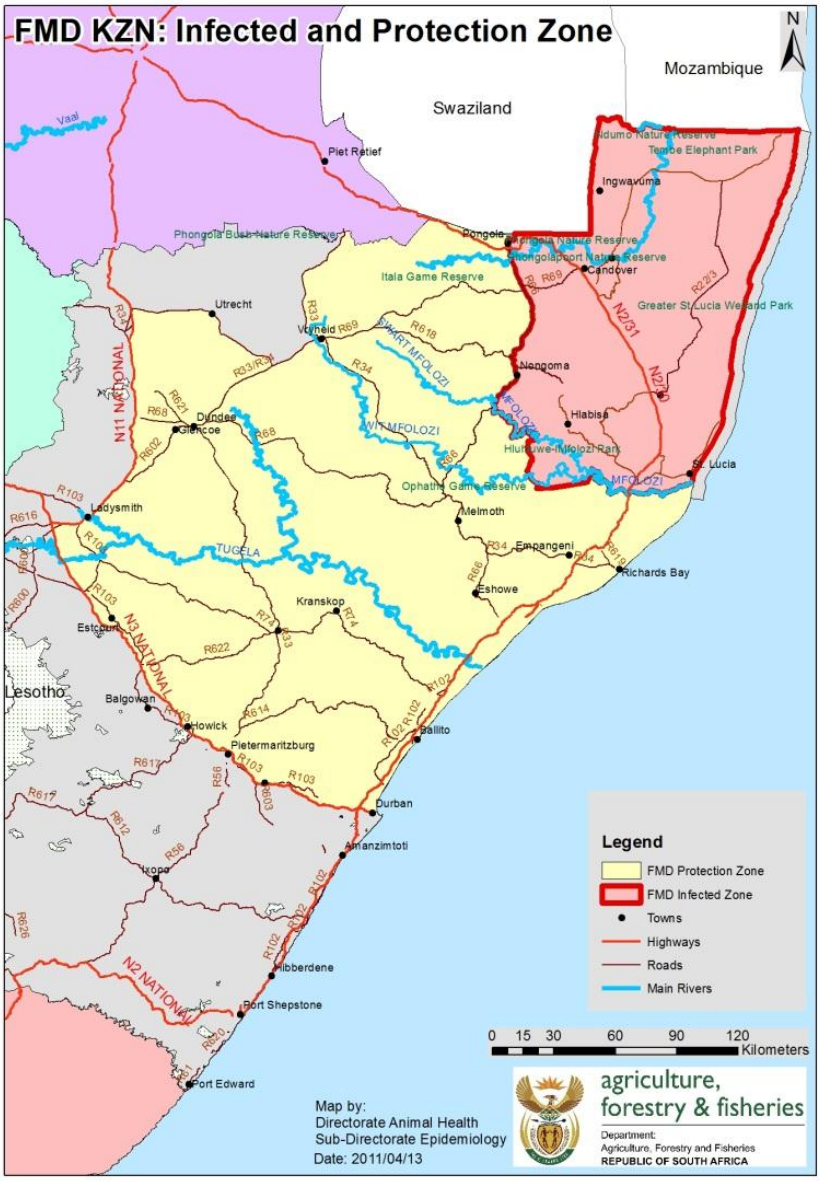

Figure 2: Initial FMD controlled areas (March 2011) in KZN after the outbreak in February 2011

SAT 1 FMD virus was isolated from cattle at one diptank in the Hluhluwe area. Later during the outbreak, the same virus was isolated from a feedlot in Gauteng Province and the origin of the cattle in the feedlot was traced back to the area with positive serology in KZN. In addition, SAT 3 FMD virus was isolated from buffalo in the Ndumo Game Reserve in the North of KZN Province on the Mozambique border. The seropositive locations are illustrated in Figure 3.

