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Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (*Syncerus caffer*) in the Kruger National and Hluhluwe-iMfolozi Parks, South Africa

by

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Submitted in partial fulfillment of the requirements for the degree of Magister Scientiae (Veterinary Tropical Diseases) in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

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Declaration

I declare that this dissertation hereby submitted to the University of Pretoria for the degree Magister Scientiae (Veterinary Tropical Diseases) has not been previously submitted by me for a degree at this or any other University, that it is my own work in design and in execution, and that all material contained therein has been duly acknowledged.

Signed:

Date:

This dissertation forms part of the requirements for a web-based MSc degree research project in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. These projects carry a weight of approximately 100 credits, and are therefore smaller than projects required for a research-based MSc degree with a weight of 240 credits. It would be appreciated if reviewers could evaluate the dissertation in that context.

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Table of contents

List of Abbreviations

ARC-OVI	Agriculture Research Council – Onderstepoort Veterinary Institute
BDC	Bovine dermis cells
CC	Conjugate control
CPE	Cytopathic effect
CV	Coefficient of variation
DFAT	Direct fluorescent antibody test
DVTD	Department of Veterinary Tropical Diseases
ELISA	Enzyme-linked immunosorbent assay
FCS	Foetal calf serum
GTPV	Goatpox virus
Hluhluwe-Imfolozi	HiP
Hrs	Hours
I-ELISA	Indirect ELISA
IFAT	Indirect fluorescent antibody test
IQC	Internal quality control
KNP	Kruger National Park
LCL	Lower control limit
LK	Lamb kidney cells
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
MEM	Minimum essential medium
OD	Optical density
OIE	Office International des Epizooties
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PP	Percentage positive
rNP	Recombinant nucleoprotein
rt-PCR	Real-time PCR
RT-PCR	Reverse transcriptase PCR
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SD	Standard deviation
SNS	Smithburn neurotropic strain
SNT	Serum virus neutralisation test
SOP	Standard operating procedure
SPPV	Sheeppox virus
$TCID_{50}$	Tissue culture infective dose 50
TMB	3,3',5,5'–TetramethylbenzidineDihydrochloride
TST	Tris-Saline-Tween
UCL	Upper control limit
VI	Virus isolation

List of Figures and Tables

List of Figures

List of Tables

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Abstract

Lumpy skin disease (LSD) and Rift Valley fever (RVF) are transboundary viral diseases occurring in Africa and the Middle East (e.g. Israel, Saudi Arabia and Yemen) with increasing potential for global spread. Although the role of wildlife in the epidemiology of these diseases is still not clearly understood, the African buffalo (*Syncerus caffer*) is thought to play a role in the epidemiology of these diseases. This study sought to expand our understanding of the role of buffalo in the maintenance of RVF and LSD by determining seroprevalence to these viral diseases in buffalo during the inter-epidemic period.

Lumpy skin disease is endemic in Africa, and has spread to the Middle East (e.g. Israel); consequently there is a high risk of lumpy skin disease virus (LSDV) expanding its geographical distribution to other areas and due to its economic importance it is included in the list of Notifiable Diseases of the World Organization of Animal Health (OIE).

The African buffalo is also suspected to play a role in the epidemiology of RVF. Like LSD, RVF was, until recently, only endemic in Africa. However, it spread to the Arabian Peninsula (Saudi Arabia and Yemen) in 2000 exacerbating concerns that it will extend to other regions of the world. Studies have already established that competent mosquito vectors for RVFV exist in North America and other parts of the world.

A total of 248 buffalo sera was tested for antibodies to capripoxviruses and neutralising antibodies against LSDV and RVFV using an indirect enzyme-linked immunosorbent assay (I-ELISA) as well as the serum neutralisation test (SNT). The samples were obtained from the Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HiP) in South Africa.

The prevalence of antibodies to LSDV and RVFV in the sera tested was 70/248 (28.2%) and 15/248 (6.1%), respectively using an I-ELISA.

The LSDV I-ELISA, using a sheeppox virus as antigen, has not been validated for use in African buffalo. The high percentage of LSDV positive antibody results obtained in this study is however a concern. Results obtained is in contrast with other published results as well as results obtained with the SNT for antibodies against LSDV. The SNT is currently the gold standard for LSDV antibody testing. Using this test for LSDV in this study, 5/66 (7.6 %) samples tested positive.

The results of the RVF I-ELISA, which had previously been validated for use in the African buffalo, correlated with the SNT results. From 12 SNT RVFV-positive sera, 3 (25%) had very high SNT titres of 1:640. Neutralising antibody titres of more than 1:80 were found in 80% of the positive sera tested. Eleven buffaloes (4.4% of the total samples) also showed evidence of antibodies to both LSDV and RVFV.

The results obtained in this study complement other reports indicating the role of African buffalo in the epidemiology of these diseases during inter-epidemic periods.

CHAPTER 1
Literature review

1.1 Introduction

Both lumpy skin disease (LSD) and Rift Valley fever (RVF) are economically important viral diseases of cattle in Africa and have on occasion spread to Israel and the Middle East (RVF; Saudi Arabia and Yemen).

Lumpy skin disease is an infectious disease of cattle caused by a poxvirus and is characterized by fever, multiple firm, circumscribed skin nodules, and necrotic plaques in the mucous membranes (mainly of the upper respiratory tract and oral cavity), mastitis, orchitis and swelling of the peripheral lymph node s (Coetzer 2004). The African buffalo (*Syncerus caffer*) is thought to play a role in the epidemiology of LSD (Bray 2007).

Rift Valley fever is a peracute or acute mosquito-borne viral disease of domestic and wild ruminants characterized by necrotic hepatitis and a haemorrhagic state, with infections often inapparent or mild in wild animals. It is a zoonotic disease and infections in humans often present as an influenza-like illness; less than one per cent of cases develop complications such as a haemorrhagic state and encephalitis that may proceed to death of the patient (Swanepoel & Coetzer 2004). It was first documented in 1931 by Daubney, Hudson & Garnham (cited by Swanepoel & Coetzer 2004) in the Rift Valley of Kenya.

It is not clear how both these disease are maintained during inter-epidemic periods.

1.2 Aetiology

Lumpy skin disease

The aetiological agent of LSD is the lumpy skin disease virus (LSDV). The prototype strain is the Neethling strain, isolated in South Africa in 1944 (Alexander *et al*. 1957, cited by Coetzer 2004). It is a large (300 nm) pleomorphic, double-stranded, unsegmented DNA virus that is classified in the genus *Capripoxvirus* of the family *Poxviridae*, subfamily *Chordopoxvirinae* (vertebrate poxviruses). It has only one serotype and is closely related to goatpox (GTPV) and sheeppox viruses (SPPV), the only other members of the genus *Capripoxvirus*. Antigenic variation of field isolates of LSDV has not been reported (Davies & Otema 1981).

The LSDV exhibit a remarkable degree of resistance to a range of environment conditions. Weiss (1968) reported that the virus can survive for at least 33 days in skin lesions. More recently, Tuppurainen *et al*. (2005), using nucleic acid detection and virus isolation techniques, reported a longer period of survival of the virus in blood and skin of experimentally infected cattle. They succeeded in isolating LSDV from skin lesions as long as 39 days post-infection and detected viral DNA in skin biopsies for up to 92 days post-infection. The virus is inactivated in 2 hours at 56℃ degrees (OIE 2010a). It is phenol-labile (2% for 15 min) and is susceptible to highly alkaline or acid pH solutions, ether (20%), chloroform and formalin (1%) and can be inactivated by sodium dodecyl sulphate (10%) (Weiss 1968).

Rift Valley fever

The aetiological agent of RVF is the Rift Valley fever virus (RVFV), a pleomorphic, negative-sense, segmented single-stranded RNA virus from the genus *Phlebovirus* in the family *Bunyaviridae*. It has a tripartite genome with a large, medium and small segment (Rice *et al.* 1980). The spherical particle is about 80-100 nm in diameter and containing an envelope (Schmaljohn & Nichol 2006).

The virus is resistant to an alkaline pH but is inactivated by a pH of less than 6.8, and is sensitive to ether and chloroform. It can be inactivated by strong solutions of sodium or calcium hypochlorite with chlorine concentrations over 5000 ppm and is inactivated in serum heated to 56 ℃ for 2 hrs. Rift Valley fever virus survives in dried discharges as well as in 0.5% phenol at 4 ℃ for 6 months and can be recovered

from serum stored at 4℃ for several months. The virus survives in aerosols maintained at 23℃ and 50–85% humidity (World Organization for Animal Health, OIE 2008c). As is characteristic of *Bunyaviridae* viruses, treatment with lipid solvents and non-ionic detergents adversely affects its infectivity in arthropods and mammals since its virus membrane are removed (Obijeski & Murphy 1977).

Initial isolation of RVFV was achieved by inoculating lambs with the serum from a moribund sheep (Daubney *et al.* 1931, cited by Swanepoel & Coetzer 2004). Although pathogenic differences occur between different isolates, there are no significant antigenic differences between RVFV field isolates and laboratory passaged strains of diverse origins (Swanepoel & Coetzer 2004). Additionally, although there is evidence for reassortment, RVFV shows little genetic variation; this low genetic diversity is due to its highly conserved genome (Sall *et al.* 1999).

1.3 Epidemiology

1.3.1 Occurrence

Lumpy skin disease

Lumpy skin disease was first reported in Zambia in the late 1930s (MacDonald 1931, cited by Coetzer 2004), and was later documented in Botswana in 1941 (Backström 1945, cited by Coetzer 2004) and by 1944, in South Africa (Thomas *et al*. 1945, cited by Coetzer 2004). This economically devastating epidemic lasted until 1949, affecting some 8 million cattle (Thomas & Mare 1945; Backström 1945, cited by Davies 1991a). The virus has spread throughout sub-Saharan Africa and its epidemiology is characterised by periodic outbreaks (Davies 1991a; Davies 1991b). Outbreaks in Egypt were reported by Ali *et al.* (1990) and another, confirmed by viral isolation, was reported by House *et al.* (1990). A more recent outbreak in Egypt in Asian water buffaloes were reported by Sharawi & Abd El-Rahim (2011).

The epidemics of 1989/90 and 2000/01 in South Africa and most other countries of southern Africa were particularly severe and affected large numbers of cattle. Unfortunately, there are no accurate statistics on these epidemics (Coetzer 2004). More recently, LSD outbreaks were reported in 2010 in the Eastern Cape,

Mpumalanga, Limpopo, Free State, Gauteng, Western Cape and North-West Provinces of South Africa (OIE 2010b).

While the updated OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2010a) indicates that recent outbreaks outside Africa occurred in the Middle East in 2006 and 2007, earlier data indicated that LSD has been reported in Israel (Abraham & Zissman 1991), and recently in Bahrain, Kuwait, Oman and United Arab Emirates (OIE 2008a). Additionally, there is a documented case of capripox infection of a captive bred Arabian Oryx (*Oryx leucoryx*) in Saudi Arabia (Greth *et al*. 1992). This report, and other similar ones in the Middle East, has been classified as unconfirmed due to lack of virus isolation (Coetzer 2004; Orlova *et al.* 2006; Babiuk *et al.* 2008a). This lack of virus isolation and the possibility of cross-reactions between capripoxviruses, raised concerns as to whether the Arabian Oryx infection was not due to GTPV or SPPV. In the past, capripoxvirus outbreaks have been reported to be endemic in sheep and goats in Oman and Yemen (Kitching *et al.* 1986). Recently, a laboratory unconfirmed outbreak of LSD on a dairy farm in Oman affecting up to 35% of the total herd with 12% fatality was reported by Kumar (2011).

Rift Valley fever

Since 1931, severe outbreaks of RVF have occurred across Africa in countries such as Kenya, South Africa, Mauritania, Senegal and Egypt (Swanepoel & Coetzer 2004). Smaller outbreaks, periodic isolations of virus or serologic evidence of the occurrence of RVF have been recorded in other African countries including Cameroun, Chad and Nigeria (Tomori *et al.* 1988; Zeller *et al.* 1995; Olaleye *et al.* 1996a; Olaleye *et al.*1996b; Durand *et al.* 2003; Ringot *et al.* 2004; Lebreton *et al.* 2006). Despite serological evidence of RVFV in livestock and humans in places such as Nigeria (Tomori *et al.* 1988), outbreaks of clinical disease have not been reported.

Madagascar also experienced animal and human outbreaks in the early 1990s and more recently in 2008/2009 (Morvan *et al.* 1992b, Morvan *et al.* 1992a; Andriamandimby *et al*. 2010). In 2000, the simultaneous RVF outbreak in Saudi Arabia and Yemen, with extensive animal and human impact, was the first time RVF was recorded in the Arabian Peninsula (Shoemaker *et al*. 2002).

A major change in the usual distribution of RVF occurred during the first major outbreak of the disease outside sub-Saharan Africa, in Egypt in 1977-78 (Imam *et al.* 1979a; Meegan *et al.* 1979). The outbreak of RVF in Egypt in 1977 was accompanied by extensive human involvement (Abdel Wahab *et al.* 1978; Imam *et al.* 1979b; Laughlin *et al.* 1979; Siam *et al.* 1980). There was also another, even more extensive outbreak in Egypt in 1993 (Arthur *et al.* 1993). Recently large outbreaks of RVF involving humans and livestock occurred in East Africa (Sall *et al.* 1998; Woods *et al.* 2002; Bird *et al.* 2008) and the Arabian Peninsula (Shoemaker *et al.* 2002; Al-Hazmi 2003).

From 2007 to 2010, outbreaks have occurred mainly in Kenya, South Africa, Sudan and Madagascar (ProMED 2007; ProMED 2008; OIE 2008b; Bird *et al.* 2008; Andriamandimby *et al*. 2010; Paweska *et al*. 2010).

In South Africa the first outbreak of RVF was recorded and confirmed in 1950 (Alexander 1951). During 1974-75 South Africa experienced the second big epidemic of RVF in sheep and cattle which was preceded by wet climatic conditions that favour the breeding and subsequent increase in the insect vector population (Coetzer 1977; Coetzer & Ishak 1982). Since 2008 until 2010, RVF outbreaks occurred every year in South Africa, affecting mainly four neighbouring provinces (Mpumalanga, Limpopo, Gauteng and North-West Provinces) (OIE 2008b; OIE 2010c).

1.3.2 Host range

Lumpy skin disease

Lumpy skin disease virus is a viral infection of cattle of all age groups. In Africa, imported *Bos taurus* breeds appear to be more susceptible than indigenous *Bos indicus* cattle (Davies 1991b). While cattle are the definitive hosts, LSDV has been associated with an outbreak of capripox infection in Kenyan sheep (Burdin & Prydie 1959) and later confirmed by Kitching *et al.* (1989). Clinical cases have been documented in Asian water buffalo (*Bubalus bubalis*) in Egypt (Ali *et al*. 1990) but these cases were not serologically or virologically confirmed. In the same year, another publication from Egypt documented clinical disease in cattle where the virus was successfully isolated (House *et al*. 1990). Antibodies have been demonstrated in black wildebeest (*Connochaetes gnou*), blue wildebeest (*Connochaetes taurinus*),

eland (*Taurotragus oryx*) giraffe (*Giraffa camelopardalis*), impala (*Aepyceros melampus*), greater kudu (*Tragelaphus strepsiceros*), reedbuck (*Redunca arundinum*), springbok (*Antidorcas marsupialis*), and waterbuck (*Kobus ellipsiprymnus*) (Hedger & Hamblin 1983; Barnard 1997). Giraffe and impala were proven to be highly susceptible by experimental infection (Young *et al*. 1970). However, these authors did not observe clinical signs or viraemia in 2 young experimentally infected African buffalo (*Syncerus caffer*) calves. These negative results, in a very small population of African buffaloes, correspond to later results obtained by Hedger & Hamblin (1983) and unpublished data of Howell & Coetzer cited by Coetzer 2004). However, seropositive African buffaloes have been detected in a LSDV-endemic area in Kenya (Davies 1982).

Rift Valley fever

This disease primarily affects domesticated ruminants (cattle, sheep and goats). Cattle indigenous to Africa are thought to be just as susceptible as imported breeds. Clinical disease, resulting in abortions and neonatal mortalities was reported in camels (Davies & Martin 2003). Clinical signs of RVF were also reported in camels in the 2006/2007 Kenyan and the 2010 Mauritania outbreaks (Munyua *et al*. 2010; El Mamy *et al*. 2011). Birds and pigs are not affected (Davies & Martin 2003). Clinical disease due to RVFV infection does occur in humans (Swanepoel & Coetzer 2004).

The role of wildlife in the epidemiology of RVF remains unresolved (Gerdes 2004; Swanepoel & Coetzer 2004; Evans *et al.* 2008). Neutralising antibodies to RVFV were demonstrated in African buffalo, black rhino (*Diceros bicornis*), lesser kudu (*Tragelaphus imberbis*), impala, African elephant (*Loxodonta africana*), kongoni (*Alcelaphus buselaphus cokii*) and waterbuck in Kenya during a time when no outbreak occurred. The highest titres observed were mainly in buffalo, from animals born during this time (Evans *et al.* 2008). These studies suggested that wild ungulates, such as the African buffalo may serve as amplifying hosts.

Experimental RVF infection of a seven month old African buffalo in Kenya produced fever and malaise (Daubney 1932, cited in Swanepoel & Coetzer 2004), and in another experiment 4 of 5 individuals exhibited transient viraemia and one of two females aborted (Davies & Karstad 1981). In 1999, a RVF outbreak caused abortion

in 6 African buffalo females held in breeding pens in the Kruger National Park (KNP), South Africa (Swanepoel 1999). More recently, an outbreak with clinical cases of RVF in African buffaloes characterized by abortions was reported in the Mpumalanga Province, South Africa (OIE 2008b).

