THE PATHOLOGY OF RINDERPEST IN COMMON WARTHOG, CATTLE AND AFRICAN CAPE BUFFALO EXPERIMENTALLY INFECTED WITH AFRICAN LINEAGES I AND II ISOLATES OF RINDERPEST VIRUS.

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DECLARATION

THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN SUBMITTED FOR A DEGREE IN ANY OTHER UNIVERSITY.

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DEDICATION

In memory of my father William Nyariki

and grandfather William Manyibe.

In gratitude to my mother Janes Nyanduko

and grandmother Anne Mokeira

for bringing me up.

and

most of all, to my wife Jane and daughter Irene

for their love and support.

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ABSTRACT

The persistence of mild rinderpest in the Eastern Africa region despite continued eradication efforts has been a subject of concern in recent years among the wildlife conservation, livestock production, and scientific communities. The current study has examined the role that wildlife play in the transmission dynamics of the disease, by characterizing the pathology of two recent isolates of rinderpest virus (RPV) in common warthogs (*Phacochoerus africanus*), African cape buffaloes (*Syncerus caffer*) and indigenous cattle (*Bos indicus*).

Six (6) wild buffaloes, 13 wild warthogs, and 19 indigenous cattle were used in this study involving kudu/Bov/BK/V2 and RBK/WP/86/1 isolates belonging to the African lineages II and I, respectively, of RPV. Four of the warthogs and four of the cattle were inoculated parenterally with $10^{4.3}$ $TCID_{50}$ of the African lineage II isolate of RPV. The inoculated warthogs were housed in contact with four (4) naive cattle and two (2) naive warthogs. The inoculated cattle were similarly kept in contact with four (4) naive warthogs and two (2) naive cattle. Two other warthogs were inoculated parenterally with a similar dose of the virulent Kabete 'O' strain of RPV belonging to the Asian type lineage to serve as positive controls. Another group of four (4) cattle was inoculated parenterally with $10^{4.8}$ $TCID_{50}$ of the African lineage I (RBK/WP/86/1) isolate of RPV and two of these cattle were kept in contact with four naive wild buffaloes and three (3) naive cattle. Two animals of each of the three species were parenterally inoculated with a placebo comprising of minimum essential medium (MEM) devoid of RPV and kept separate as uninfected controls.

Cattle were clinically examined and sampled daily starting from the initial day to the time of necropsy on days 5 to13, or at convalescence on day 22. Buffaloes and warthogs were chemically immobilized, examined, and sampled on the initial day and then on alternate days starting from days 3 to 9 depending on the group. Blood samples were taken in tubes coated with ethylene-diamine tetra-acetic acid (EDTA) for haematological analysis. The inoculated animals were euthanised for necropsy at the early and late stages of the disease from day 5 to day 13 after inoculation. One each of the uninfected control cattle and warthogs were also euthanised for necropsy along with the inoculated ones. One sick in-contact warthog was euthanised for necropsy 22 days after being housed with others inoculated with the African lineage II isolate of RPV. Representative tissue samples were obtained from euthanised animals and preserved in 10% neutral buffered formalin. The tissues were then processed, stained with haematoxylin and eosin (H&E), and examined under the light microscope using the standard procedure.

The African lineage II isolate of RPV induced a mild disease in the inoculated groups of warthogs and cattle as well as in the warthogs that were kept in contact with the latter. However, the virus induced a moderately severe disease in the group of warthogs that were kept in contact with inoculated ones. The African lineage I isolate of RVP induced a mild clinical disease in both the inoculated and in-contact cattle as well as in the in-contact buffaloes. The virulent Kabete "O" strain on the other hand induced a severe, rapidly fatal disease in the inoculated warthogs. The disease induced by the various strains was clinically characterized by oculonasal discharges, stomatitis of varying degrees, fever in a few cases, and rarely, diarrhoea. In addition, cyanosis of the skin,

vescicular dermatitis and inflammation of joints were observed in warthogs at varying degrees of frequency. Transient leucopaenia was the main haematological change induced by the different isolates while the main gross lesions were congestion of the gastrointestinal mucosa with ulceration in two cases, and congestion of lymph nodes and Peyers' patches. The main histological lesions induced in warthogs and cattle by the three isolates of RPV were varying degrees of epithelial necrosis mostly affecting the mucosa of the gut and lymphocytolysis in lymphoid tissues. These were occassionally accompanied by formation of syncytia and eosinophilic intracytoplasmic inclusion bodies and often infiltrated with neutrophils and macrophages.

These findings showed that warthogs are susceptible to the wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of RPV developing mild to moderately severe disease depending on the mode of transmission. They are capable of transmitting the virus to cattle as well as contracting it from cattle resulting in mild disease. They are also capable of transmitting it to other warthogs resulting in moderately severe disease that may be fatal in a proportion of infected warthogs due to secondary complications.

The results from this study suggest that wildlife plays an important role in the epidemiology of rinderpest but further research is needed to clarify the dynamics involved in the maintenance and persistence of the disease.