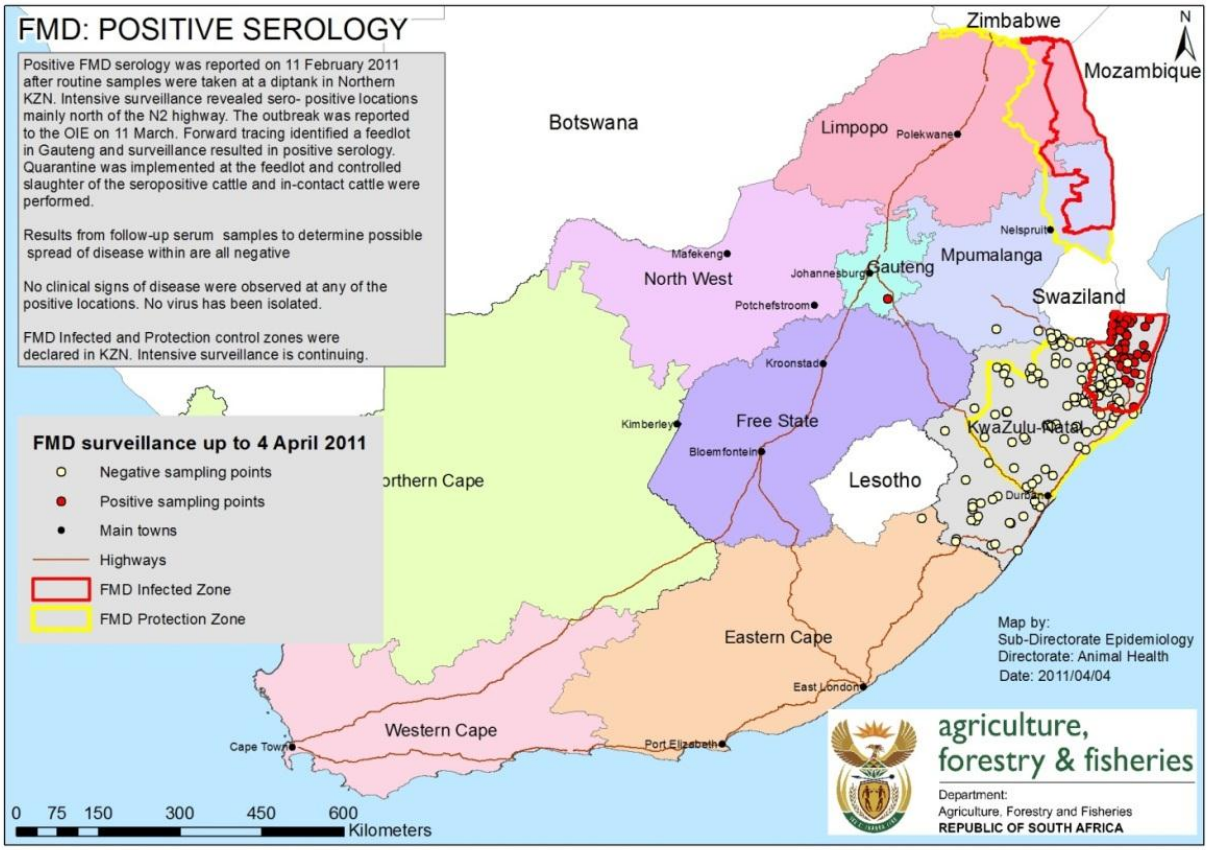

Figure 3: Location of the positive FMD serology in KZN and Gauteng

Cattle in the infected zone north of the N2 highway were vaccinated during May 2011. A second round of vaccination was conducted in the northern part of the infected zone during June 2011. No systematic vaccination was conducted south of the N2 highway at any stage of the outbreak.

After the first round of sero-surveillance in the initial infected and protection zones, it became clear that mainly serological reactions were seen. No clear evidence of active clinical infection was found and there was no evidence that the outbreak was spreading. It was therefore proposed that the protection zone and infected zone borders be moved northwards to make these areas smaller – and to ensure that the new Infected Zone was demarcated by clear geographic and physical boundaries. This decision was taken in a joint meeting between the KZN Provincial Veterinary Service and DAFF on 6th of June, 2011.