1.3.3 Transmission

Lumpy skin disease

The results of transmission studies are not clear; while one study showed that *Aedes aegypti* is capable of mechanical transmission of LSDV (Chihota *et al.* 2001), another failed to achieve LSDV transmission from infected to susceptible cattle using mosquitoes (*Anopheles stephensi*), the stable fly (*Stomoxys calcitrans*) and the biting midge (*Culicoides nubeculosus*) (Chihota *et al.* 2003). These inconclusive results may be due to low levels of viraemia in the blood of infected animals that contribute to the inefficient transmission of LSDV by biting flies feeding on blood alone (Carn & Kitching 1995). Recently, Tuppurainen *et al.* (2010) reported the potential role of ixodid ticks in the transmission of LSDV.

It is thought that weather changes such as cold spells may adversely affect insect vector populations and thus reduce LSDV transmission, but although LSD may spread in the absence of insects, direct transmission by contact between animals is also thought to be inefficient (Davies 1991a). Infected saliva may, however, contribute towards the spread of the disease (Haig 1957). The disease is also rarely transmitted to suckling calves through infected milk (Weiss 1968). Other sources of LSDV are infected skin lesions where the virus may persist for more than 38 days (Weiss 1968) and more recently it was demonstrated for more than 90 days (Tuppurainen *et al.* 2005). The virus has also been shown to be present in semen: however, the role of semen in the transmission of the virus is not clear (Tuppurainen *et al.* 2005; Bagla *et al*. 2006; Annandale *et al.* 2010).

Rift Valley fever

Rift Valley fever is an arboviral disease transmitted by mosquitoes, mainly *Aedes* spp. of the *Neomelaniconium* group. Transovarial transmission has been established and the eggs of aedine mosquitoes serve as the ‘reservoir’ of RVFV (Linthicum *et al.* 1985). Several other genera of haematophagous mosquitoes such as *Anopheles,*

Culex, Eretmapodites and Mansonia can transmit RVFV as biological competent vectors. Mechanical spread by biting insects is also possible (Davies & Martin 2003). In humans, raw milk consumption and processing of meat has been associated with RVFV transmission (Mohamed *et al.* 2010; Labeaud *et al.* 2011). Vertical transmission from mother to neonate has been reported in humans (Arishi *et al.* 2006).

Outbreaks of RVF in sub-Saharan Africa are usually associated with abnormally heavy rainfall with sustained flooding and the simultaneous emergence of large numbers of aedine mosquitoes (Linthicum *et al.* 1985). After virus amplification in vertebrates, mosquito species such as *Culex* and *Anopheles* spp. act as secondary vectors to sustain the epidemic (Linthicum *et al.* 1985; Gerdes 2004). In South Africa, severe RVF epidemics in the 1950's and mid 1970's followed periods of above average rainfall (McIntosh & Jupp 1981; Jupp 2004). In northern and western Africa, outbreaks have occurred independent of rainfall; transmission have been by mosquitoes that breed in large rivers and dams, rather than floodwater aedines (Gerdes 2004).

The role of wildlife in the transmission of RVFV remains not clear (Gerdes 2004; Swanepoel & Coetzer 2004; Evans *et al.* 2008). As discussed earlier, studies have suggested that wild ungulates may serve as amplifying hosts and may therefore play a role in the transmission of the disease.

Virus activity and transmission of virus during inter-epidemic periods have been described in Kenya by isolating the virus from mosquitoes and IgG-positive animals during this period (Linthicum *et al.* 1985; Rostal *et al*. 2010). Studies on archived human sera collected during an inter-epidemic period from defined populations in Kenya were tested and seroconversion in humans could be demonstrated. IgG was detected in young children born after the 1997/98 epidemic, suggesting low level RVFV transmission possibly from wild ruminants and mediated by mosquito vectors during the inter-epidemic periods (LaBeaud *et al*. 2007; LaBeaud *et al*. 2008).

1.4 Clinical signs

Lumpy skin disease

The clinical manifestations of LSDV in experimental and naturally occurring infections have been documented (Prozesky & Barnard 1982; Davies 1991b; Carn & Kitching 1995; Tuppurainen *et al*. 2005; Babiuk *et al*. 2008b) and reviewed by Coetzer (2004) and Babiuk *et al.* (2008a).

Under field conditions, the incubation period is 2 to 4 weeks (Haig 1957); with experimental inoculation, it is between 7 and 14 days (Carn & Kitching 1995, Tuppurainen *et al*. 2005). Clinical disease is characterized by a biphasic febrile reaction that can reach 41 ℃. This may persist for 7 days (OIE 2009). Clinical signs observed during this stage include salivation, lachrymation and mucopurulent nasal discharge. Ocular lesions in some cases may become advanced including conjunctivitis followed by lachrymation and may eventually lead to blindness (Coetzer 2004). In most animals, the superficial lymph nodes are enlarged (Davies 1991a).

Skin nodules are classical manifestations of LSD and have been well described (Coetzer 2004; Babiuk *et al*. 2008b). These nodules are usually widespread and may include the genitalia, udder, perineum, vulva, ears, limbs and skin around the head. These nodules can be 2-5 cm in diameter and necrotic skin lesions may extend from the dermis and hypodermis into the surrounding tissues (Prozesky & Barnard 1982).

Rift Valley fever

With severe RVF, affecting mainly sheep and goats, lethargy, inappetence as well as abortions are present. Neonate lambs are most susceptible followed by neonate kids and calves. The disease is short-lived in lambs: the incubation period, usually between 12-36 hours, is followed by pyrexia of over 41 ℃ that progresses to death within 36-72 hours (Erasmus & Coetzer 1981). Mortality in neonate lambs less than a week old can be over 90%. Icterus, lachrymation, salivation and dysgalactia can be observed in calves and in adult cattle, although not always apparent in adult cattle. Mortality can reach 10% in cattle and 20% in calves (Coetzer & Swanepoel 2004). Abortion has been reported in experimentally and naturally infected African buffaloes (Davies & Karstad 1981; OIE 2008b). It is not known whether other clinical signs occur in this species in the wild.

1.5 Diagnosis

Lumpy skin disease

A presumptive diagnosis of the disease can be made based on clinical signs. It has a variable morbidity rate, ranging between 5-85%; mortality rates are variable but usually less than 10% (OIE 2009).

Various diagnostic techniques are used to identify the virus in samples including transmission electron microscopy, immunohistochemistry, virus isolation in cell cultures, direct and indirect fluorescent antibody test (DFAT/IFAT), agar gel immunodiffusion and enzyme-linked immunosorbent assay (ELISA), Western blot and serum neutralisation test (SNT). Molecular diagnostic methods being used include conventional PCR (Ireland & Binepal 1998; Tuppurainen *et al.* 2005; Orlova *et al*. 2006; Stram *et al.* 2008), real-time PCR (Babiuk *et al.* 2008b) and dot blot hybridization (Awad *et al.* 2010).

Immunohistochemistry, using immunoperoxidase staining, can be used to visualise LSDV antigens in infected tissues (Babiuk *et al.* 2008b; Annandale *et al.* 2010). This method is laborious, time-consuming and not a high throughput assay and therefore not easily used to screen large animal populations.

Transmission electron microscopy is the most rapid diagnostic technique and permits reliable detection of LSDV particles in fresh or formalin-preserved samples (Woods 1988). It has been used in outbreaks (Nawathe *et al.*1978; Khalafalla *et al.* 1993) as well as experimental infections (Aspden *et al.* 2003; Tuppurainen *et al.* 2005). It has the advantage of not requiring specific reagents, which is not the case with serological and molecular tests (Goldsmith & Miller 2009). However, access to a transmission electron microscopy as well as a competent microscopist may not be available in most LSD endemic countries (Zheng *et al.* 2007). Unlike serological and molecular tests, it is not suitable for primary screening of large number of samples. Furthermore, it cannot differentiate between SPPV, GTPV and LSDV (Kitching & Smale 1986). Lastly, where orthopoxviruses are endemic in cattle (Yeruham *et al.* 1996; Singh *et al.* 2008), transmission electron microscopy can only differentiate between these viruses and LSDV when specific immunological staining techniques are used (Babiuk *et al.* 2008a).

The use of virus isolation (VI) to detect LSDV and the cell lines used has been summarised in the literature (Binepal *et al*. 2001). The LSDV is commonly isolated using primary lamb kidney (LK) or primary lamb testis cells. Foetal lung, skin, muscle and endothelial cells can also be used (Davies 1991a; Binepal *et al*. 2001). Growth is indicated by the development of cytopathic effect (CPE) which may become evident after 4 to 10 days in most cell cultures (Davies 1991a). Primary cell culture of bovine dermis cells (BDC) prepared from a foetal calf's ear can be used to isolate LSDV (Tuppurainen *et al*. 2005; Bagla *et al*. 2006). An ovine testis cell line (OA3.Ts) for LSDV isolation was recently evaluated and the observed CPE were similar to those obtained with the commonly used primary LK cells (Babiuk *et al.* 2007). Distinct viral plaques indicative of LSDV growth could be detected in this cell line by immunostaining with capripoxvirus-specific antiserum. Lumpy skin disease virus can be isolated from nodular skin lesions, ocular, nasal and saliva swabs and buffy coat (Carn & Kitching 1995). Although the use of VI techniques to isolate LSDV from semen is not very sensitive (Irons *et al*. 2005), VI tends to be in general more sensitive than rapid antigen assays and less expensive than molecular tests (Leland & Ginocchio 2007).

Various PCR protocols for detecting capripoxvirus nucleic acid material are available: some use conventional PCR (Ireland & Binepal 1998) while others use real-time PCR (Balinsky *et al.* 2008; Bowden *et al.* 2008). Studies have shown that real-time PCR detects capripoxvirus viraemia (viral DNA) earlier than VI (Bowden *et al.* 2008). Published data suggests that real-time PCR is more sensitive than conventional PCR in detecting capripoxviruses (Babiuk *et al*. 2008a; Balamurugan *et al.* 2009) and specifically for the detection of LSDV (Babiuk *et al*. 2008b). A disadvantage of PCR protocols is the detection of false positives due to reagent contamination with traces of capripox DNA from various sources (Ireland & Binepal 1998). The PCR technique has been used to detect LSDV in semen and testicular tissue (Irons *et al*. 2005) and it has been shown that PCR is much more sensitive than VI in detecting LSDV in semen as bovine semen is toxic to cell cultures (Tuppurainen *et al.* 2005; Bagla *et al*. 2006). In general, the literature shows that PCR can be used for high throughput work: although not cheaper than ELISA's, it is more sensitive and can detect LSDV nucleic acid in skin samples post viraemia.

The SNT is the most specific serological test and gold standard for detecting antibodies against LSDV (Babiuk *et al*. 2008a; OIE 2010a). Although it can be used to perform retrospective serosurveillance, it is very time consuming to perform. The sensitivity of the SNT in the presence of low levels of neutralizing antibodies in tested sera has been reported and should always be considered when interpreting results (Babiuk *et al*. 2008a). Therefore, a negative result does not necessarily indicate the animal has not been exposed to the virus. This is due to the fact that LSDV infection predominantly provokes a cell-mediated immune response (OIE 2010a). At present, a SNT utilising a recombinant protein is being evaluated: preliminary indications are that it reduces virus neutralisation detection from 6 to 2 days (Babiuk *et al*. 2008a).

Fluorescent antibody techniques can be used to detect LSDV (Davies & Otema 1978; Gari *et al.* 2008). However, these techniques are prone to cross reaction with other parapoxviruses. Such cross reactions have however not been observed with SNT (Davies & Otema 1981). The indirect fluorescent antibody technique is more time consuming than ELISAs (Gari *et al.* 2008); it is also less specific than the SNT (OIE 2010a). Additionally, Western blotting may be used to detect LSDV with reliable specificity and sensitivity: however, these assays are expensive and need specialized equipment and training to be performed (Chand *et al.* 1994; OIE 2010a).

Various ELISA protocols have been developed for use in detecting LSDV infection: An indirect ELISA (I-ELISA) was developed using an expressed recombinant capripoxvirus protein as antigen (Carn *et al.* 1994). Carn (1995) later designed an antigen trapping ELISA used to detect LSDV in the supernatant of cell cultures and skin biopsy samples. The detector system was a guinea-pig polyclonal antiserum raised against the recombinant capripoxvirus structural protein, P32. The advantages of this ELISA, especially in developing countries in Africa where LSD is endemic, include reduced costs, stability of reagents as well as easy handling. It can also be used to differentiate between buffalopox virus and LSDV in water buffalo *(Bubalus bubalis)*. The results of this ELISA correlated well with VI, though it was less sensitive. Another similar ELISA protocol developed in Australia using recombinant P32 protein as coating antigen (Heine *et al.* 1999) permits discrimination between capripox-, parapox (orf) and orthopoxvirus (vaccinia virus) infections. These protocols use non-infectious antigens and can therefore be used in non-endemic countries.

Recently, Babiuk *et al.* (2009) validated an ELISA that detects LSDV antibodies using an inactivated SPPV virus. Compared to the SNT and Western blotting, it is easier to perform and less time consuming. Unlike the SNT, it does not require live LSDV and BSL-3 facilities in LSD-free countries. Results can also be obtained within a day as opposed to the 6 days it takes to read the results from SNT. It compares well with the SNT detecting LSDV antibodies in experimentally tested cattle as early as 21 days post-infection. However, the SNT proved to be slightly more specific. To avoid the cost and complex quality issues associated with producing the inactivated antigen used in this new ELISA, it is envisaged that these antigens will soon be replaced by recombinant immunodominant capripoxvirus proteins (Babiuk *et al.* 2009).

Rift Valley fever

A presumptive diagnosis of RVFV infection is based on abortions in goats, sheep and cattle and fatalities in especially young animals. Acute febrile conditions in livestock workers may also be observed simultaneously (Swanepoel & Coetzer 2004). Necrotic hepatitis and widespread haemorrhages are evident. The histopathological lesions in the liver are highly pathognomonic (Coetzer 1977; Coetzer 1982; Coetzer & Ishak 1982). Using immunohistochemistry, RVFV can be demonstrated in tissues obtained from dead animals (Van der Lugt *et al.* 1996).

Virus isolation procedures, considered the method of choice for the identification of RVFV, can be achieved using various cell lines – Vero, mosquito cell lines, primary calf, lamb and goat kidney cells or in suckling or weaned mice (Swanepoel 1981; Shimshony & Barzilai 1983; Digoutte *et al.* 1989; Swanepoel *et al.* 1986). Virus growth in cell cultrures can be rapidly identified using IFAT (Davies 1975). Mice are inoculated via the intracerebral route with field material and die 2-5 days post-inoculation (Swanepoel 1981).

Various approaches for the detection of RVFV nucleic acid in human, animal and vector samples have been reported (Drosten *et al.* 2003; Bird *et al.* 2009). These protocols include the use of reverse transcriptase-polymerase chain reaction (RT-PCR) (Ibrahim *et al.* 1997; Jupp *et al.* 2000); nested-PCR (Ibrahim *et al.* 1997; Sall *et al.* 2001; Sall *et al.* 2002; Sánchez-Seco *et al.* 2003); quantitative real-time reverse transcriptase-PCR [qrt RT-PCR] (Garcia *et al.* 2001; Drosten *et al.* 2002; Bird *et al.*

2007; Näslund *et al.* 2008; Njenga *et al.* 2009); real-time reverse-transcription loop-mediated isothermal amplification (RT-LAMP) (Peyrefitte *et al.* 2008; Le Roux *et al.* 2009) and MassTag PCR (Palacios *et al.* 2006).

During the Kenyan outbreak of 2006-2007, a qRT-PCR protocol was successfully used to correlate high viraemia with RVF fatality, thereby identifying almost all fatal cases (Njenga *et al.* 2009). A nested RVFV RT-PCR was used to evaluate samples from the 1998 Mauritania outbreak and gave a sensitivity of 70.6% (Sall *et al.* 2002). A RT-LAMP assay was recently used and obtained similar results as with VI (Le Roux *et al.* 2009). This assay detects RVFV nucleic acid in samples within 30 minutes and it can be operated as a portable device or in less well-equipped laboratories.

Rift Valley fever virus activity can also be demonstrated by the detection of RVFV-specific IgM or IgG in animal or human sera. To achieve this, various modifications of the ELISA are commonly used (Paweska *et al.* 2003a; Paweska *et al.* 2003b; Paweska *et al.* 2005b). The ELISA's have largely replaced previously established methods such as the agar gel immunodiffusion, complement fixation, haemagglutination inhibition and plaque-reduction neutralisation (Flick & Bouloy 2005). Various ELISA's with new highly specific monoclonal antibodies (Zaki *et al.* 2006) or recombinant proteins (Jansen van Vuren *et al.* 2007) have been used in large serosurveys. In particular, a recombinant nucleoprotein (rNP) of RVFV has served as a diagnostic antigen in a validated I-ELISA for humans, domestic ruminants and the African buffalo (Paweska *et al.* 2007; Fafetine *et al.* 2007; Paweska *et al.* 2008). This rNP has been successfully used in an I-ELISA to detect RVFV antibodies in buffaloes in Kenya during an inter-epidemic period (Evans *et al.* 2008). Additionally, to establish whether there was RVF transmission in humans in the same inter-epidemic period, another group of workers in Kenya successfully used an IgG ELISA, with the MP-12 RVFV vaccine strain as test antigen. All the ELISA positive samples were confirmed using a plaque reduction neutralisation test (LaBeaud *et al.* 2008).