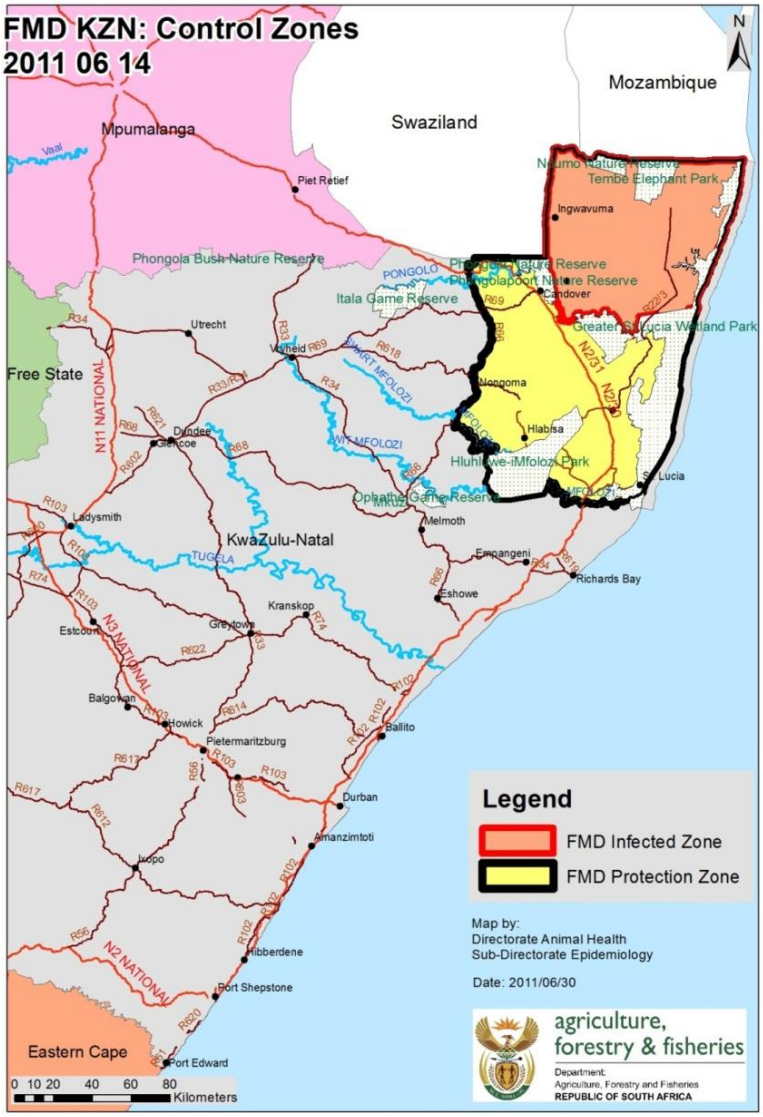

Figure 4: Smaller FMD protection and infection zones as implemented in KZN in June 2011

Continuing serological and clinical surveillance demonstrated no further seropositive locations or spread of disease. The outbreak was thus officially terminated on 17 July 2011.

A small FMD prevalence survey in the protection zone of KZN was planned and designed in December 2011 and preparation for the execution was done in January 2012.

2. Purpose of the survey

It became necessary to determine the status of the FMD protection zone (dated 6th June 2011) in KZN. The purpose of the survey was to determine the FMD sero-prevalence of cattle in the FMD protection zone (refer to Figure 4 for the location of the protection zone) in order to make recommendations regarding the future inclusion of this area into the free zone. A few of the diptanks in this area had been vaccinated during the outbreak in the adjacent infected zone but no FMD vaccination had been administered since the beginning of June.

The expectation was to show that a sero-prevalence of below 5% exists. If the sero-prevalence of this protection zone is below 5%, without any indication of virus circulation or FMD infection, a recommendation would be made for the area to revert to being part of the FMD free zone. This would decrease the size of the final FMD controlled zones in KZN and make FMD control more manageable because of clear geographic and physical boundaries of the zones.

The information obtained in this survey was to be used as background information in the design of a countrywide survey to prove FMD freedom in preparation for a dossier to the OIE to apply for an FMD free zone status internationally.

3. Location of the survey

The survey was conducted in the FMD protection zone (June 2011) in KZN and included locations in the following local municipalities:

- Nongoma
- Uphongola
- The Big Five False Bay
- Hlabisa
- Mtubatuba

The survey area included parts of the Zululand and Umkhanyakude State Veterinary areas as can be seen in Figure 5.

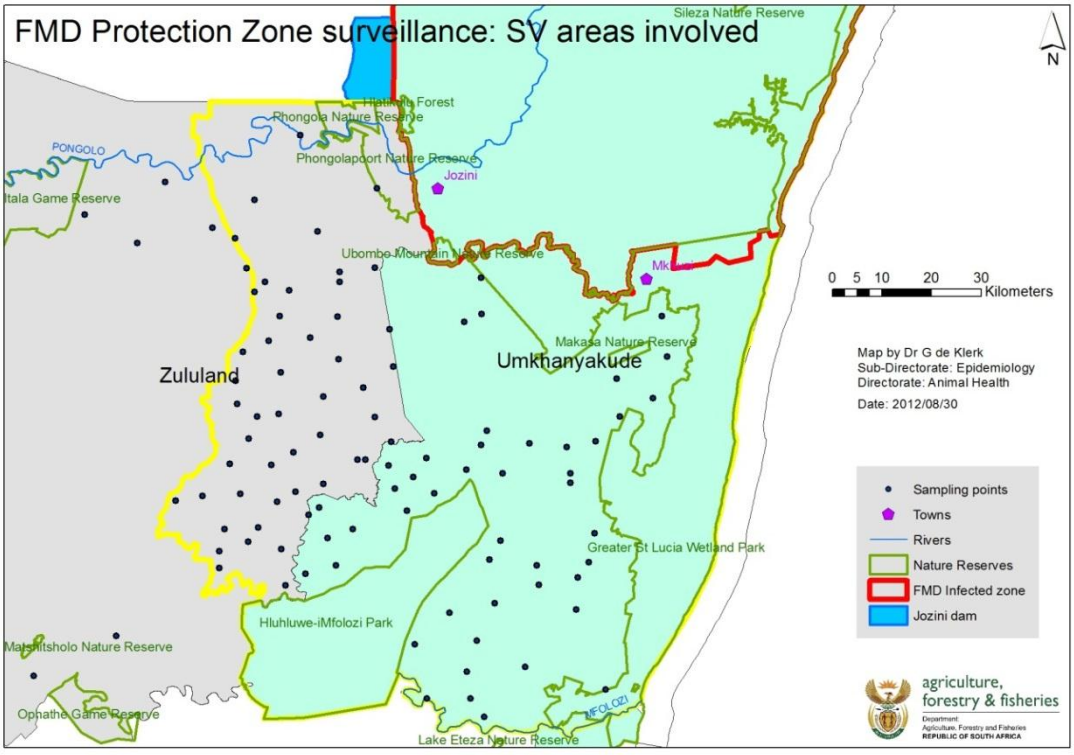

Figure 5: State Veterinary areas and sampling points included in the survey

4. Survey design

4.1 Selection of sampling points and samples

4.1.1 Population parameters

Information on the number of diptanks and farms and the number of animals at each location was provided by the KZN Veterinary Services.

Survey Toolbox© (Cameron 1999) was used to calculate the number of points to be sampled. The calculations were done for a prevalence survey by using the probability proportional to size (PPS) option. The selection was done randomly without replacement and was stratified by using the farming type (diptank or farm).

The following parameter were used in the calculation:

Estimated prevalence of seropositive cattle:	5%
Within diptank/farm variance	0.55
Between diptank/farm variance	0.03
Average diptank/farm population	928
Total farms/diptanks in sampling frame	129

The between diptank/farm variance is a measure of the level of difference there is between the herds or villages and the within diptank/farm variance is a measure of the level of difference there is between the individual animals. Sample size needs to be higher when the variance in the population is higher. The between diptank/farm variance was estimated as low and the within diptank/farm variance was estimated as medium in this population.

4.1.2 Cost parameters

An approximate cost per village and per animal was used in the calculations:

Cost per village	R4 000
Cost per animal	R350

4.1.3 Precision and confidence parameters

The following parameters were chosen:

Fixed width confidence interval	±5%
Confidence level	95%

The width of the confidence interval indicates how good the estimate of this survey is. You choose a narrow confidence interval if you are sure about where the true value, in this case the prevalence of FMD seropositive cattle, lies. The confidence level means that you are 95% sure that the value falls in this interval.

According to the calculations, 46 locations had to be sampled with 15 randomly selected samples per location. To compensate for a possible loss of sampling points or samples during transport and testing, 50 locations were chosen and collection of 16 samples was

requested. The sampling points included diptanks in communal areas, as well as commercial farms and were randomly selected from the sampling frame of all commercial farms and communal areas with cattle in the survey area. (n=129).