From the literature, it is clear that the role of wildlife, specifically buffalo, in the epidemiology of LSD and RVF in endemic areas requires further investigation.

Therefore the objective of this study was:

- To detect the presence of antibodies to RVFV and LSDV in stored buffalo sera obtained from the KNP and HiP, South Africa using ELISA and SNT

CHAPTER 2
Materials and Methods

2.1 Sample collection

Serum samples were collected between 2003 and 2004 from buffaloes during a routine examination of animals in the KNP and Hluhluwe-iMfolozi, South Africa. Samples were collected from three areas in the KNP; Lower Sabie (twice sampled, once in 2003 and 2004), Gudzani Dam and Satara and from the HiP in KZN Province. These samples, (Table 1), were stored at -20 ℃ since 2003 at the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, University of Pretoria and were tested for the presence of LSDV and RVFV antibodies.

Table 1. Information on the buffalo sera used in this study

Location	Number of samples	GPS coordinate	Month/Year Sampled
Lower Sabie 2003	51	**25^0.12' S; 31^0 92' E**	07/2003
Lower Sabie 2004	41	**25^0.12' S; 31^0 92' E**	09/2004
Gudzani Dam	25	**24^0.37' S; 31^0 93' E**	08/2003
Satara	21	**24^0.39' S; 31^0 78' E**	04/2003
Hluhluwe-iMfolozi	110	**28°.28' S; 30°86' E**	09/2004
Total	**248**		

The KNP is the largest wildlife reserve in South Africa, having an area of almost 20 000 km^2. From its northern most point, at the Zimbabwean border, to its southern tip, the KNP is 320 km long. From its western limits to the eastern border, shared with Mozambique, it is up to 65 km wide. The vegetational cover and precipitation of the KNP have been described in the literature (Gertenbach 1983; Venter & Gertenbach 1986). The KNP as a whole is located in the Transvaal Lowveld and characterised as being a deciduous savanna (Venter & Gertenbach 1986). The vegetation of the Lower Sabie area consists of the Knobthorn Savanna and Lebombo Bushveld ecozones (Gertenbach 1983). In the Lower Sabie region, the average annual amount of rainfall is between 400-500 mm. The rainfall and temperature patterns are

unimodal in distribution: as such, the area experiences a warm wet season that occurs between October and March and a cool dry season between April and September (Gertenbach 1983). It provides habitat for more than 147 mammalian species including about 2500 African buffaloes (South African National Parks - SANPARK). The Lower Sabie rest camp is named after the Sabie River, one of the perennial rivers that flows through the KNP (Rogers & Biggs 1999).

The HiP is the third largest game reserve in South Africa and is about 900 km^2 in size. The land is covered mainly by savanna grassland and its buffalo population (roughly about 3000), form stable herds (Dora 2004).

The sites where samples were collected for this study are outlined in Figure 1.

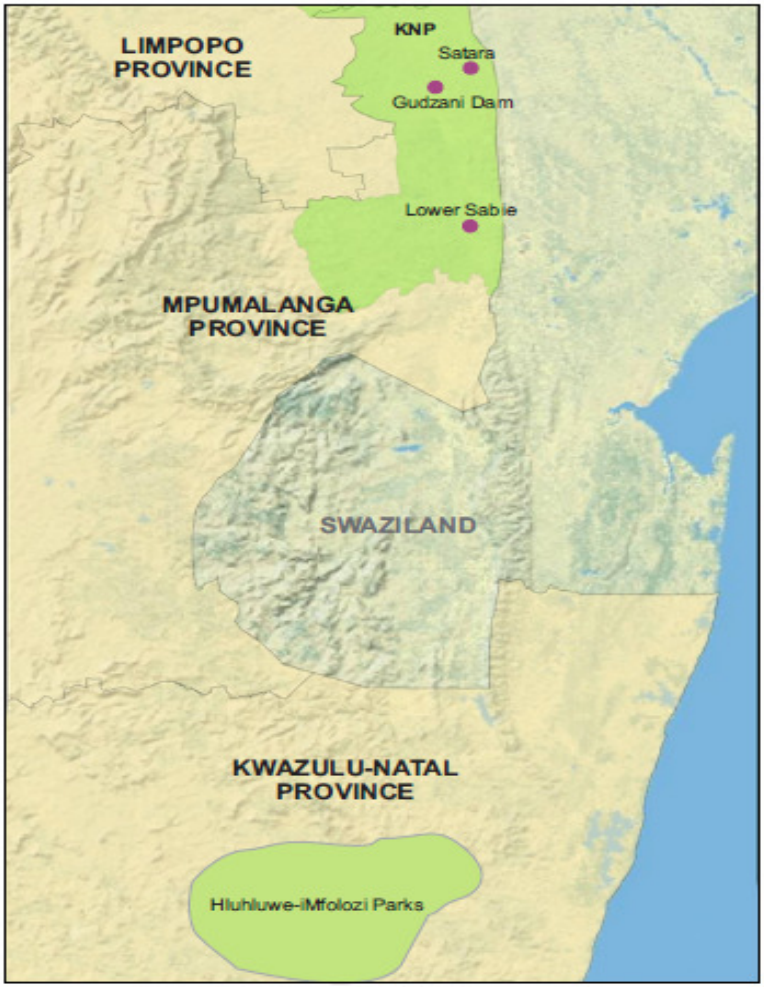

Figure 1. Sites where samples were collected in the KNP and the HiP. The dots indicate areas of sampling.

2.2 Laboratory tests

2.2.1 I-ELISA

2.2.1.1 Lumpy skin disease virus IgG Indirect ELISA

The I-ELISA used was carried out as described by Babiuk *et al.* (2009) with minor modifications.

The NUNC polysorb ELISA plates were coated with 100 µl /well of antigen diluted in 0.05 M carbonate-bicarbonate ELISA coating buffer. The antigen used was an inactivated Nigerian SPPV virus purified by sucrose gradient centrifugation and heat inactivated at 60 ℃ and used at a dilution of 1: 100. The plates were sealed and incubated overnight at 4 ℃ and manually washed thrice with Tris-Saline-Tween (TST) [provided by the Agriculture Research Council – Onderstepoort Veterinary Institute (ARC-OVI)] using dispensed distilled water. The plates were then blocked by adding 200 µl/well of blocking buffer (skim milk), consisting of 6% milk made up in TST buffer. This was followed by incubation for 1 h at 37 ℃ on a plate shaker (Merck, SA) and another washing cycle.

To each plate well, a 1:100 dilution of test sera and controls were added. The plates were sealed and incubated for 1 h at 37 ℃ on a plate shaker and washed as mentioned earlier. Protein G-HRP (horseradish peroxidise- Zymed, San Francisco, CA) conjugate, diluted 1:5000 in blocking buffer, was then added to the plates at 100 µl per well. The sealed plates were incubated for 1 h at 37 ℃ on a plate shaker and again washed as before. A volume of 100 µl/well of the substrate - 3,3',5,5'–TetramethylbenzidineDihydrochloride (TMB)- was added and the plates were incubated at room temperature in the dark for 10 minutes. The reaction was stopped using 100 µl/well 1 M sulphuric acid (H_2S0_4., Merck, SA) and the optical density (OD) values determined at 450 nm on a plate reader. The cut-off value for the I-ELISA was +3SD (standard deviations) of the mean negative control.

Each buffalo serum sample was tested twice to achieve quality control. For each plate, there were three replicates of the positive and negative controls and four replicates of the conjugate controls (CC) - (see Appendix 1). The internal quality control (IQC) parameters -upper control limits (UCL) and lower control limits (LCL) -

were derived from the mean ± 2SD for replicates of each control (Paweska *et al.* 2003b). Furthermore, the coefficient of variation (CV) of the positive control (C++) on each plate was monitored not to exceed 20%.

2.2.1.2 Rift Valley fever virus IgG Indirect ELISA

This protocol was based on the I-ELISA developed by Jansen van Vuren *et al.* (2007) using a recombinant nucleocapsid protein and the kits were provided by the ARC-OVI. Briefly: All the reagents were allowed to reach room temperature prior to use. The milk buffer solution was prepared from milk powder (6%) diluted in 100 ml TST. The test and control sera were then diluted in a 1: 100 dilution using the milk buffer as is. Each serum was then tested in duplicate and 100 µl of diluted serum added to each well. The plate was incubated for 30 minutes at 37 ℃ and washed 3 x manually using a 1:100 dilution of TST buffer in a squash bottle. This was followed by the addition of 100 µl of conjugate solution to each well. The plate was then incubated for 30 minutes at 37 ℃ and washed as described above. A volume of 100 µl substrate solution was then added to each well, the plate was covered and incubated at room temperature in the dark, until the absorbance of the C++ wells reached a value of between 0.400 and 0.600 at 630 nm wavelength in a spectrophotometer.

To stop the reaction, 100 µl stop solution (1% sodium dodecyl sulphate) was added to each well and the plate was gently tapped. After a waiting period of 2 minutes, the plate was read using a spectrophotometer equipped with a 450 nm filter. The mean OD values were calculated into percentage positivity (PP) values for interpretation.

$$\%PP = \frac{\text{Mean OD of test sample-median OD of negative sample}}{\text{Median OD of positive sample-median OD of negative sample}} \times 100$$

Samples with PP values > 10 were regarded as positive. Lower PP values were interpreted to mean that lesser antibodies were detected in the samples

The IQC procedure previously described by Paweska *et al*. (2008) was used with minor adjustments. Each buffalo serum was tested twice. Each plate had four

replicates of each high-positive (C++), negative serum (C-) and conjugate (CC) controls- see Appendix 1. The following IQC rules had to be fulfilled: Firstly, at least 3 of the 4 net OD values of C++ replicates must fall within the valid IQC range of 0.700 (lower control limit) and 3.000 (upper control limit). If 2 or more of the 4 replicates of C++ fall outside IQC limits, the plate must be rejected and repeated. Secondly, the CV for the OD values of the two intermediate replicates of C++ must both be less than 20% (OVI- Shirley Smith-personal communication). The means and SD of the OD values and percentage positivity (PP) of internal controls for replicates on each plate are determined and used to evaluate intra- and inter-plate variation.

The CVs for the C++ controls were calculated using the formula

$$CV = \frac{\text{standard deviation of replicates}}{\text{mean of replicates}} \times 100$$

2.2.2 Serum virus neutralisation test

Selected positive and negative sera from the ELISA were tested by the SNT. The SNT procedures for both viruses were carried out following the SOP of the DVTD.

2.2.2.1 Lumpy skin disease virus

The procedure was carried out using 96-well flat-bottomed cell culture microtitre plates (NUNC). A 1:5 dilution of the test sera was made using phosphate buffered saline (PBS) containing Mg^{2+} and Ca^{2+} (PBS+) (Merck) and sera were inactivated at 56 ℃ for 30 minutes in a water bath. A series of six, two-fold dilutions was then made using MEM containing 5% foetal calf serum (Highveld Biological; Cat #3080) and 0.05 mg/ml gentamicin (Virbac Animal Health). A volume of 100 µl of each dilution was then added to the plate. To obtain a working virus concentration of $100TCID_{50}$, the stock virus was diluted (1:150) in MEM containing 5% foetal calf serum. A series of four, ten-fold dilutions was made from the $100TCID_{50}$ antigen, to be used as the virus control. From this dilution, 100 µl was added to each of the wells containing the diluted test sera. The plates were incubated for 1 h at 37 ℃ in a humid atmosphere of 5% CO_2. Bovine dermis cells (BDC) were harvested at 80% confluency and counted using a haemocytometer. The cell concentration was

modified to achieve a concentration of 480 000 cells/ml and 80 µl of this concentration was then added to each of the wells. To determine if the antigen titre of 100$TCID_{50}$ was correctly used, a back titration of the antigen was made as discussed in 2.2.2.3. The plates were then incubated at 37 ℃ in a humid atmosphere of 5% CO_2 with daily observations for 7 days using an inverted microscope. The end point was determined to be the dilution at which 50% of the cells were infected.

2.2.2.2 Rift Valley fever virus

The same procedure was used as described in 2.2.2.1 with minor modifications. Vero cells instead of bovine dermis cells were used and the stock virus was diluted (1:500) in MEM containing 5% foetal calf serum to obtain a 100$TCID_{50}$ of RVFV. To determine if the antigen titre of 100$TCID_{50}$ was correctly used, a back titration of the antigen was made as discussed in 2.2.2.3. The plates were then incubated at 37 ℃ in a humid atmosphere of 5% CO_2 and observed daily for 4 days.

2.2.2.3 Quality control of serum neutralisation test for Rift Valley fever and lumpy skin disease viruses

The cell culture control was set up in duplicate rows of 200 µl MEM containing 5% foetal calf serum and 80 µl of the cell suspension. The virus control was set up in two rows and six wells as follows: 100 µl MEM containing 5% foetal calf serum was added to all the wells; 100 µl of the 100$TCID_{50}$ virus was added to the first two wells; and 100 µl of the different 10 fold dilutions was added accordingly to the remaining four wells, starting with the highest virus dilution. Since there were 8 test sera per microplate, the virus control was done on the same plate as some, but not all, of the samples.

CHAPTER 3
Results

3.1 Indirect ELISA

A total of 248 serum samples were tested using the I-ELISA. Lumpy skin disease virus antibodies were detected in 28.2% (70/248) of samples. The highest prevalence was recorded in HiP where 35.5% (39/110) were positive. From a total of 138 samples taken in the KNP, Lower Sabie (2004) had the highest percentage of LSDV I-ELISA IgG (10/41; 24.4%) followed by Satara with 5 positive samples (5/21; (23.8%).

A total of 6.1% (15/248) of samples were positive using the RVF I-ELISA. The prevalence rate was the highest for samples collected at Lower Sabie in 2003 (6/51; 11.8%), followed by Satara (2/21; 9.5%).

A summary of the results is shown in Table 2.

Table 2. Indirect ELISA results for LSDV and RVFV

Location	Total number of samples	LSDV positive samples (%)	RVFV positive samples (%)
Lower Sabie 2003	51	13 (25.5)	6 (11.8)
Lower Sabie 2004	41	10 (24.4)	1 (2.4)
Gudzani Dam	25	3 (12.0)	1 (4.0)
Satara	21	5 (23.8)	2 (9.5)
Hluhluwe-iMfolozi Park	110	39 (35.5)	5 (4.5)
Total	**248**	**70** (28.2)	**15** (6.1)

3.2 Serum virus neutralisation test

A total of 66 samples for LSDV and 57 samples for RVF were tested for neutralising antibodies using the SNT. This included all ELISA positive samples, samples with borderline (PP) values and selected negative samples. Some samples were not retested by the SNT due to insufficient sera. Results are presented in Table 3. The raw data are provided in Appendix 1.

A total of 110 sera from HiP were tested for both viruses using the I-ELISA, but only 31(28.2%) were tested for LSDV and 19 (17.3%) for RVFV using SNT: none of these had LSDV neutralising antibodies; however 3 had RVFV antibodies.

From the total of 138 sera from the KNP tested by I-ELISA, only 35 (25.4%) and 38 (27.5%) were tested by SNT for LSDV and RVFV, respectively. In all, 5 sera were LSDV positive: 3 taken from Lower Sabie in 2003 and 2 from Gudzani dam. With the RVFV SNT test, only 9 sera were positive at KNP, most (n=7; 58.3%) of which were samples taken at Lower Sabie in 2003. None of the sera from Satara tested positive for LSDV (n=7) or RVFV (n=4) neutralising antibodies.

Table 3: Serum virus neutralisation results for LSDV and RVFV

	LSDV		RVFV	
Location	**Total number of samples***	**Positive samples (%)**	**Total number of samples****	**Positive samples (%)**
Lower Sabie 2003	14	3 (21.4)	16	7 (53.9)
Lower Sabie 2004	10	0 (0)	11	1 (9.1)
Gudzani Dam	4	2 (50)	7	1 (14.3)
Satara	7	0 (0)	4	0 (0)
Hluhluwe-iMfolozi Park	31	0 (0)	19	3 (15.8)
Total	**66**	**5** (7.6)	**57**	**12** (21.1)

*9 samples not tested due to insufficient sera- all were ELISA positive

**3 samples not tested due to insufficient sera- one ELISA positive and 2 ELISA negatives

3.3 Site based differences in prevalence of LSDV and RVFV IgG antibodies

The prevalence of LSDV and RVFV IgG antibodies varied according to the sampling sites as shown in Table 4. The lowest prevalence for LSDV and RVFV IgG were recorded around the Gudzani Dam site. The highest percentage of LSDV and RVFV positive sera came from HiP (58.2%) and Lower Sabie [2003] (25.5%), respectively. While 35.5% of the samples from HiP were LSDV positive, only 4.54% were found to have RVFV IgG antibodies. The lowest number of positive samples for both the LSDV and RVF I-ELISA was recorded for the samples obtained at Guzdani Dam, the site with the second smallest sample size.

Table 4. Prevalence of LSDV and RVFV IgG antibodies at different sites

	Site prevalence (%)		% of total positive samples	
Location	**LSDV**	**RVFV**	**LSDV (n=70)**	**RVFV (n=15)**
Lower Sabie (2003)	25.5	11.8	18.6	40.0
Lower Sabie (2004)	24.4	2.4	14.9	6.7
Gudzani Dam	12.0	4.0	4.5	6.7
Satara	23.8	9.5	7.5	13.3
Hluhluwe-iMfolozi Park	35.5	4.5	58.2	33.3
% of total samples	**27.0**	**6.1**	**100**	**100**

Lower Sabie was sampled twice and results for the two different years are shown in Table 5. In total for 2003 and 2004 Lower Sabie had a prevalence of 49.9% for LSDV and 14.2% for RVFV. The prevalence for LSDV between the years is insignificant, but a marked difference is observed for the presence of antibodies against RVFV.