Three diptanks, situated in the southern part of the Jozini local municipality, were included in the sampling frame, but not selected in the random sampling point selection process. The location of the sampling points can be seen in Figure 6.

Figure 6: Geographic location of the sampling points

4.1.4 Selection of samples at each location:

Animals had to be selected randomly in order to give all the animals at each location an equal chance to be sampled. It was therefore neccessary to calculate the interval between cattle to ensure that the sixteen samples are selected throughout the herd. The interval calculation was done for 80% of the cattle census at the diptank to compensate for the fact that not all cattle will appear at the diptank on any inspection/dipping day. The sampling

interval calculated for each location is given in Table 1. If the interval was for example 13, this means that every 13th animal going through the crush had to be sampled.

Although other cloven-hoofed domestic animals are also susceptible, only cattle were sampled.

Table 1: Selected sampling points and sampling intervals at each sampling point

Sampling Point	Type	Local Municipality	80% of cattle at location	Interval between samples
Amatis	Farm	Big Five False Bay	268	13
Glen Gweni	Farm	Big Five False Bay	457	23
HH Ranch	Farm	Big Five False Bay	183	9
Koorsboom	Farm	Big Five False Bay	113	6
Mzinene Estate	Farm	Big Five False Bay	125	6
Ngweni	Farm	Big Five False Bay	150	8
Silvasands	Farm	Big Five False Bay	1599	80
Waterloo	Farm	Big Five False Bay	107	5
Gunjaneni	Diptank	Lower Umkhanyakude	1200	60
Machibini	Diptank	Lower Umkhanyakude	990	50
Mahiya	Diptank	Lower Umkhanyakude	1004	50
Masakeni	Diptank	Lower Umkhanyakude	1500	75
Matshamhlophe	Diptank	Lower Umkhanyakude	939	47
Mpempe	Diptank	Lower Umkhanyakude	1800	90
Mquthungu	Diptank	Lower Umkhanyakude	1241	62
Mvutshini	Diptank	Lower Umkhanyakude	2033	102
Mzinene A	Diptank	Lower Umkhanyakude	1850	93
Ngwenyambili A	Diptank	Lower Umkhanyakude	671	34
Nhlwathi	Diptank	Lower Umkhanyakude	1233	62
Nibela	Diptank	Lower Umkhanyakude	2433	122
Nkomo	Diptank	Lower Umkhanyakude	1535	77
Nomathiya	Diptank	Lower Umkhanyakude	1193	60
Sovane	Diptank	Lower Umkhanyakude	1051	53
Uhlanga	Diptank	Lower Umkhanyakude	881	44
Boomerang	Farm	Mtuba	60	3
Baxa	Diptank	Nongoma	766	38
Cwabini	Diptank	Nongoma	1232	62
Maduma	Diptank	Nongoma	1700	85
Madwaleni	Diptank	Nongoma	1292	65
Manzaneni	Diptank	Nongoma	910	46
Manzawayo	Diptank	Nongoma	679	34
Manzimakhulu	Diptank	Nongoma	1094	55
Mduna	Diptank	Nongoma	1213	61
Mngeni	Diptank	Nongoma	934	47
Mona	Diptank	Nongoma	1093	55
Mpuphusi	Diptank	Nongoma	1337	67
Mthonjaneni	Diptank	Nongoma	1844	92
Mtikini	Diptank	Nongoma	1371	69
Ngongoma	Diptank	Nongoma	418	21
Ngwenyama	Diptank	Nongoma	936	47
Nswempe	Diptank	Nongoma	627	31
Ntweni	Diptank	Nongoma	1899	95
Nxwala	Diptank	Nongoma	1052	53

Siphethwini	Diptank	Nongoma	1555	78
Wela	Diptank	Nongoma	1640	82
Candover	Diptank	uPongola	1000	50
Dwarsland farm	Farm	uPongola	350	18
Nkunzana	Farm	uPongola	30	2
Nyaliza	Diptank	uPongola	700	35
Panbuilt	Farm	uPongola	100	5

5. Preparation and training

A standard operational procedure (SOP) for the survey was compiled and presented at a monthly veterinary meeting with the Provincial Director, State Veterinarians and Animal Health Technicians (AHTs).

Gel-bleeding tubes, bleeding sleeves and submission forms printed on green paper were procured from TADP. This, together with animal counters and clip boards were pre-packed and dispatched to the Zululand (Vryheid office) and Umkhanyakude (Mtubatuba office) State Veterinary areas.