Table 5. Prevalence of LSDV and RVFV IgG antibodies: Lower Sabie (2003 and 2004)

Location	Sample Size	LSDV % positive	RVFV % positive
Lower Sabie (2003)	51	25.5	11.8
Lower Sabie (2004)	41	24.4	2.4

3.4 Presence of antibodies to both LSDV and RVFV

From the 248 sera tested, 11 sera (4.4%) were positive for antibodies to both LSDV and RVFV by either the I-ELISA and/or SNT as shown in Table 6.

Table 6. Sera positive for both LSDV and RVFV IgG antibodies

		LSDV		RVF	
Sample	**Location**	**IgG**	**SNT**	**IgG**	**SNT**
1.	Satara	(+)	(-)	(+)	(-)
2.	Satara	(+)	(-)	(+)	(-)
3.	Sabie 2003	(+)	(+)	(+)	(+)
4.	Sabie 2003	(+)	(-)	(-)	(+)
5.	Sabie 2003	(+)	(+)	(-)	(+)
6.	Sabie 2003	(+)	(-)	(+)	(+)
7.	Sabie 2004	(+)	(-)	(+)	(+)
8.	HiP	(+)	(-)	(-)	(+)
9.	HiP	(+)	(-)	(+)	(-)
10.	HiP	(+)	(-)	(+)	(+)
11.	HiP	(+)	not tested	(+)	not tested

(+) - positive; (-) negative

From the 11 LSDV IgG-positive samples, 7 were RVFV SNT positive. Two LSDV SNT positive samples, both taken in 2003, were also RVFV SNT positive. Five of the eleven samples (5/11; 45.5%) were collected at Lower Sabie. Additionally, 4 of the 5 sera were positive for at least 3 of the 4 tests done: 1 was positive for all 4 tests (Table 6, highlighted in green). Three samples were only LSDV and RVFV IgG ELISA positive with negative SNT results. A fourth sample, from HiP, was not tested by SNT as the serum was insufficient. The 2 samples from Satara, both negative by LSDV and RVFV SNT, were taken from buffalo heifers. None of the samples from Gudzani dam had evidence of coinfection.

3.5 Internal quality control for the Indirect ELISA

The IQC data for the LSDV and RVFV IgG ELISA and the calculated CV (data not shown) indicate that intra-plate repeatability was maintained and the tests were thus successfully carried out.

CHAPTER 4
Discussion

Prior to 1989, LSD epidemics were rare and sporadic outbreaks involved a small number of cattle in a herd. Since 1990, this pattern changed with major outbreaks occurring in different areas of Africa and South Africa. Although clinical signs have not been reported in wildlife, evidence of previous infection with LSDV has been demonstrated in African buffaloes (Davies 1982). Wildlife has also been shown to be susceptible to experimental infection (Young *et al*. 1970).

During the past few years extensive (large) outbreaks of RVF have been recorded in Africa and the Middle East (Saudi Arabia and Yemen), and the burning question is still the maintenance of the virus during the inter-epidemic period. Previous research has demonstrated evidence of past RVFV infection in a wide range of wild and domestic animals in RVF-endemic regions of Africa (Gerdes 2004; Swanepoel & Coetzer 2004; Evans *et al.* 2008). Additionally, clinical disease associated with abortions has been recorded in African buffaloes in the KNP, South Africa (OIE 2008b).

The importance of these viral diseases is emphasized by the fact that their potential spread to naïve areas can adversely affect trade and in the case of RVF, human health (Australian Quarantine & Inspection Service 1999; EFSA 2005). Consequently, there is a pressing need to accurately delineate the probable sources of introduction of these viruses into such areas. To determine the prevalence of antibodies to both LSDV and RVFV, sera were collected in this study from buffaloes during the inter-epidemic period in the KNP and HiP, regions endemic for both diseases (Swanepoel 1999; National Department of Agriculture 2008), and were assayed for IgG antibodies against LSDV and RVFV using I-ELISA protocols. Selected positive and negative sera from the ELISA were then tested using the SNT.

Samples and habitat

Buffalo samples from the present study were obtained from the KNP and HiP of which the habitat of both areas is well described as being mainly savanna grassland.

The African buffaloes in the KNP have been shown to preferentially stay near water sources (Redfern *et al.* 2003). It is well known that their feeding behaviour is sensitive to access to food and water and that fluctuations in vegetation quality and water access do influence the ranging and eating behaviour of buffaloes (Funston *et al.* 1994). Additionally, one recent study of herds in the Lower Sabie showed that the average daily distance covered by herds is about 3.35 km (Ryan & Jordaan 2005). Apart from feeding and ranging behaviour that might affect exposure to LSDV and RVFV infections in buffaloes within the KNP, their nocturnal activities such as grazing may also determine exposure to arthropods (especially mosquitoes) that vector RVFV.

Indirect ELISA

Lumpy skin disease

From a total of 248 buffalo sera tested, 70 (28.2%) were positive using the I-ELISA published by Babiuk *et al.* (2009). Previous studies on the prevalence of antibodies against LSDV in African buffaloes include the studies of Davies (1982); Hedger & Hamblin (1983); Hamblin *et al.* (1990) and Barnard (1997). Data for these studies was collected over a period from 1963 to 1996. Apart from the study by Barnard that used an I-ELISA, all the other workers used the SNT, with Davies combining the SNT with IFAT.

Comparing the results of this study with previous studies mentioned, it could be suggested that the observed differences in ELISA results may be due to one or more of the following factors: sample size; sampling frequency; the true endemicity of LSD in the area sampled; the type of wildlife sampled; time during infection when the animals were sampled; and most importantly the testing platform employed.

The buffaloes tested by Barnard (1997) were the smallest sample size (n = 15) of all the studies. Since the buffaloes in his study were from the KNP, the buffaloes in this

study share the same vegetative ecology of the KNP. In contrast to the 70 positive buffalo sera in the present study, all the buffaloes tested by Barnard were negative. Of note is the fact that other wildlife (black wildebeest, blue wildebeest, impala, springbok and eland) tested by Barnard in the same study were positive for LSDV antibodies, indicating that LSD was endemic in the sample area. However, it is difficult to conclude that the buffalo sera tested by Barnard were truly representative of the potentially infected buffaloes in the area or that buffaloes are not susceptible to LSDV. Apart from the small sample size, Barnard also noted that the below average rainfall the years before and during his sampling period may have resulted in lowered insect populations.

Rift Valley fever

In contrast to the few studies that attempted to detect LSDV infection in buffaloes, the literature documents several studies on RVFV antibody detection using ELISA protocols (Barnard 1997; Anderson & Rowe 1998; Wolhuter *et al.* 2009). As with the present study, most of the previous studies (n=8) attempted to detect RVFV IgG antibodies in field collected buffalo sera.

A prevalence of 6.1% (15/248) was observed in the present study in the KNP and 4.5% of samples were RVFV IgG positive in HiP. Only three (Barnard 1997; Anderson & Rowe 1998; Wolhuter *et al.* 2009) of the previous studies are similar to the present study in regards to buffalo sera being taken during the inter-epidemic period. Of these three studies, the one from Anderson and Rowe (1998) was done outside the KNP (in Zimbabwe) and a total of 514 buffaloes were tested of which 34 (6.3%) had evidence of previous RVFV infection. The study by Wolhuter and co-workers (2009) did not describe the specific ELISA used and reported a higher seroprevalence of 57.6%. All 71 buffaloes tested by Barnard (1997) were negative.

In a study by Paweska *et al.* (2005b), 258 South African buffaloes were sampled (epidemic or inter-epidemic period were not indicated) and RVFV IgG antibodies could be detected in 53/258 (20.5%) of the samples using an I-ELISA. A sandwich ELISA was also used to detect RVFV in various tissues from 3 aborted buffalo foetuses associated with the 2008 RVF outbreak in South Africa (Jansen van Vuren & Paweska 2009).

Bird *et al*. (2008) could detect 4/26 (15.3%) buffalo specimens positive for IgM antibodies in a study where human, livestock and buffalo sera were collected during the 2006-2007 Kenyan outbreak.

In addition to the IgG ELISA, Anderson & Rowe (1998) also used a haemagglutination inhibition (HAI) protocol. Though the HAI is laborious and requires pre-treatment of serum, it has proven to be reliable (Swanepoel *et al.* 1986). It has been extensively used in the diagnosis of RVF (Swanepoel 1981), but this is the only record where buffalo samples were used. Anderson & Rowe (1998) concluded that the ELISA was more sensitive than HAI.

The circulation of RVFV in animals such as a range of wildlife species and sometimes in cattle may go unnoticed except when clinical cases are observed, as was the case when a RVF outbreak causing abortions occurred in captured buffalo females held in breeding pens in the KNP (Swanepoel 1999). Abortion has also been reported in a population of captive female buffaloes on a game farm in Ngwenya, (southern boundary of the KNP), Mpumalanga Province, South Africa (OIE 2008a).

Serum neutralisation test

Lumpy skin disease

In the present study, the SNT detected neutralising antibodies in 5/66 (7.6%) of samples, some of them with low titres of e.g. 1:20. All previous studies (n=4) evaluating the prevalence of LSD in African buffalo used the SNT, except the study of Davies (1982) who tested buffalo samples with the SNT and the IFAT. He detected neutralising antibodies in buffalo sera collected during epidemic and inter-epidemic periods in Kenya, Tanzania and Uganda. While he indicated that only a subset of the IFAT positive samples (150/254, 59% positive with IFAT) were positive for neutralising antibodies, the exact number is not given. However, similar to his work, the present study detected neutralising antibodies also only in a subset of samples that were positive by I-ELISA.

The study by Hamblin *et al.* (1990) had a much larger sample size and was more diverse than the present study. It involved more wild ruminants (n=8) from different game areas in Tanzania. The 370 sera tested for LSDV neutralising antibodies were

all negative. They were not equivocal as to whether their samples were collected during an epidemic or in an inter-epidemic period.

The largest documented LSDV survey is that of Hedger & Hamblin (1983). They tested samples collected between 1963 and 1982 from 11 sub-Saharan African countries and all samples were negative; only neutralising antibodies were tested and the authors did not state the prevalence of LSD during the various sampling periods.

In the fourth study, done by Barnard (1997), discussed above, no positive results were obtained.

It is well known that the SNT is not very sensitive in detecting LSDV neutralising antibodies (Babiuk *et al.* 2009). One reason is due to the predominantly cell-mediated immune response to LSDV infection (OIE 2010a). Additionally, LSDV does not easily grow in cell cultures, which makes the SNT difficult to perform. As mentioned above different cell types have been used to isolate the virus in the laboratory and the use of a primary cell culture like bovine dermis cells, used in this study, may contribute to the sensitivity of the SNT. These cells have previously been used to detect LSDV in blood and semen of experimentally infected bulls (Tuppurainen *et al*. 2005; Bagla *et al*. 2006) and have now been described in this study to detect LSDV neutralising antibodies in African buffalo sera. Other cell types that have been used include: lamb foetal kidney cells (Hedger & Hamblin 1983), secondary calf kidney cells (Hamblin *et al.* 1990) and bovine foetal muscle cells (Davies 1982).

Unpublished data from a study performed at the ARC-OVI on wildlife in South Africa indicates that LSDV antibodies were detected in sera from springbok using only the SNT. Though other wildlife was tested in this study, the African buffalo was not included (Truuske Gerdes, 2011 personal communication).

Rift Valley fever

Of the previous studies done on RVF in buffaloes (n=10) 5 were published within the last 7 years using mainly samples from eastern African countries and South Africa.

Studies by Davies (1975); Barnard (1997); Paweska *et al.* (2003b); Paweska *et al.* (2008) and Evans *et al.* (2008) used only the SNT while the SNT as well as other tests including IgG ELISA and I-ELISA were used in studies by Paweska *et al.* (2003b), Paweska *et al.* (2008) and Evans *et al.* (2008).

In the present study 21.1% (12/57) of samples tested RVFV positive by SNT. Results obtained by Paweska *et al*. (2003b); 5.8% (54/928), Paweska *et al.* (2008); 7.5% (77/1023) and Evans *et al.* (2008); 15.6% (37/237) where larger sample sizes were used, were lower than obtained in this study. Results obtained by the study of Paweska *et al.* (2005a); 20.5% (53/258) are similar to those of the present study. However his study included samples obtained from an outbreak during 1997 – 1998 in East Africa: the portion of samples taken from the outbreak was not stated in the paper.

The earliest study, by Davies (1975), also with a small sample size (n=62), used both the SNT and DFAT, but did not obtain any positive results.

Evaluation of ELISA and SNT results

The I-ELISA and SNT detects two different types of antibodies as has been noted in the literature (Babiuk *et al.* 2009; Bowden *et al.* 2009). The difference between these antibodies in time frame of disease and function is not clearly understood.

Lumpy skin disease

It is apparent that more samples (70) were positive for LSDV antibodies by I-ELISA than with the SNT (5) (nine ELISA positive samples were not tested by SNT due to insufficient sera).

In general, currently available serological diagnostic tests for capripoxviruses and LSDV in particular i.e. SNT, Western blotting and the whole virus ELISA used in this study, may not be sensitive or specific (false positive results) enough to detect anti-capripoxvirus antibodies (Babiuk *et al.* 2009). One reason, as been discussed before, may be that these tests do not detect the predominantly cell-mediated immunity that occurs in LSDV infection (Babiuk *et al.* 2008a, OIE 2010a). In their study, Babiuk *et al.* (2008b) observed that the ELISA was inconsistently detecting LSDV antibodies in

experimentally infected calves. They tested sequential sera from two calves experimentally infected with a virulent isolate of LSDV (Neethling) using SNT and ELISA. With the SNT, neutralising antibodies were detected 3 weeks post-inoculation. However, the ELISA could only detect antibodies in one of the calves. The ELISA positive animal had low levels of antibodies that persisted for a short period.

Carn *et al.* (1994), used a cloned capripoxvirus structural protein (P32) as antigen, and showed that the ELISA was more sensitive than the SNT in detecting LSDV antibodies. This study tested only bovine sera. In the present study using sera from African buffaloes, it was observed that the SNT, though the gold standard, was also less sensitive in detecting LSDV infection than the I-ELISA. This study had a fairly large sample size and the I-ELISA detected a high percentage (28.2%) positive samples. This I-ELISA used purified, heat-inactivated, Nigerian SPPV as coating antigen (Babiuk *et al*. 2009) and was not specifically validated for wildlife sera.

Difficulties encountered with development and evaluation of serodiagnostic tests for capripoxviruses has been to obtaining sufficiently large numbers of well-characterised sera from different host species (e.g. sheep, goats, cattle and buffalo) to facilitate validation (Timothy Bowden, 2011 personal communication). There are also a large number of host (including breed, age, sex, previous infection / vaccination history, quality of sera etc) and laboratory factors that might affect the performance characteristics (diagnostic sensitivity and diagnostic specificity) of any antibody detecting ELISA. Determining the true exposure status of naturally infected animals is therefore often difficult. It is therefore not unusual to obtain a high percentage of seropositive animals, like in this study, using the ELISA (Timothy Bowden, 2011 personal communication).

The high percentage of LSDV positive antibody results obtained in this study is however a concern. Results are in contrast with other published results as well as results obtained with the SNT for antibodies against LSDV (as discussed previously). Samples were obtained from the field and the possibility of the results by the I-ELISA to be false positives can not be excluded. A validated LSDV-specific ELISA, although difficult to establish, should be used for testing of buffalo and other wildlife sera.

Rift Valley fever

The data in Table 7 indicate that 11 of the 15 ELISA positive sera (73%) had neutralising antibodies. (Two ELISA positive samples were not tested by SNT due to insufficient sera).

Table 7: Comparison of RVFV SNT and indirect ELISA results

Test	SNT positive	ELISA positive*	SNT and ELISA positive	ELISA negative & SNT positive	ELISA positive & SNT negative
Number of samples	12	15	11	6	4

* 2 ELISA +ve samples were not tested by SNT due to insufficient sera

The results obtained by the I-ELISA and the SNT compared well with that obtained in other recent studies. Evans *et al*. (2008) used a IgG validated ELISA to test sera derived from African buffalo and sera from other wild ruminants in Kenya. About 35% of these sera were collected during an inter-epidemic period. A total of 265 buffalo sera were tested, 30 (11.3%) were positive by SNT and 49 (18.5%) by IgG ELISA. There was a high correlation between the I-ELISA test results and the SNT (R^2=0.86, Pearson's correlation coefficient). Similarly, Paweska *et al.* (2008), also obtained a high correlation (R^2 = 0.882 Spearman test) between the SNT titres and the I-ELISA PP values. Additionally, there was a high correlation in this study between the SNT titres and the PP values from the I-ELISA. The Pearson's correlation coefficient was R^2=0.75. The result of this study provides additional evidence that the rNP-based IgG ELISA is a valuable diagnostic tool in the sero-epidemiological monitoring of RVFV infection in African buffalo.

In conclusion, this study provides data indicating previous infection by LSDV and RVFV in an African buffalo population in the KNP and HiP during an inter-epidemic period. The role of buffaloes in the epidemiology of these diseases is however still not clear. From the results obtained, both the SNT and ELISA tests used for RVFV are sensitive and provide reproducible results. However, further studies are required to evaluate the performance characteristics (sensitivity and especially the specificity)

of the I-ELISA assay for the detection of antibodies against LSDV in African buffalo serum in order to detect the true prevalence of the LSDV antibodies in African buffalo. In particular a large number of known LSD-negative African buffalo serum samples should be tested with I-ELISA.

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Appendix 1

Raw Data – LSDV Indirect ELISA

Plate 1

LSD- Indirect ELISA

Antigen-
Nigerian SPV

PLATE 1

Date: 03.12.2008

lot #06-08-15

Antigen:	Coat with Carbonate Buffer 1:100 overnight 4 C		
Blocking:	10% Milk		1h
Sera:	6% Milk	1:100	1h
Conjugate:	1:5000 Protein G		1h
TMB:	Substrate ready-to-use		1h
STOP:	2M H2SO4		1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.037	0.481	0.057	0.07	0.07	0.056	0.134	0.062	0.063	0.068	0.061	0.075
B	0.039	0.482	0.059	0.078	0.064	0.056	0.051	0.057	0.066	0.063	0.056	0.074
C	0.895	1.48	0.056	0.068	0.074	0.059	0.059	0.072	0.06	0.077	0.066	0.126
D	0.929	1.519	0.056	0.069	0.067	0.062	0.058	0.073	0.057	0.075	0.07	0.12
E	0.082	0.051	0.096	0.058	0.058	0.052	0.062	0.066	0.055	0.091	0.068	0.118
F	0.111	0.053	0.091	0.081	0.059	0.055	0.066	0.074	0.06	0.089	0.086	0.082
G	0.047	0.067	0.08	0.057	0.052	0.056	0.061	0.079	0.084	0.055	0.06	0.069
H	0.041	0.059	0.075	0.064	0.055	0.057	0.064	0.082	0.091	0.054	0.058	0.075

cc	0.04	0.00	0.04	0.04	0.04	0.04	0.04	0.03
Pos no 1	0.91	0.02	0.94	0.96	0.98	0.89	0.86	0.84
Neg no. 1	0.10	0.02	0.12	0.14	0.16	0.08	0.06	0.03
cc	0.04	0.00	0.05	0.05	0.06	0.04	0.04	0.03
Pos no. 2	0.48	0.00	0.48	0.48	0.48	0.48	0.48	0.48
Pos no 3	1.50	0.03	1.53	1.55	1.58	1.47	1.44	1.42
Neg no 2	0.05	0.00	0.05	0.05	0.06	0.05	0.05	0.05
Neg no 3	0.06	0.01	0.07	0.07	0.08	0.06	0.05	0.05
			2sd cut off					

Cut off
0.08888

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.057	0.059	0.058	0.00	2.44	-5	Neg
2	0.07	0.078	0.074	0.01	7.64	-3	Neg
3	0.07	0.064	0.067	0.00	6.33	-4	Neg
4	0.056	0.056	0.056	0.00	0.00	-5	Neg
5	0.134	0.051	0.0925	0.06	63.45	0	POS
6	0.062	0.057	0.0595	0.00	5.94	-5	Neg
7	0.063	0.066	0.0645	0.00	3.29	-4	Neg
8	0.068	0.063	0.0655	0.00	5.40	-4	Neg
9	0.061	0.056	0.0585	0.00	6.04	-5	Neg
10	0.075	0.074	0.0745	0.00	0.95	-3	Neg
11	0.056	0.056	0.056	0.00	0.00	-5	Neg
12	0.068	0.069	0.0685	0.00	1.03	-3	Neg
13	0.074	0.067	0.0705	0.00	7.02	-3	Neg
14	0.059	0.062	0.0605	0.00	3.51	-4	Neg
15	0.059	0.058	0.0585	0.00	1.21	-5	Neg
16	0.072	0.073	0.0725	0.00	0.98	-3	Neg
17	0.06	0.057	0.0585	0.00	3.63	-5	Neg
18	0.077	0.075	0.076	0.00	1.86	-3	Neg
19	0.066	0.07	0.068	0.00	4.16	-3	Neg
20	0.126	0.12	0.123	0.00	3.45	3	POS
21	0.096	0.091	0.0935	0.00	3.78	0	POS
22	0.058	0.081	0.0695	0.02	23.40	-3	Neg
23	0.058	0.059	0.0585	0.00	1.21	-5	Neg
24	0.052	0.055	0.0535	0.00	3.97	-5	Neg
25	0.062	0.066	0.064	0.00	4.42	-4	Neg
26	0.066	0.074	0.07	0.01	8.08	-3	Neg
27	0.055	0.06	0.0575	0.00	6.15	-5	Neg
28	0.091	0.089	0.09	0.00	1.57	-1	POS
29	0.068	0.086	0.077	0.01	16.53	-2	Neg
30	0.118	0.082	0.1	0.03	25.46	0	POS
31	0.08	0.075	0.0775	0.00	4.56	-2	Neg
32	0.057	0.064	0.0605	0.00	8.18	-4	Neg
33	0.052	0.055	0.0535	0.00	3.97	-5	Neg
34	0.056	0.057	0.0565	0.00	1.25	-5	Neg
35	0.061	0.064	0.0625	0.00	3.39	-4	Neg
36	0.079	0.082	0.0805	0.00	2.64	-2	Neg
37	0.084	0.091	0.0875	0.00	5.66	-1	Neg
38	0.055	0.054	0.0545	0.00	1.30	-5	Neg
39	0.06	0.058	0.059	0.00	2.40	-5	Neg
40	0.069	0.075	0.072	0.00	5.89	-3	Neg

Plate 2

LSD- Indirect ELISA

Antigen-
Nigerian SPV

PLATE 2

Date: 03.12.2008

lot #06-08-15

Antigen:	Coat with Carbonate Buffer 1:100 overnight 4 C		
Blocking:	10% Milk		1h
Sera:	6% Milk	1:100	1h
Conjugate:	1:5000	Protein G	1h
TMB:	Substrate ready-to-use		1h
STOP:	2M H2SO4		1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.045	0.627	0.075	0.056	0.078	0.054	0.068	0.061	0.066	0.109	0.07	0.093
B	0.042	0.732	0.069	0.057	0.06	0.056	0.067	0.062	0.068	0.105	0.074	0.103
C	1.199	1.775	0.071	0.067	0.069	0.075	0.074	0.069	0.066	0.064	0.114	0.117
D	0.949	1.914	0.074	0.065	0.075	0.075	0.08	0.074	0.089	0.116	0.101	0.113
E	0.095	0.064	0.114	0.066	0.067	0.056	0.063	0.064	0.107	0.065	0.074	0.1
F	0.097	0.068	0.112	0.063	0.068	0.056	0.063	0.07	0.101	0.064	0.074	0.091
G	0.072	0.069	0.072	0.061	0.129	0.142	0.074	0.07	0.157	0.15	0.078	0.178
H	0.038	0.075	0.073	0.065	0.128	0.136	0.081	0.082	0.167	0.155	0.088	0.188

cc	0.04	0.00	0.05	0.05	0.05	0.04	0.04	0.04
Pos no 1	1.07	0.18	1.25	1.43	1.60	0.90	0.72	0.54
Neg no. 1	0.10	0.00	0.10	0.10	0.10	0.09	0.09	0.09
cc	0.06	0.02	0.08	0.10	0.13	0.03	0.01	-0.02
Pos no. 2	0.68	0.07	0.75	0.83	0.90	0.61	0.53	0.46
Pos no 3	1.84	0.10	1.94	2.04	2.14	1.75	1.65	1.55
Neg no 2	0.07	0.00	0.07	0.07	0.07	0.06	0.06	0.06
Neg no 3	0.07	0.00	0.08	0.08	0.08	0.07	0.06	0.06

Cut off
0.08366

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.075	0.069	0.072	0.00	5.89	-2	Neg
2	0.056	0.057	0.0565	0.00	1.25	-4	Neg
3	0.078	0.06	0.069	0.01	18.45	-3	Neg
4	0.054	0.056	0.055	0.00	2.57	-4	Neg
5	0.068	0.067	0.0675	0.00	1.05	-3	Neg
6	0.061	0.062	0.0615	0.00	1.15	-4	Neg
7	0.066	0.068	0.067	0.00	2.11	-3	Neg
8	0.109	0.105	0.107	0.00	2.64	1	POS
9	0.07	0.074	0.072	0.00	3.93	-2	Neg
10	0.093	0.103	0.098	0.01	7.22	0	POS
11	0.071	0.074	0.0725	0.00	2.93	-2	Neg
12	0.067	0.065	0.066	0.00	2.14	-3	Neg
13	0.069	0.075	0.072	0.00	5.89	-2	Neg
14	0.075	0.075	0.075	0.00	0.00	-2	Neg
15	0.074	0.08	0.077	0.00	5.51	-2	Neg
16	0.069	0.074	0.0715	0.00	4.94	-3	Neg
17	0.066	0.089	0.0775	0.02	20.99	-2	Neg
18	0.064	0.116	0.09	0.04	40.86	-1	POS
19	0.114	0.101	0.1075	0.01	8.55	1	POS
20	0.117	0.113	0.115	0.00	2.46	2	POS
21	0.114	0.112	0.113	0.00	1.25	2	POS
22	0.066	0.063	0.0645	0.00	3.29	-3	Neg
23	0.067	0.068	0.0675	0.00	1.05	-3	Neg
24	0.056	0.056	0.056	0.00	0.00	-4	Neg
25	0.063	0.063	0.063	0.00	0.00	-3	Neg
26	0.064	0.07	0.067	0.00	6.33	-3	Neg
27	0.107	0.101	0.104	0.00	4.08	1	POS
28	0.065	0.064	0.0645	0.00	1.10	-3	Neg
29	0.074	0.074	0.074	0.00	0.00	-2	Neg
30	0.1	0.091	0.0955	0.01	6.66	0	POS
31	0.072	0.073	0.0725	0.00	0.98	-2	Neg
32	0.061	0.065	0.063	0.00	4.49	-3	Neg
33	0.129	0.128	0.1285	0.00	0.55	3	POS
34	0.142	0.136	0.139	0.00	3.05	4	POS
35	0.074	0.081	0.0775	0.00	6.39	-2	Neg
36	0.07	0.082	0.076	0.01	11.16	-2	Neg
37	0.157	0.167	0.162	0.01	4.36	7	POS
38	0.15	0.155	0.1525	0.00	2.32	6	POS
39	0.078	0.088	0.083	0.01	8.52	-1	Neg
40	0.178	0.188	0.183	0.01	3.86	9	POS

duplicate samples not used in the final analysis

Plate 3

LSD- Indirect ELISA

Antigen-
Nigerian SPV

Date:

lot #06-08-15

Antigen:	Coat with Carbonate Buffer 1:100 overnight 4 C		
Blocking:	10% Milk		1h
Sera:	6% Milk	1:100	1h
Conjugate:	1:5000	Protein G	1h
TMB:	Substrate ready-to-use		1h
STOP:	2M H2SO4		1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.043	0.564	0.079	0.078	0.065	0.07	0.075	0.085	0.15	0.074	0.098	0.116
B	0.042	0.547	0.075	0.073	0.061	0.07	0.071	0.077	0.155	0.063	0.092	0.111
C	1.045	1.527	0.066	0.141	0.134	0.06	0.105	0.11	0.067	0.082	0.083	0.309
D	0.949	1.616	0.065	0.135	0.139	0.066	0.109	0.113	0.063	0.073	0.093	0.414
E	0.063	0.057	0.084	0.075	0.067	0.065	0.08	0.09	0.097	0.078	0.154	0.132
F	0.084	0.076	0.099	0.07	0.068	0.067	0.076	0.087	0.098	0.076	0.112	0.131
G	0.056	0.073	0.11	0.125	0.072	0.081	0.067	0.077	0.063	0.095	0.086	0.154
H	0.054	0.074	0.12	0.127	0.078	0.092	0.071	0.083	0.067	0.101	0.096	0.172

cc	0.04	0.00	0.04	0.04	0.04	0.04	0.04	0.04
Pos no 1	1.00	0.07	1.06	1.13	1.20	0.93	0.86	0.79
Neg no. 1	0.07	0.01	0.09	0.10	0.12	0.06	0.04	0.03
cc	0.06	0.00	0.06	0.06	0.06	0.05	0.05	0.05
Pos no. 2	0.56	0.01	0.57	0.58	0.59	0.54	0.53	0.52
Pos no 3	1.57	0.06	1.63	1.70	1.76	1.51	1.45	1.38
Neg no 2	0.07	0.01	0.08	0.09	0.11	0.05	0.04	0.03
Neg no 3	0.07	0.00	0.07	0.07	0.08	0.07	0.07	0.07
			2sd cut off					

Cut off
0.09049

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.079	0.075	0.077	0.00	3.67	0	Neg
2	0.078	0.073	0.0755	0.00	4.68	0	Neg
3	0.065	0.061	0.063	0.00	4.49	-1	Neg
4	0.07	0.07	0.07	0.00	0.00	0	Neg
5	0.075	0.071	0.073	0.00	3.87	0	Neg
6	0.085	0.077	0.081	0.01	6.98	1	Neg
7	0.15	0.155	0.1525	0.00	2.32	9	POS
8	0.074	0.063	0.0685	0.01	11.35	-1	Neg
9	0.098	0.092	0.095	0.00	4.47	2	POS
10	0.116	0.111	0.1135	0.00	3.12	4	POS
11	0.066	0.065	0.0655	0.00	1.08	-1	Neg
12	0.141	0.135	0.138	0.00	3.07	7	POS
13	0.134	0.139	0.1365	0.00	2.59	7	POS
14	0.06	0.066	0.063	0.00	6.73	-1	Neg
15	0.105	0.109	0.107	0.00	2.64	4	POS
16	0.11	0.113	0.1115	0.00	1.90	4	POS
17	0.067	0.063	0.065	0.00	4.35	-1	Neg
18	0.082	0.073	0.0775	0.01	8.21	0	Neg
19	0.083	0.093	0.088	0.01	8.04	2	Neg
20	0.309	0.414	0.3615	0.07	20.54	31	POS
21	0.084	0.099	0.0915	0.01	11.59	2	POS
22	0.075	0.07	0.0725	0.00	4.88	0	Neg
23	0.067	0.068	0.0675	0.00	1.05	-1	Neg
24	0.065	0.067	0.066	0.00	2.14	-1	Neg
25	0.08	0.076	0.078	0.00	3.63	0	Neg
26	0.09	0.087	0.0885	0.00	2.40	2	Neg
27	0.097	0.098	0.0975	0.00	0.73	3	POS
28	0.078	0.076	0.077	0.00	1.84	0	Neg
29	0.154	0.112	0.133	0.03	22.33	6	POS
30	0.132	0.131	0.1315	0.00	0.54	6	POS
31	0.11	0.12	0.115	0.01	6.15	4	POS
32	0.125	0.127	0.126	0.00	1.12	6	POS
33	0.072	0.078	0.075	0.00	5.66	0	Neg
34	0.081	0.092	0.0865	0.01	8.99	1	Neg
35	0.067	0.071	0.069	0.00	4.10	0	Neg
36	0.077	0.083	0.08	0.00	5.30	1	Neg
37	0.063	0.067	0.065	0.00	4.35	-1	Neg
38	0.095	0.101	0.098	0.00	4.33	3	POS
39	0.086	0.096	0.091	0.01	7.77	2	POS
40	0.154	0.172	0.163	0.01	7.81	10	POS

duplicate samples not used in the final analysis

Plate 4

LSD- Indirect ELISA

Antigen-
Nigerian SPV

lot #06-08-15

Date: 04.12.08 PLATE 4

Antigen:	Coat with Carbonate Buffer 1:100 37℃ / 1hr		
Blocking:	10% Milk		1h
Sera:	6% Milk	1:100	1h
Conjugate:	1:5000 Protein G		1h
TMB:	Substrate ready-to-use		1h
STOP:	2M H2SO4		1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.04	0.441	0.092	0.078	0.098	0.073	0.078	0.063	0.066	0.093	0.093	0.137
B	0.037	0.496	0.096	0.097	0.127	0.074	0.083	0.076	0.068	0.087	0.097	0.591
C	0.768	1.388	0.072	0.067	0.062	0.057	0.074	0.077	0.079	0.076	0.107	0.14
D	1.172	1.593	0.068	0.076	0.071	0.06	0.074	0.075	0.081	0.073	0.226	0.459
E	0.11	0.077	0.078	0.118	0.098	0.067	0.096	0.077	0.071	0.27	0.081	0.249
F	0.234	0.074	0.082	0.122	0.091	0.069	0.09	0.081	0.075	0.09	0.094	0.094
G	0.043	0.067	0.078	0.106	0.093	0.082	0.063	0.095	0.121	0.135	0.155	0.084
H	0.043	0.068	0.084	0.121	0.106	0.091	0.083	0.096	0.129	0.175	0.139	0.088

cc	0.04	0.00	0.04	0.04	0.04	0.04	0.03	0.03
Pos no 1	0.97	0.29	1.26	1.54	1.83	0.68	0.40	0.11
Neg no. 1	0.17	0.09	0.26	0.35	0.44	0.08	0.00	-0.09
cc	0.04	0.00	0.04	0.04	0.04	0.04	0.04	0.04
Pos no. 2	0.47	0.04	0.51	0.55	0.59	0.43	0.39	0.35
Pos no 3	1.49	0.14	1.64	1.78	1.93	1.35	1.20	1.06
Neg no 2	0.08	0.00	0.08	0.08	0.08	0.07	0.07	0.07
Neg no 3	0.07	0.00	0.07	0.07	0.07	0.07	0.07	0.07