6. Testing of samples

Samples were analysed by the Transboundary Animal Diseases Program (TADP) at the Onderstepoort Veterinary Institute. SAT1, SAT 2 and SAT 3 Liquid Phase Blocking ELISA (LPBE) tests were performed on all samples. Four point titrations were performed on all samples and a result of ≥1.6 was considered as test positive and had to be followed up by a full clinical and epidemiological evaluation and a report to the Director Animal Health.

7. Time frame of the survey

The survey started on the 31 January 2012 and was completed well before the cut-off date of 6 March 2012 as can be seen in Figure 7.

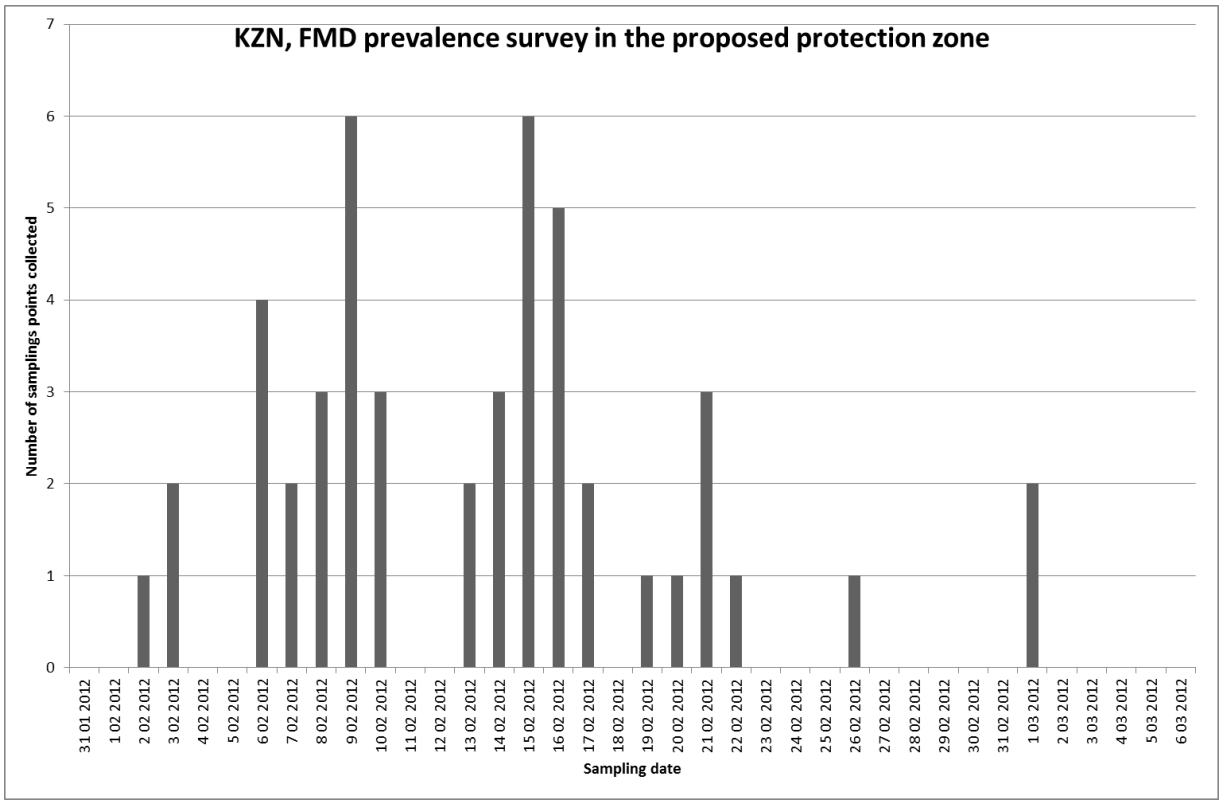

Figure 7: Distribution of the sampling point collection dates

8. Data collection and cleaning

The two State Veterinarians responsible for the areas where the survey was conducted were requested to summarise the sampling point information and the results obtained in a provided Excel spreadsheet. The spreadsheet had to be submitted by e-mail to the Sub-Directorate Epidemiology, Directorate, Animal Health, DAFF. This turned out to be challenging and the information was therefore captured at the Sub-Directorate Epidemiology, DAFF instead. The submission forms and the laboratory result sheets were obtained from the TADP laboratory and a data capturer was appointed on contract to assist in the data capturing process. The general quality of the submitted information was good but because most of the submission forms were hand-written instead of electronically completed, some were illegible and information had to be verified by contacting the sender.