2sd cut off

Cut off
0.16534

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.092	0.096	0.094	0.00	3.01	-10	Neg
2	0.078	0.097	0.0875	0.01	15.35	-11	Neg
3	0.098	0.127	0.1125	0.02	18.23	-7	Neg
4	0.073	0.074	0.0735	0.00	0.96	-12	Neg
5	0.078	0.083	0.0805	0.00	4.39	-11	Neg
6	0.063	0.076	0.0695	0.01	13.23	-13	Neg
7	0.066	0.068	0.067	0.00	2.11	-13	Neg
8	0.093	0.087	0.09	0.00	4.71	-10	Neg
9	0.093	0.097	0.095	0.00	2.98	-10	Neg
10	0.137	0.591	0.364	0.32	88.19	24	POS
11	0.072	0.068	0.07	0.00	4.04	-13	Neg
12	0.067	0.076	0.0715	0.01	8.90	-13	Neg
13	0.062	0.071	0.0665	0.01	9.57	-13	Neg
14	0.057	0.06	0.0585	0.00	3.63	-14	Neg
15	0.074	0.074	0.074	0.00	0.00	-12	Neg
16	0.077	0.075	0.076	0.00	1.86	-12	Neg
17	0.079	0.081	0.08	0.00	1.77	-12	Neg
18	0.076	0.073	0.0745	0.00	2.85	-12	Neg
19	0.107	0.226	0.1665	0.08	50.54	-1	POS
20	0.14	0.459	0.2995	0.23	75.31	16	POS
21	0.078	0.082	0.08	0.00	3.54	-12	Neg
22	0.118	0.122	0.12	0.00	2.36	-7	Neg
23	0.098	0.091	0.0945	0.00	5.24	-10	Neg
24	0.067	0.069	0.068	0.00	2.08	-13	Neg
25	0.096	0.09	0.093	0.00	4.56	-10	Neg
26	0.077	0.081	0.079	0.00	3.58	-12	Neg
27	0.071	0.075	0.073	0.00	3.87	-12	Neg
28	0.27	0.09	0.18	0.13	70.71	1	POS
29	0.081	0.094	0.0875	0.01	10.51	-11	Neg
30	0.249	0.094	0.1715	0.11	63.91	0	POS
31	0.078	0.084	0.081	0.00	5.24	-11	Neg
32	0.106	0.121	0.1135	0.01	9.35	-7	Neg
33	0.093	0.106	0.0995	0.01	9.24	-9	Neg
34	0.082	0.091	0.0865	0.01	7.36	-11	Neg
35	0.063	0.083	0.073	0.01	19.37	-12	Neg
36	0.095	0.096	0.0955	0.00	0.74	-10	Neg
37	0.121	0.129	0.125	0.01	4.53	-6	Neg
38	0.135	0.175	0.155	0.03	18.25	-2	Neg
39	0.155	0.139	0.147	0.01	7.70	-3	Neg
40	0.084	0.088	0.086	0.00	3.29	-11	Neg

Plate 5

LSD- Indirect ELISA

Antigen-
Nigerian SPV

Date: 04.12.2008 PLATE 5

lot #06-08-15

Antigen:	Coat with Carbonate Buffer 1:100 37℃ / 1hr		
Blocking:	10% Milk		1h
Sera:	6% Milk	1:100	1h
Conjugate:	1:5000 Protein G		1h
TMB:	Substrate ready-to-use		1h
STOP:	2M H2SO4		1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.038	0.571	0.127	0.09	0.113	0.157	0.14	0.104	0.074	0.095	0.078	0.11
B	0.052	0.599	0.118	0.088	0.11	0.148	0.122	0.084	0.067	0.097	0.089	0.132
C	0.963	1.608	0.077	0.085	0.107	0.085	0.095	0.095	0.091	0.097	0.117	0.435
D	1.013	1.615	0.078	0.082	0.091	0.095	0.104	0.092	0.085	0.17	0.118	0.173
E	0.124	0.066	0.077	0.098	0.087	0.106	0.079	0.092	0.084	0.102	0.086	0.092
F	0.075	0.073	0.074	0.107	0.086	0.109	0.08	0.087	0.084	0.092	0.075	0.092
G	0.091	0.074	0.115	0.121	0.063	0.083	0.123	0.076	0.115	0.07	0.06	0.077
H	0.039	0.068	0.104	0.114	0.062	0.091	0.119	0.073	0.109	0.076	0.065	0.088

cc	0.05	0.01	0.05	0.06	0.07	0.04	0.03	0.02
Pos no 1	0.99	0.04	1.02	1.06	1.09	0.95	0.92	0.88
Neg no. 1	0.10	0.03	0.13	0.17	0.20	0.06	0.03	0.00
cc	0.07	0.04	0.10	0.14	0.18	0.03	-0.01	-0.05
Pos no. 2	0.59	0.02	0.60	0.62	0.64	0.57	0.55	0.53
Pos no 3	1.61	0.00	1.62	1.62	1.63	1.61	1.60	1.60
Neg no 2	0.07	0.00	0.07	0.08	0.08	0.06	0.06	0.05
Neg no 3	0.07	0.00	0.08	0.08	0.08	0.07	0.06	0.06
			2sd cut off					

Cut off
0.10923

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.127	0.118	0.1225	0.01	5.20	3	POS
2	0.09	0.088	0.089	0.00	1.59	-1	Neg
3	0.113	0.11	0.1115	0.00	1.90	1	POS
4	0.157	0.148	0.1525	0.01	4.17	6	POS
5	0.14	0.122	0.131	0.01	9.72	4	POS
6	0.104	0.084	0.094	0.01	15.04	-1	Neg
7	0.074	0.067	0.0705	0.00	7.02	-3	Neg
8	0.095	0.097	0.096	0.00	1.47	0	Neg
9	0.078	0.089	0.0835	0.01	9.32	-2	Neg
10	0.11	0.132	0.121	0.02	12.86	2	POS
11	0.077	0.078	0.0775	0.00	0.91	-2	Neg
12	0.085	0.082	0.0835	0.00	2.54	-2	Neg
13	0.107	0.091	0.099	0.01	11.43	0	Neg
14	0.085	0.095	0.09	0.01	7.86	-1	Neg
15	0.095	0.104	0.0995	0.01	6.40	0	Neg
16	0.095	0.092	0.0935	0.00	2.27	-1	Neg
17	0.091	0.085	0.088	0.00	4.82	-1	Neg
18	0.097	0.17	0.1335	0.05	38.67	4	POS
19	0.117	0.118	0.1175	0.00	0.60	2	POS
20	0.435	0.173	0.304	0.19	60.94	23	POS
21	0.077	0.074	0.0755	0.00	2.81	-3	Neg
22	0.098	0.107	0.1025	0.01	6.21	0	Neg
23	0.087	0.086	0.0865	0.00	0.82	-1	Neg
24	0.106	0.109	0.1075	0.00	1.97	1	Neg
25	0.079	0.08	0.0795	0.00	0.89	-2	Neg
26	0.092	0.087	0.0895	0.00	3.95	-1	Neg
27	0.084	0.084	0.084	0.00	0.00	-2	Neg
28	0.102	0.092	0.097	0.01	7.29	0	Neg
29	0.086	0.075	0.0805	0.01	9.66	-2	Neg
30	0.092	0.092	0.092	0.00	0.00	-1	Neg
31	0.115	0.104	0.1095	0.01	7.10	1	POS
32	0.121	0.114	0.1175	0.00	4.21	2	POS
33	0.063	0.062	0.0625	0.00	1.13	-4	Neg
34	0.083	0.091	0.087	0.01	6.50	-1	Neg
35	0.123	0.119	0.121	0.00	2.34	2	POS
36	0.076	0.073	0.0745	0.00	2.85	-3	Neg
37	0.115	0.109	0.112	0.00	3.79	1	POS
38	0.07	0.076	0.073	0.00	5.81	-3	Neg
39	0.06	0.065	0.0625	0.00	5.66	-4	Neg
40	0.077	0.088	0.0825	0.01	9.43	-2	Neg

Plate 6

LSD- Indirect ELISA

Antigen-
Nigerian SPV

lot #06-08-15

Date: 04.12.2008 PLATE 6
Antigen: Coat with Carbonate Buffer 1:100 37℃ / 1hr
Blocking: 10% Milk 1h
Sera: 6% Milk 1:100 1h
Conjugate: 1:5000 Protein G 1h
TMB: Substrate ready-to-use 1h
STOP: 2M H2SO4 1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.037	0.43	0.088	0.078	0.092	0.092	0.063	0.062	0.071	0.067	0.085	0.104
B	0.04	0.525	0.059	0.075	0.08	0.083	0.065	0.067	0.065	0.065	0.078	0.107
C	1.093	1.621	0.084	0.088	0.065	0.078	0.068	0.072	0.072	0.083	0.072	0.089
D	0.9	1.558	0.085	0.09	0.064	0.086	0.084	0.079	0.073	0.072	0.073	0.076
E	0.087	0.07	0.149	0.091	0.071	0.06	0.085	0.09	0.078	0.083	0.067	0.146
F	0.104	0.077	0.105	0.112	0.076	0.066	0.093	0.104	0.086	0.085	0.066	0.118
G	0.124	0.089	0.064	0.093	0.126	0.113	0.086	0.118	0.109	0.148	0.106	0.072
H	0.058	0.074	0.069	0.095	0.141	0.11	0.082	0.104	0.116	0.188	0.111	0.062

cc	0.04	0.00	0.04	0.04	0.04	0.04	0.03	0.03
Pos no 1	1.00	0.14	1.13	1.27	1.41	0.86	0.72	0.59
Neg no. 1	0.10	0.01	0.11	0.12	0.13	0.08	0.07	0.06
cc	0.09	0.05	0.14	0.18	0.23	0.04	0.00	-0.05
Pos no. 2	0.48	0.07	0.54	0.61	0.68	0.41	0.34	0.28
Pos no 3	1.59	0.04	1.63	1.68	1.72	1.54	1.50	1.46
Neg no 2	0.07	0.00	0.08	0.08	0.09	0.07	0.06	0.06
Neg no 3	0.08	0.01	0.09	0.10	0.11	0.07	0.06	0.05
			2sd cut off					

Cut off
0.10188

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.088	0.059	0.0735	0.02	27.90	-2	Neg
2	0.078	0.075	0.0765	0.00	2.77	-2	Neg
3	0.092	0.08	0.086	0.01	9.87	-1	Neg
4	0.092	0.083	0.0875	0.01	7.27	-1	Neg
5	0.063	0.065	0.064	0.00	2.21	-3	Neg
6	0.062	0.067	0.0645	0.00	5.48	-3	Neg
7	0.071	0.065	0.068	0.00	6.24	-3	Neg
8	0.067	0.065	0.066	0.00	2.14	-3	Neg
9	0.085	0.078	0.0815	0.00	6.07	-2	Neg
10	0.104	0.107	0.1055	0.00	2.01	1	POS
11	0.084	0.085	0.0845	0.00	0.84	-1	Neg
12	0.088	0.09	0.089	0.00	1.59	-1	Neg
13	0.065	0.064	0.0645	0.00	1.10	-3	Neg
14	0.078	0.086	0.082	0.01	6.90	-1	Neg
15	0.068	0.084	0.076	0.01	14.89	-2	Neg
16	0.072	0.079	0.0755	0.00	6.56	-2	Neg
17	0.072	0.073	0.0725	0.00	0.98	-3	Neg
18	0.083	0.072	0.0775	0.01	10.04	-2	Neg
19	0.072	0.073	0.0725	0.00	0.98	-3	Neg
20	0.089	0.076	0.0825	0.01	11.14	-1	Neg
21	0.149	0.105	0.127	0.03	24.50	3	POS
22	0.091	0.112	0.1015	0.01	14.63	1	Neg
23	0.071	0.076	0.0735	0.00	4.81	-2	Neg
24	0.06	0.066	0.063	0.00	6.73	-4	Neg
25	0.085	0.093	0.089	0.01	6.36	-1	Neg
26	0.09	0.104	0.097	0.01	10.21	0	Neg
27	0.078	0.086	0.082	0.01	6.90	-1	Neg
28	0.083	0.085	0.084	0.00	1.68	-1	Neg
29	0.067	0.066	0.0665	0.00	1.06	-3	Neg
30	0.146	0.118	0.132	0.02	15.00	4	POS
31	0.064	0.069	0.0665	0.00	5.32	-3	Neg
32	0.093	0.095	0.094	0.00	1.50	0	Neg
33	0.126	0.141	0.1335	0.01	7.95	4	POS
34	0.113	0.11	0.1115	0.00	1.90	2	POS
35	0.086	0.082	0.084	0.00	3.37	-1	Neg
36	0.118	0.104	0.111	0.01	8.92	2	POS
37	0.109	0.116	0.1125	0.00	4.40	2	POS
38	0.148	0.188	0.168	0.03	16.84	8	POS
39	0.106	0.111	0.1085	0.00	3.26	1	POS
40	0.072	0.062	0.067	0.01	10.55	-3	Neg

Plate 7

LSD- Indirect ELISA

Antigen-
Nigerian SPV

Date: 04.12.2008 PLATE 7

lot #06-08-15

Antigen:	Coat with Carbonate Buffer 1:100 37℃ / 1hr		
Blocking:	10% Milk		1h
Sera:	6% Milk	1:100	1h
Conjugate:	1:5000 Protein G		1h
TMB:	Substrate ready-to-use		1h
STOP:	2M H2SO4		1h

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	pos No 2	1	2	3	4	5	6	7	8	9	10
B	cc	pos No 2										
C	pos No 1	pos No 3	11	12	13	14	15	16	17	18	19	20
D	pos No 1	pos No 3										
E	neg No 1	neg No. 2	21	22	23	24	25	26	27	28	29	30
F	neg No 1	neg No. 2										
G	cc	neg No. 3	31	32	33	34	35	36	37	38	39	40
H	cc	neg No. 3										

	Raw Data											
A	0.041	0.691	0.092	0.15	0.1	0.094	0.081	0.128	0.21	0.179	0.156	0.237
B	0.039	0.675	0.089	0.135	0.115	0.089	0.094	0.123	0.202	0.154	0.265	0.199
C	1.202	1.875	0.076	0.122	0.089	0.094	0.103	0.084	0.085	0.073	0.072	0.217
D	1.198	1.868	0.084	0.229	0.08	0.1	0.097	0.045	0.048	0.078	0.082	0.074
E	0.071	0.06	0.037	0.039	0.038	0.036	0.036	0.038	0.038	0.037	0.036	0.037
F	0.07	0.068	0.039	0.037	0.045	0.038	0.039	0.038	0.037	0.038	0.038	0.039
G	0.102	0.074	0.038	0.038	0.038	0.037	0.038	0.038	0.037	0.037	0.037	0.037
H	0.169	0.069	0.038	0.037	0.037	0.035	0.037	0.038	0.037	0.038	0.039	0.04

cc	0.04	0.00	0.04	0.04	0.04	0.04	0.04	0.04
Pos no 1	1.20	0.00	1.20	1.21	1.21	1.20	1.19	1.19
Neg no. 1	0.07	0.00	0.07	0.07	0.07	0.07	0.07	0.07
cc	0.14	0.05	0.18	0.23	0.28	0.09	0.04	-0.01
Pos no. 2	0.68	0.01	0.69	0.71	0.72	0.67	0.66	0.65
Pos no 3	1.87	0.00	1.88	1.88	1.89	1.87	1.86	1.86
Neg no 2	0.06	0.01	0.07	0.08	0.08	0.06	0.05	0.05
Neg no 3	0.07	0.00	0.08	0.08	0.08	0.07	0.06	0.06
			2sd cut off					

Cut off
0.07527

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.092	0.089	0.0905	0.00	2.34	2	POS
2	0.15	0.135	0.1425	0.01	7.44	6	POS
3	0.1	0.115	0.1075	0.01	9.87	3	POS
4	0.094	0.089	0.0915	0.00	3.86	2	POS
5	0.081	0.094	0.0875	0.01	10.51	2	POS
6	0.128	0.123	0.1255	0.00	2.82	5	POS
7	0.21	0.202	0.206	0.01	2.75	12	POS
8	0.179	0.154	0.1665	0.02	10.62	8	POS
9	0.156	0.265	0.2105	0.08	36.62	12	POS
10	0.237	0.199	0.218	0.03	12.33	13	POS
11	0.076	0.084	0.08	0.01	7.07	1	POS
12	0.122	0.229	0.1755	0.08	43.11	9	POS
13	0.089	0.08	0.0845	0.01	7.53	1	POS
14	0.094	0.1	0.097	0.00	4.37	2	POS
15	0.103	0.097	0.1	0.00	4.24	3	POS
16	0.084	0.045	0.0645	0.03	42.76	-1	Neg
17	0.085	0.048	0.0665	0.03	39.34	0	Neg
18	0.073	0.078	0.0755	0.00	4.68	0	POS
19	0.072	0.082	0.077	0.01	9.18	1	POS
20	0.217	0.074	0.1455	0.10	69.50	7	POS
21	0.037	0.039	0.038	0.00	3.72	-3	Neg
22	0.039	0.037	0.038	0.00	3.72	-3	Neg
23	0.038	0.045	0.0415	0.00	11.93	-3	Neg
24	0.036	0.038	0.037	0.00	3.82	-3	Neg
25	0.036	0.039	0.0375	0.00	5.66	-3	Neg
26	0.038	0.038	0.038	0.00	0.00	-3	Neg
27	0.038	0.037	0.0375	0.00	1.89	-3	Neg
28	0.037	0.038	0.0375	0.00	1.89	-3	Neg
29	0.036	0.038	0.037	0.00	3.82	-3	Neg
30	0.037	0.039	0.038	0.00	3.72	-3	Neg
31	0.038	0.038	0.038	0.00	0.00	-3	Neg
32	0.038	0.037	0.0375	0.00	1.89	-3	Neg
33	0.038	0.037	0.0375	0.00	1.89	-3	Neg
34	0.037	0.035	0.036	0.00	3.93	-3	Neg
35	0.038	0.037	0.0375	0.00	1.89	-3	Neg
36	0.038	0.038	0.038	0.00	0.00	-3	Neg
37	0.037	0.037	0.037	0.00	0.00	-3	Neg
38	0.037	0.038	0.0375	0.00	1.89	-3	Neg
39	0.037	0.039	0.038	0.00	3.72	-3	Neg
40	0.037	0.04	0.0385	0.00	5.51	-3	Neg

non samples

Plate 1

Anti-RVF IgG Indirect ELISA P1

Date: 01.12.08

Sera: 1:100

Conjugate: **1:10 000 AS IS**

TMB: AS IS

STOP: 1M H2SO4

Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.086	0.041	0.056	0.054	0.13	0.049	0.045	0.055	0.055	0.196	0.047	0.062
B	0.045	0.04	0.048	0.054	0.17	0.055	0.048	0.056	0.054	0.192	0.052	0.058
C	1.441	1.681	0.147	0.051	0.051	0.05	0.049	0.098	0.066	0.054	0.047	0.049
D	1.339	1.502	0.158	0.049	0.048	0.048	0.052	0.085	0.065	0.063	0.048	0.047
E	0.052	0.062	0.07	0.076	0.056	0.105	0.058	0.051	0.052	0.047	1.22	0.184
F	0.047	0.044	0.078	0.083	0.056	0.103	0.05	0.056	0.051	0.051	1.273	0.171
G	0.037	0.039	0.088	0.06	0.048	0.087	0.087	0.053	0.078	0.074	0.057	0.059
H	0.044	0.054	0.06	0.056	0.081	0.092	0.054	0.069	0.066	0.082	0.055	0.054

	Median	PP value
cc	0.043	0
c++	1.472	100
c--	0.050	0
CC	0.042	-1

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.056	0.048	0.052	0.01	11	0	NEG
2	0.054	0.054	0.054	0.00	0	0	NEG
3	0.13	0.17	0.15	0.03	19	7	SUSP
4	0.049	0.055	0.052	0.00	8	0	NEG
5	0.045	0.048	0.0465	0.00	5	0	NEG
6	0.055	0.056	0.0555	0.00	1	0	NEG
7	0.055	0.054	0.0545	0.00	1	0	NEG
8	0.196	0.192	0.194	0.00	1	10	POS
9	0.047	0.052	0.0495	0.00	7	0	NEG
10	0.062	0.058	0.06	0.00	5	1	NEG
11	0.147	0.158	0.1525	0.01	5	7	SUSP
12	0.051	0.049	0.05	0.00	3	0	NEG
13	0.051	0.048	0.0495	0.00	4	0	NEG
14	0.05	0.048	0.049	0.00	3	0	NEG
15	0.049	0.052	0.0505	0.00	4	0	NEG
16	0.098	0.085	0.0915	0.01	10	3	NEG
17	0.066	0.065	0.0655	0.00	1	1	NEG
18	0.054	0.063	0.0585	0.01	11	1	NEG
19	0.047	0.048	0.0475	0.00	1	0	NEG
20	0.049	0.047	0.048	0.00	3	0	NEG
21	0.07	0.078	0.074	0.01	8	2	NEG
22	0.076	0.083	0.0795	0.00	6	2	NEG
23	0.056	0.056	0.056	0.00	0	0	NEG
24	0.105	0.103	0.104	0.00	1	4	NEG
25	0.058	0.05	0.054	0.01	10	0	NEG
26	0.051	0.056	0.0535	0.00	7	0	NEG
27	0.052	0.051	0.0515	0.00	1	0	NEG
28	0.047	0.051	0.049	0.00	6	0	NEG
29	1.22	1.273	1.2465	0.04	3	84	POS
30	0.184	0.171	0.1775	0.01	5	9	SUSP
31	0.088	0.06	0.074	0.02	27	2	NEG
32	0.06	0.056	0.058	0.00	5	1	NEG
33	0.048	0.081	0.0645	0.02	36	1	NEG
34	0.087	0.092	0.0895	0.00	4	3	NEG
35	0.087	0.054	0.0705	0.02	33	1	NEG
36	0.053	0.069	0.061	0.01	19	1	NEG
37	0.078	0.066	0.072	0.01	12	2	NEG
38	0.074	0.082	0.078	0.01	7	2	NEG
39	0.057	0.055	0.056	0.00	3	0	NEG
40	0.059	0.054	0.0565	0.00	6	0	NEG

Plate 2

Anti-RVF IgG Indirect ELISA P2

Date: 02.12.2008
Sera: 1:100
Conjugate: **1:10 000 AS IS**

TMB: AS IS
STOP: 1M H2SO4
Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.038	0.036	0.073	0.076	0.046	0.061	0.055	0.102	0.079	0.096	0.139	0.104
B	0.038	0.037	0.074	0.069	0.044	0.07	0.065	0.09	0.119	0.102	0.123	0.095
C	1.055	1.136	0.072	0.059	0.059	0.066	0.096	0.09	0.058	0.062	0.094	0.215
D	1.103	1.258	0.067	0.062	0.06	0.057	0.089	0.072	0.057	0.067	0.074	0.103
E	0.042	0.043	0.064	0.05	0.052	0.069	0.063	0.099	0.092	0.046	0.06	0.163
F	0.108	0.059	0.058	0.051	0.053	0.052	0.059	0.102	0.06	0.05	0.052	0.181
G	0.073	0.04	0.095	0.081	0.725	0.689	0.085	0.084	0.079	0.088	0.136	0.075
H	0.071	0.041	0.094	0.071	0.692	0.57	0.084	0.069	0.076	0.071	0.13	0.07

	Median	PP value
cc	0.038	-1
c++	1.120	100
c--	0.051	0
CC	0.056	0

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values	Results	
1	0.073	0.074	0.0735	0.00	1	2	NEG	
2	0.076	0.069	0.0725	0.00	7	2	NEG	
3	0.046	0.044	0.045	0.00	3	-1	NEG	
4	0.061	0.07	0.0655	0.01	10	1	NEG	
5	0.055	0.065	0.06	0.01	12	1	NEG	
6	0.102	0.09	0.096	0.01	9	4	SUSP	
7	0.079	0.119	0.099	0.03	29	4	SUSP	
8	0.096	0.102	0.099	0.00	4	4	SUSP	
9	0.139	0.123	0.131	0.01	9	7	SUSP	
10	0.104	0.095	0.0995	0.01	6	5	SUSP	
11	0.072	0.067	0.0695	0.00	5	2	NEG	
12	0.059	0.062	0.0605	0.00	4	1	NEG	
13	0.059	0.06	0.0595	0.00	1	1	NEG	
14	0.066	0.057	0.0615	0.01	10	1	NEG	
15	0.096	0.089	0.0925	0.00	5	4	NEG	
16	0.09	0.072	0.081	0.01	16	3	NEG	
17	0.058	0.057	0.0575	0.00	1	1	NEG	
18	0.062	0.067	0.0645	0.00	5	1	NEG	
19	0.094	0.074	0.084	0.01	17	3	NEG	
20	0.215	0.103	0.159	0.08	50	10	POS	
21	0.064	0.058	0.061	0.00	7	1	NEG	
22	0.05	0.051	0.0505	0.00	1	0	NEG	
23	0.052	0.053	0.0525	0.00	1	0	NEG	
24	0.069	0.052	0.0605	0.01	20	1	NEG	
25	0.063	0.059	0.061	0.00	5	1	NEG	
26	0.099	0.102	0.1005	0.00	2	5	SUSP	
27	0.092	0.06	0.076	0.02	30	2	NEG	
28	0.046	0.05	0.048	0.00	6	0	NEG	
29	0.06	0.052	0.056	0.01	10	0	NEG	
30	0.163	0.181	0.172	0.01	7	11	POS	
31	0.095	0.094	0.0945	0.00	1	4	SUSP	
32	0.081	0.071	0.076	0.01	9	2	NEG	
33	0.725	0.692	0.7085	0.02	3	62	POS	
34	0.689	0.57	0.6295	0.08	13	54	POS	
35	0.085	0.084	0.0845	0.00	1	3	NEG	
36	0.084	0.069	0.0765	0.01	14	2	NEG	
37	0.079	0.076	0.0775	0.00	3	2	NEG	
38	0.088	0.071	0.0795	0.01	15	3	NEG	
39	0.136	0.13	0.133	0.00	3	8	SUSP	
40	0.075	0.07	0.0725	0.00	5	2	NEG	

duplicate samples not used in the final analysis

Plate 3

Anti-RVF IgG Indirect ELISA P3

Date: 02.12.2008

Sera: 1:100
Conjugate: **1:10 000 AS IS**

TMB: AS IS
STOP: 1M H2SO4
Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.051	0.043	0.965	0.094	0.097	0.085	0.119	0.181	0.074	0.072	0.075	0.121
B	0.049	0.041	0.814	0.085	0.107	0.089	0.12	0.16	0.071	0.077	0.069	0.159
C	1.349	1.332	0.162	0.123	0.125	0.105	0.659	0.663	0.104	0.093	0.117	0.152
D	1.257	1.447	0.165	0.145	0.132	0.088	0.806	0.865	0.103	0.095	0.09	0.139
E	0.055	0.048	0.999	0.079	0.863	0.125	0.068	0.075	0.087	0.08	0.093	0.081
F	0.062	0.051	1.085	0.099	0.819	0.127	0.062	0.084	0.099	0.069	0.097	0.073
G	0.066	0.044	0.136	0.118	0.098	0.065	0.071	0.144	0.193	0.093	0.077	0.089
H	0.053	0.044	0.157	0.119	0.097	0.078	0.07	0.095	0.187	0.094	0.078	0.114

	Median	PP value
cc	0.046	-1
c++	1.341	100
c--	0.053	0
CC	0.049	0

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values	Results	
1	0.965	0.814	0.8895	0.11	12	65	POS	
2	0.094	0.085	0.0895	0.01	7	3	NEG	
3	0.097	0.107	0.102	0.01	7	4	NEG	
4	0.085	0.089	0.087	0.00	3	3	NEG	
5	0.119	0.12	0.1195	0.00	1	5	SUSP	
6	0.181	0.16	0.1705	0.01	9	9	SUSP	
7	0.074	0.071	0.0725	0.00	3	2	NEG	
8	0.072	0.077	0.0745	0.00	5	2	NEG	
9	0.075	0.069	0.072	0.00	6	1	NEG	
10	0.121	0.159	0.14	0.03	19	7	SUSP	
11	0.162	0.165	0.1635	0.00	1	9	SUSP	
12	0.123	0.145	0.134	0.02	12	6	SUSP	
13	0.125	0.132	0.1285	0.00	4	6	SUSP	
14	0.105	0.088	0.0965	0.01	12	3	NEG	
15	0.659	0.806	0.7325	0.10	14	53	POS	
16	0.663	0.865	0.764	0.14	19	55	POS	
17	0.104	0.103	0.1035	0.00	1	4	NEG	
18	0.093	0.095	0.094	0.00	2	3	NEG	
19	0.117	0.09	0.1035	0.02	18	4	NEG	
20	0.152	0.139	0.1455	0.01	6	7	SUSP	
21	0.999	1.085	1.042	0.06	6	77	POS	
22	0.079	0.099	0.089	0.01	16	3	NEG	
23	0.863	0.819	0.841	0.03	4	61	POS	
24	0.125	0.127	0.126	0.00	1	6	SUSP	
25	0.068	0.062	0.065	0.00	7	1	NEG	
26	0.075	0.084	0.0795	0.01	8	2	NEG	
27	0.087	0.099	0.093	0.01	9	3	NEG	
28	0.08	0.069	0.0745	0.01	10	2	NEG	
29	0.093	0.097	0.095	0.00	3	3	NEG	
30	0.081	0.073	0.077	0.01	7	2	NEG	
31	0.136	0.157	0.1465	0.01	10	7	SUSP	
32	0.118	0.119	0.1185	0.00	1	5	SUSP	
33	0.098	0.097	0.0975	0.00	1	3	NEG	
34	0.065	0.078	0.0715	0.01	13	1	NEG	
35	0.071	0.07	0.0705	0.00	1	1	NEG	
36	0.144	0.095	0.1195	0.03	29	5	SUSP	
37	0.193	0.187	0.19	0.00	2	11	POS	
38	0.093	0.094	0.0935	0.00	1	3	NEG	
39	0.077	0.078	0.0775	0.00	1	2	NEG	
40	0.089	0.114	0.1015	0.02	17	4	NEG	

duplicate samples not used in the final analysis

Plate 4

Anti-RVF IgG Indirect ELISA P4

Date: 02.12.2008
Sera: 1:100
Conjugate: **1:10 000 AS IS**

TMB: AS IS
STOP: 1M H2SO4
Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.048	0.044	0.073	0.063	0.184	0.083	0.123	0.088	0.223	0.153	0.166	0.087
B	0.041	0.043	0.077	0.066	0.199	0.084	0.084	0.075	0.279	0.143	0.155	0.082
C	1.29	1.477	0.076	0.086	0.07	0.066	0.076	0.08	0.069	0.084	0.094	0.08
D	1.566	1.695	0.07	0.08	0.068	0.072	0.07	0.074	0.069	0.087	0.086	0.073
E	0.047	0.047	0.061	0.069	0.062	0.066	0.066	0.098	0.069	0.079	0.068	0.096
F	0.056	0.05	0.065	0.071	0.067	0.068	0.066	0.1	0.071	0.079	0.076	0.104
G	0.063	0.046	0.095	0.089	0.075	0.089	0.159	0.146	0.074	0.072	0.125	0.069
H	0.056	0.043	0.085	0.085	0.07	0.082	0.199	0.158	0.081	0.068	0.116	0.067

	Median	PP value
cc	0.044	0
c++	1.522	100
c--	0.049	0
CC	0.051	0

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.073	0.077	0.075	0.00	4	2	NEG
2	0.063	0.066	0.0645	0.00	3	1	NEG
3	0.184	0.199	0.1915	0.01	6	10	SUSP
4	0.083	0.084	0.0835	0.00	1	2	NEG
5	0.123	0.084	0.1035	0.03	27	4	NEG
6	0.088	0.075	0.0815	0.01	11	2	NEG
7	0.223	0.279	0.251	0.04	16	14	POS
8	0.153	0.143	0.148	0.01	5	7	SUSP
9	0.166	0.155	0.1605	0.01	5	8	SUSP
10	0.087	0.082	0.0845	0.00	4	2	NEG
11	0.076	0.07	0.073	0.00	6	2	NEG
12	0.086	0.08	0.083	0.00	5	2	NEG
13	0.07	0.068	0.069	0.00	2	1	NEG
14	0.066	0.072	0.069	0.00	6	1	NEG
15	0.076	0.07	0.073	0.00	6	2	NEG
16	0.08	0.074	0.077	0.00	6	2	NEG
17	0.069	0.069	0.069	0.00	0	1	NEG
18	0.084	0.087	0.0855	0.00	2	3	NEG
19	0.094	0.086	0.09	0.01	6	3	NEG
20	0.08	0.073	0.0765	0.00	6	2	NEG
21	0.061	0.065	0.063	0.00	4	1	NEG
22	0.069	0.071	0.07	0.00	2	1	NEG
23	0.062	0.067	0.0645	0.00	5	1	NEG
24	0.066	0.068	0.067	0.00	2	1	NEG
25	0.066	0.066	0.066	0.00	0	1	NEG
26	0.098	0.1	0.099	0.00	1	3	NEG
27	0.069	0.071	0.07	0.00	2	1	NEG
28	0.079	0.079	0.079	0.00	0	2	NEG
29	0.068	0.076	0.072	0.01	8	2	NEG
30	0.096	0.104	0.1	0.01	6	3	NEG
31	0.095	0.085	0.09	0.01	8	3	NEG
32	0.089	0.085	0.087	0.00	3	3	NEG
33	0.075	0.07	0.0725	0.00	5	2	NEG
34	0.089	0.082	0.0855	0.00	6	3	NEG
35	0.159	0.199	0.179	0.03	16	9	SUSP
36	0.146	0.158	0.152	0.01	6	7	SUSP
37	0.074	0.081	0.0775	0.00	6	2	NEG
38	0.072	0.068	0.07	0.00	4	1	NEG
39	0.125	0.116	0.1205	0.01	5	5	SUSP
40	0.069	0.067	0.068	0.00	2	1	NEG

Plate 5

Anti-RVF IgG Indirect ELISA P5

Date: 02.12.2008
Sera: 1:100
Conjugate: **1:10 000 AS IS**

TMB: AS IS
STOP: 1M H2SO4

Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.16	0.099	0.099	0.087	0.121	0.085	0.105	0.085	0.077	0.09	0.129	0.119
B	0.052	0.073	0.124	0.134	0.123	0.102	0.119	0.086	0.073	0.085	0.178	0.127
C	1.398	1.434	0.092	0.081	0.103	0.109	0.104	0.084	0.117	0.114	0.17	0.387
D	1.543	1.656	0.085	0.081	0.138	0.092	0.108	0.082	0.126	0.117	0.168	0.317
E	0.057	0.054	0.084	0.114	0.088	0.1	0.088	0.081	0.111	0.095	0.074	0.088
F	0.058	0.054	0.082	0.11	0.08	0.103	0.09	0.086	0.112	0.095	0.077	0.082
G	0.048	0.05	0.119	0.093	0.065	0.105	0.123	0.084	0.302	0.117	0.092	0.077
H	0.053	0.049	0.103	0.087	0.069	0.114	0.123	0.089	0.29	0.11	0.094	0.084