Two commercial farms in the uPhongola local municipality (Panbult and Nkunzana) were not sampled as requested because the locations could not be found. This was only discovered after the cut-off point of the survey had been reached. Forty-eight sampling points were therefore included in the survey, two more than the required 46 sampling points as per survey design.

9. Verification of the survey

An audit was performed in the two State Veterinary areas involved in the survey while the survey was underway to verify that the samples were collected according to the prescribed procedure. The following was observed:

- Not all cattle were present at the inspection points on the day of dipping/inspection in the non-commercial areas. Three diptanks were visited and the following strike rates were observed:

Table 2: Strike rates of animals at the visited sampling points

Name of diptank	SV area	Cattle registered at the diptank	Number of cattle present	% of animals present (strike rate).
Baxa	Zululand	766	255	33
Nswempe	Zululand	627	502	80
Nhlwathi	Umkhanyakude	1200	1200	100
Total		2593	1957	76

- Sampling procedure was done according to the instructions but if fewer animals appeared at the diptank, more animals were sampled out of the last few herds (therefore a smaller sampling interval than the calculated interval). It is not possible to determine the final number present before the sampling on the day, as animals will come and go over a period of 2 to 3 hours.
- It was not possible to verify if all animals in the area were registered at the diptank. No signs of disease were observed at any of the diptanks and the condition of the animals was good.

10. Results of the survey

Five out of the 48 sampling points tested had one or more result ≥1.6 as illustrated in Figure 5. Most of the samples tested positive for SAT 1 (n=16), a single sample tested positive for SAT 2 and 4 samples tested positive for SAT 3. Some samples tested positive for more than one SAT type.