	Median	PP value
cc	0.086	2
c++	1.489	100
c--	0.056	0
CC	0.050	0

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values	Results
1	0.099	0.124	0.1115	0.02	16	4	NEG
2	0.087	0.134	0.1105	0.03	30	4	NEG
3	0.121	0.123	0.122	0.00	1	5	SUSP
4	0.085	0.102	0.0935	0.01	13	3	NEG
5	0.105	0.119	0.112	0.01	9	4	NEG
6	0.085	0.086	0.0855	0.00	1	2	NEG
7	0.077	0.073	0.075	0.00	4	1	NEG
8	0.09	0.085	0.0875	0.00	4	2	NEG
9	0.129	0.178	0.1535	0.03	23	7	SUSP
10	0.119	0.127	0.123	0.01	5	5	SUSP
11	0.092	0.085	0.0885	0.00	6	2	NEG
12	0.081	0.081	0.081	0.00	0	2	NEG
13	0.103	0.138	0.1205	0.02	21	5	SUSP
14	0.109	0.092	0.1005	0.01	12	3	NEG
15	0.104	0.108	0.106	0.00	3	4	NEG
16	0.084	0.082	0.083	0.00	2	2	NEG
17	0.117	0.126	0.1215	0.01	5	5	SUSP
18	0.114	0.117	0.1155	0.00	2	4	SUSP
19	0.17	0.168	0.169	0.00	1	8	SUSP
20	0.387	0.317	0.352	0.05	14	21	POS
21	0.084	0.082	0.083	0.00	2	2	NEG
22	0.114	0.11	0.112	0.00	3	4	NEG
23	0.088	0.08	0.084	0.01	7	2	NEG
24	0.1	0.103	0.1015	0.00	2	3	NEG
25	0.088	0.09	0.089	0.00	2	2	NEG
26	0.081	0.086	0.0835	0.00	4	2	NEG
27	0.111	0.112	0.1115	0.00	1	4	NEG
28	0.095	0.095	0.095	0.00	0	3	NEG
29	0.074	0.077	0.0755	0.00	3	1	NEG
30	0.088	0.082	0.085	0.00	5	2	NEG
31	0.119	0.103	0.111	0.01	10	4	NEG
32	0.093	0.087	0.09	0.00	5	2	NEG
33	0.065	0.069	0.067	0.00	4	1	NEG
34	0.105	0.114	0.1095	0.01	6	4	NEG
35	0.123	0.123	0.123	0.00	0	5	SUSP
36	0.084	0.089	0.0865	0.00	4	2	NEG
37	0.302	0.29	0.296	0.01	3	17	POS
38	0.117	0.11	0.1135	0.00	4	4	SUSP
39	0.092	0.094	0.093	0.00	2	3	NEG
40	0.077	0.084	0.0805	0.00	6	2	NEG

Plate 6

Anti-RVF IgG Indirect ELISA P6

Date: 02.12.2008
Sera: 1:100
Conjugate: **1:10 000 AS IS**

TMB: AS IS
STOP: 1M H2SO4
Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.057	0.05	0.064	0.071	0.084	0.071	0.069	0.092	0.102	0.065	0.075	0.087
B	0.058	0.058	0.069	0.073	0.086	0.071	0.075	0.085	0.101	0.066	0.071	0.078
C	1.285	1.423	0.071	0.069	0.074	0.151	0.079	0.167	0.079	0.07	0.064	0.069
D	1.314	1.481	0.075	0.07	0.071	0.154	0.086	0.158	0.083	0.072	0.066	0.072
E	0.063	0.057	0.07	0.081	0.074	0.075	0.082	0.078	0.171	0.088	0.066	0.085
F	0.064	0.059	0.076	0.077	0.074	0.078	0.069	0.088	0.183	0.098	0.074	0.102
G	0.054	0.053	0.07	0.079	0.11	0.092	0.082	0.085	0.09	0.153	0.27	0.073
H	0.053	0.049	0.067	0.075	0.096	0.075	0.067	0.09	0.089	0.16	0.256	0.068

	Median	PP value
cc	0.058	0
c++	1.369	100
c--	0.061	0
CC	0.053	-1

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values
1	0.064	0.069	0.0665	0.00	5	0
2	0.071	0.073	0.072	0.00	2	1
3	0.084	0.086	0.085	0.00	2	2
4	0.071	0.071	0.071	0.00	0	1
5	0.069	0.075	0.072	0.00	6	1
6	0.092	0.085	0.0885	0.00	6	2
7	0.102	0.101	0.1015	0.00	1	3
8	0.065	0.066	0.0655	0.00	1	0
9	0.075	0.071	0.073	0.00	4	1
10	0.087	0.078	0.0825	0.01	8	2
11	0.071	0.075	0.073	0.00	4	1
12	0.069	0.07	0.0695	0.00	1	1
13	0.074	0.071	0.0725	0.00	3	1
14	0.151	0.154	0.1525	0.00	1	7
15	0.079	0.086	0.0825	0.00	6	2
16	0.167	0.158	0.1625	0.01	4	8
17	0.079	0.083	0.081	0.00	3	2
18	0.07	0.072	0.071	0.00	2	1
19	0.064	0.066	0.065	0.00	2	0
20	0.069	0.072	0.0705	0.00	3	1
21	0.07	0.076	0.073	0.00	6	1
22	0.081	0.077	0.079	0.00	4	1
23	0.074	0.074	0.074	0.00	0	1
24	0.075	0.078	0.0765	0.00	3	1
25	0.082	0.069	0.0755	0.01	12	1
26	0.078	0.088	0.083	0.01	9	2
27	0.171	0.183	0.177	0.01	5	9
28	0.088	0.098	0.093	0.01	8	2
29	0.066	0.074	0.07	0.01	8	1
30	0.085	0.102	0.0935	0.01	13	2
31	0.07	0.067	0.0685	0.00	3	1
32	0.079	0.075	0.077	0.00	4	1
33	0.11	0.096	0.103	0.01	10	3
34	0.092	0.075	0.0835	0.01	14	2
35	0.082	0.067	0.0745	0.01	14	1
36	0.085	0.09	0.0875	0.00	4	2
37	0.09	0.089	0.0895	0.00	1	2
38	0.153	0.16	0.1565	0.00	3	7
39	0.27	0.256	0.263	0.01	4	15
40	0.073	0.068	0.0705	0.00	5	1

Plate 7

Anti-RVF IgG Indirect ELISA P7

Date: 02.12.2008
Sera: 1:100
Conjugate: **1:10 000 AS IS**

TMB: AS IS
STOP: 1M H2SO4
Work sheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	cc	cc	1	2	3	4	5	6	7	8	9	10
B	cc	cc	1	2	3	4	5	6	7	8	9	10
C	c++	c++	11	12	13	14	15	16	17	18	19	20
D	c++	c++	11	12	13	14	15	16	17	18	19	20
E	c--	c--	21	22	23	24	25	26	27	28	29	30
F	c--	c--	21	22	23	24	25	26	27	28	29	30
G	cc	cc	31	32	33	34	35	36	37	38	39	40
H	cc	cc	31	32	33	34	35	36	37	38	39	40

OD values Raw Data

A	0.058	0.057	0.08	0.107	0.1	0.109	0.073	0.343	0.156	0.086	0.074	0.074
B	0.064	0.057	0.084	0.106	0.11	0.119	0.084	0.404	0.153	0.081	0.076	0.076
C	0.948	0.969	0.09	0.103	0.107	0.085	0.087	0.067	0.067	0.061	0.06	0.06
D	0.909	1.021	0.084	0.094	0.095	0.084	0.091	0.06	0.067	0.066	0.065	0.06
E	0.068	0.065	0.067	0.064	0.07	0.064	0.066	0.061	0.064	0.077	0.067	0.061
F	0.068	0.068	0.066	0.067	0.057	0.072	0.066	0.062	0.061	0.076	0.061	0.062
G	0.058	0.057	0.059	0.059	0.065	0.061	0.063	0.064	0.06	0.058	0.063	0.061
H	0.058	0.057	0.058	0.058	0.058	0.065	0.061	0.061	0.061	0.058	0.063	0.061

	Median	PP value
cc	0.058	-1
c++	0.959	100
c--	0.068	0
CC	0.058	-1

Interpretation of Results

<	4		neg
4.01	9.99		SUSP
>		10	POS

Sample	OD	OD	AV	SD	%CV	PP Values	Results	
1	0.08	0.084	0.082	0.00	3	2	NEG	
2	0.107	0.106	0.1065	0.00	1	4	SUSP	
3	0.1	0.11	0.105	0.01	7	4	SUSP	
4	0.109	0.119	0.114	0.01	6	5	SUSP	
5	0.073	0.084	0.0785	0.01	10	1	NEG	
6	0.343	0.404	0.3735	0.04	12	34	POS	
7	0.156	0.153	0.1545	0.00	1	10	SUSP	
8	0.086	0.081	0.0835	0.00	4	2	NEG	
9	0.074	0.076	0.075	0.00	2	1	NEG	
10	0.074	0.076	0.075	0.00	2	1	NEG	
11	0.09	0.084	0.087	0.00	5	2	NEG	
12	0.103	0.094	0.0985	0.01	6	3	NEG	
13	0.107	0.095	0.101	0.01	8	4	NEG	
14	0.085	0.084	0.0845	0.00	1	2	NEG	
15	0.087	0.091	0.089	0.00	3	2	NEG	
16	0.067	0.06	0.0635	0.00	8	-1	NEG	
17	0.067	0.067	0.067	0.00	0	0	NEG	
18	0.061	0.066	0.0635	0.00	6	-1	NEG	
19	0.06	0.065	0.0625	0.00	6	-1	NEG	
20	0.06	0.06	0.06	0.00	0	-1	NEG	
21	0.067	0.066	0.0665	0.00	1	0	NEG	
22	0.064	0.067	0.0655	0.00	3	0	NEG	
23	0.07	0.057	0.0635	0.01	14	-1	NEG	
24	0.064	0.072	0.068	0.01	8	0	NEG	
25	0.066	0.066	0.066	0.00	0	0	NEG	
26	0.061	0.062	0.0615	0.00	1	-1	NEG	
27	0.064	0.061	0.0625	0.00	3	-1	NEG	
28	0.077	0.076	0.0765	0.00	1	1	NEG	
29	0.067	0.061	0.064	0.00	7	0	NEG	
30	0.061	0.062	0.0615	0.00	1	-1	NEG	
31	0.059	0.058	0.0585	0.00	1	-1	NEG	
32	0.059	0.058	0.0585	0.00	1	-1	NEG	
33	0.065	0.058	0.0615	0.00	8	-1	NEG	
34	0.061	0.065	0.063	0.00	4	-1	NEG	
35	0.063	0.061	0.062	0.00	2	-1	NEG	
36	0.064	0.061	0.0625	0.00	3	-1	NEG	
37	0.06	0.061	0.0605	0.00	1	-1	NEG	
38	0.058	0.058	0.058	0.00	0	-1	NEG	
39	0.063	0.063	0.063	0.00	0	-1	NEG	
40	0.061	0.061	0.061	0.00	0	-1	NEG	

non samples

SNT RESULTS - LSDV

Sample number	ELISA number	Original number	Result
1	Plate 1: 5	26	Negative
2	Plate 1: 20	65	(1:20)
3	Plate 1: 21	53	Negative
4	Plate1: 24	41	Negative
5	Plate1: 28	189	(1:20)
6	Plate1: 30	201	Negative
7	Plate 2: 4	191	Negative
8	Plate 2: 8	195	(1:10)
9	Plate 2: 10	252	Negative
10	Plate 2: 18	267	Negative
11	Plate 2: 19	268	Negative
12	Plate 2: 20	269	Negative
13	Plate 2: 21	270	Negative
14	Plate 2: 27	277	Negative
15	Plate 2: 30	281	Negative
16	Plate 2: 33	4a	(1:10)
17	Plate 2: 34	4b	Negative
18	Plate 2: 37	37a	Negative
19	Plate 2: 38	37b	Negative
20	Plate 2: 40	44	Negative
21	Plate 3: 7	44	Negative
22	Plate 3: 9	51	Negative
23	Plate 3: 10	46	Negative
24	Plate 3: 12	56a	Negative
25	Plate 3: 13	56b	Negative
26	Plate 3: 15	50a	Negative
27	Plate 3: 16	50b	(1:10)
28	Plate 3: 20	33	Negative
29	Plate 3: 21	42	Negative
30	Plate 3: 27	B37	Negative
31	Plate 3: 29	B39	Negative
32	Plate 3: 30	B40	Negative
33	Plate 3: 31	B41	Negative
34	Plate 3: 32	B42	Negative
35	Plate 3: 38	B48	Negative
36	Plate 3: 39	B49	Negative

Negative control

Not enough serum

Duplicate samples not used in final analysis

SNT RESULTS - LSDV contd

37	Plate 3: 40	B50	Negative
38	Plate 4: 10	B60	Negative
39	Plate 4: 19	B70	Negative
40	Plate 4: 20	B71	Negative
41	Plate 4: 28	B79	Negative
42	Plate 4: 30	B81	Negative
43	Plate 5: 1	D11	Negative
44	Plate 5: 3	D13	Negative
45	Plate 5: 4	D14	Negative
46	Plate 5: 5	D15	Negative
47	Plate 5: 10	D20	Negative
48	Plate 5: 18	D28	Negative
49	Plate 5: 19	D29	Negative
50	Plate 5: 20	D30	Negative
51	Plate 5: 31	D41	Negative
52	Plate 5: 32	D42	Negative
53	Plate 5: 35	B4	Negative
54	Plate 5: 37	B6	Negative
55	Plate 6: 10	B19	Negative
56	Plate 6: 13	B22	Negative
57	Plate 6: 21	B30	Negative
58	Plate 6: 30	A6	Negative
59	Plate 6: 33	A11	Negative
60	Plate 6: 36	A14	Negative
61	Plate 6: 37	A15	Negative
62	Plate 6: 39	A17	Negative
63	Plate 7: 1	A19	Negative
64	Plate 7: 3	A22	Negative
65	Plate 7: 4	A25	Negative
66	Plate 7: 5	A27	Negative
67	Plate 7: 9	A31	Negative
68	Plate 7: 10	A33	Negative
69	Plate 7: 11	A34	Negative
70	Plate 7: 15	A45	Negative
	Plate 6: 34	A12	
	Plate 6: 38	A16	
	Plate 7: 2	A20	
	Plate 7: 6	A28	
	Plate 7: 7	A29	
	Plate 7: 8	A30	
	Plate 7: 12	A36	
	Plate 7: 13	A37	
	Plate 7: 14	A41	

Negative control

Not enough serum

Duplicate samples not used in final analysis

SNT RESULTS - RVFV

Sample number	ELISA number	Original number	Result
1	Plate 1: 3	95	Negative
2	Plate 1: 8	35	Negative
3	Plate 1: 11	96	Negative
4	Plate 1: 29	206	(1:640)
5	Plate 1: 30	201	Negative
6	Plate 2: 4	191	Negative
7	Plate 2: 6	192	Negative
8	Plate 2: 7	197	Negative
9	Plate 2: 8	195	Negative
10	Plate 2: 9	193	Negative
11	Plate 2: 10	252	Negative
12	Plate 2: 20	269	Negative
13	Plate 2: 26	276	Negative
14	Plate 2: 30	281	Negative
15	Plate 2: 31	39	Negative
16	Plate 2: 33	4a	(1:320)
17	Plate 2: 34	4b	(1:640)
18	Plate 2: 39	28	Negative
19	Plate 3: 1	42	(1:320)
20	Plate 3: 5	52	Negative
21	Plate 3: 6	2	(1:80)
22	Plate 3: 10	46	Negative
23	Plate 3: 11	2	(1:80)
24	Plate 3: 12	56a	(1:10)
25	Plate 3: 13	56b	Negative
26	Plate 3: 15	50a	(1:320)
27	Plate 3: 16	50b	(1:640)
28	Plate 3: 20	33	Negative
29	Plate 3: 21	42	(1:160)
30	Plate 3: 23	49	(1:640)
31	Plate 3: 24	52	Negative
32	Plate 3: 31	B41	Negative
33	Plate 3: 32	B42	Negative

Negative control

Not enough serum

Duplicate samples not used in final analysis

34	Plate 3: 36	B46	Negative
35	Plate 3: 37	B47	(1:160)
36	Plate 4: 3	B53	Negative
37	Plate 4: 7	B57	Negative
38	Plate 4: 8	B58	Negative
39	Plate 4: 9	B59	Negative
40	Plate 4: 35	D5	Negative
41	Plate 4: 36	D6	Negative
42	Plate 4: 39	D9	Negative
43	Plate 5: 3	D13	Negative
44	Plate 5: 9	D19	Negative
45	Plate 5: 10	D20	Negative
46	Plate 5: 13	D23	Negative
47	Plate 5: 17	D27	Negative
48	Plate 5: 18	D28	Negative
49	Plate 5: 19	D29	Negative
50	Plate 5: 20	D30	(1:20)
51	Plate 5: 35	D35	Negative
52	Plate 5: 37	D37	Negative
53	Plate 5: 38	D38	Negative
54	Plate 6: 14	B23	Negative
55	Plate 6: 16	B25	Negative
56	Plate 6: 27	A3	(1:20)
57	Plate 6: 38	A16	Negative
58	Plate 6: 39	A17	(1:80)
59	Plate 7: 3	A22	Negative
60	Plate 7: 4	A25	Negative
61	Plate 6: 13	B22	Negative
	Plate 7: 2	A20	
	Plate 7: 6	A28	
	Plate 7: 7	A29	

Negative control

Not enough serum

Duplicate samples not used in final analysis