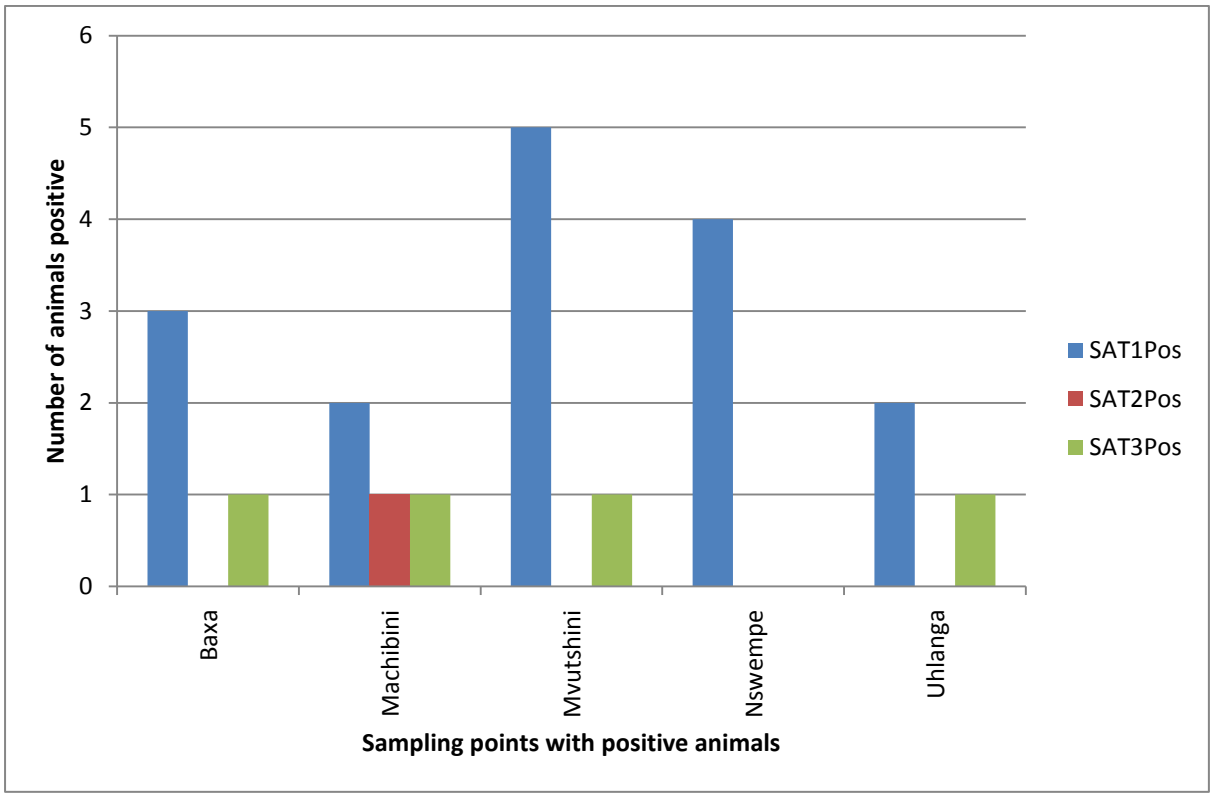

Figure 8: Positive sampling points

11. Analysis of the survey

The results were analysed in two strata; the commercial farms and the non-commercial diptanks/ inspection points. Precision of the outcome of the survey is measured as the width of the calculated confidence interval (a fixed width of the confidence interval was used). The confidence level describes how confident we are that the true value lies within the calculated confidence intervals.

11.1 Stratum 1: Non-commercial diptanks / inspection points sampled:

38 points and 606 animals were sampled. 17 animals tested positive.

Prevalence: 2.8053 %
Variance: 0.000152
95% CI: 0.3860 to 5.2246 (=<1% to 5.2%)
Thus, we are 95% confident that the prevalence of seropositive animals in the non-commercial sector lies between 0.3% and 5.2%.

11.2 Stratum 2: Commercial farms sampled:

10 points and 152 animals were sampled. None of the animals tested positive.

Prevalence: 0.0000 %
Variance: 0.000000
95% CI: 0.0000 to 0.0000

Because of the 0% prevalence found you have to refer to the overall result. The discrepancy between the commercial and non-commercial farms may be due to increased biosecurity and less movement of animals between herds in the commercial sector. It also may or may not relate to the performance of the laboratory test.

11.3 Overall Results of the stratified analysis

48 points and 758 animals were sampled. Five sampling points with 17 animals tested positive.

Prevalence: 2.2208 %
Variance: 0.000095
95% CI: 0.3056 to 4.1361
Average within Village Variance: 0.008227
Average between Village Variance: 0.002078

Thus, we are 95% confident that the prevalence of seropositive animals in the survey area lies between 0.3% and 4.1%.

12. Discussion

The survey was executed in accordance with the planning procedures and the instructions issued and completed satisfactorily. Follow-up investigatons did not indicate any circulation of FMD virus or active disease. The test positive animals found in the survey could be a result of previously vaccinated animals and/or previously infected animals or false positive test results.

Previously vaccinated animals

No systemic vaccination was ever conducted in the survey area. However, it is possible that previously vaccinated animals were introduced into the survey area.

Previously infected animals

During the outbreak no active infection was detected in the survey area despite heightened clinical and serological surveillance. It cannot be excluded that some previously infected and/or vaccinated animals might have been introduced from the infected zone. However, it can be concluded that these animals did not cause active infection in the survey area.

False positive test results

Subsequent experience has shown that the performance of the LPBE test may not be optimal at all times and under all sircumstances. The accurate sensitivity and the specificity of this LPBE test conducted at TADP is currently not known. It is thus not possible to determine the percentage of false test positive animals.

13. Conclusion

Clinical surveillance at all positive sampling points and ongoing routine inspections at diptanks in the area gave no indication of circulating FMD virus or active infection.

This area is not suitable to be declared as part of a permanent FMD protection zone due to the absence of physical and geographical borders to assist in FMD control measures as described in the FMD VPN. The outcome of the survey, together with the above, indicates that this area should be included into the FMD free zone.

14. References

CAMERON, AR. 1999. Survey Toolbox; A practical manual and software package for active surveillance of livestock diseases.

KZN VETERINARY SERVICES. 2011. Stock figures and diptank locations

15. Acknowledgements

Provincial State Veterinarians and Animal Health Technicians

Laboratory officials of TADP at Onderstepoort Veterinary Institute

Animal Health Forum