CHAPTER 1

1.1 Introduction

Rinderpest is the world's most serious disease of cattle and domestic buffaloes. Natural infections are restricted to the even toed animals of the order *Artiodactyla.* In virgin outbreaks, the disease has the capacity to spread rapidly between animals in plague proportions that cause catastrophic losses. Its classical form, 'cattle plague', can clear cattle from large areas, leading to poverty and great hardships in rural communities (Rossiter, 1999). Other domestic ungulates affected include sheep, goats and the Asian sway-backed pig. Wild ungulates suffer from peracute to mild infections (Anderson *et al*; 1996; Kock *et al.,* 1999). Between the 1960s and 1980s, about two million cattle and buffalo were lost each year due to the disease (Barrett *et al*., 1998).

Rinderpest is considered eradicated from the rest of the world except in a few eastern African countries (Rossiter, 1994; Bessin, 1999; Klooster and Roeder, 2002). In recent years, outbreaks have occurred in Kenya between 1993 and 1997, and in 2001, mainly affecting wildlife in national parks (Kock *et al*., 1999; Kock 2002).

Molecular typing of virus isolates from past rinderpest outbreaks showed that the virus causing the recent outbreaks in wildlife in Kenya was distinct from earlier isolates of the virus recovered from outbreaks of the disease in Kenya during the 1980's and early 1990's (Barret *et al*., 1998). The virus in the recent outbreaks was isolated from a lésser kudu from Tsavo East National Park during an outbreak of rinderpest in 1995 (Kock *et*

al., 1999, Wamwayi *et al.*, 2002). It causes severe disease in natural outbreaks in some species of wildlife such as buffalo (*Syncerus caffer*) and lesser kudu (*Tragelaphus imberbis*) but the infection is unusually mild in experimentally infected cattle ((Kock *et al.*, 1999; Wamwayi *et al.*, 2002). The sequence of nucleotides in a highly conserved fragment of the fusion protein gene of the kudu-derived isolate of rinderpest virus (RPV) is very similar to that of the RGK/1 strain that was isolated in 1962 from a reticulated giraffe (*Giraffa reticulata*) in north eastern Kenya but differs from that of other isolates in the region (Leiss and Plowright, 1964; Barret *et al.*, 1998). Based on the degree of similarity of this nucleotide sequence, the kudu-derived isolate and the RGK/1 strain have been classified together as African lineage II whereas the other recent east African isolates have been classified together as African lineage I (Chamberlain *et al*, 1993; Wamwayi *et al.*, 1995a). A third genotypic group, Asian lineage III, comprises of rinderpest viruses of Asian origin including the virulent Kabete 'O' strain. (Chamberlain *et al.*, 1993) The genotyping of RPV has revealed a correlation between virus lineage type and geographical origin of isolates thus providing a basis for molecular epidemiology (Chamberlain *et al.*, 1993).

The re-emergence of a wildlife-derived African lineage II strain of RPV in eastern Africa that has mild clinical manifestations in susceptible cattle has important implications for the epidemiology of the disease as well as its control and eradication (Barrett *et al.*, 1998). Field observations and experimental studies show that the virus causes no overt disease in cattle, but it causes serious disease in susceptible wildlife. Wildlife in contact with infected cattle may suffer great losses and thus serve as a sensitive indicator of the

presence of the disease. The disease can clear some rare wildlife species from large areas thereby increasing their risk of extinction (Kock *et al.*, 1999; Rossiter, 1999; Wamwayi, 2002).

The pathogenicity of the kudu-derived lineage II isolate of RPV in warthogs has not been investigated. Field observations indicate that warthogs are highly susceptible to RPV and die in large numbers during outbreaks (Anderson *et al.*, 1996). More recent field observations, during outbreaks in areas where the recently recovered African lineage II isolate of RPV was circulating indicate that warthogs intermingled with sick buffaloes without themselves getting the disease (Rossiter, 1999). This could mean that either warthogs are not highly susceptible to the African lineage II virus responsible for the outbreaks or that there are other epidemiological factors that were not favourable for cross-transmission to take place. Warthogs are an important part of the Somali ecosystem where they interact closely with livestock around the Somali homesteads (Personal observations).

There are fresh calls to re-examine the role of wildlife in maintaining and transmitting rinderpest in view of these recent observations (Wamwayi *et al.*, 2002). Among aspects that require investigation are transmissibility of the kudu-derived African lineage II isolate between wildlife and livestock and its pathology in wildlife and livestock.

The pathology and transmission of rinderpest in cattle have been extensively studied (Huncheon, 1902; Littlewood, 1905; Mettan, 1937; Robey and Hale, 1946;Plowright,

1964; Leiss and Plowright, 1964;Tajima and Ushijima 1970; Gathumbi, 1988; Wamwayi *et al*., 1995b; Wamwayi *et al*, 2002; Wohlsein *et al*, 1995). Wamwayi *et al* (1995 b) and Wohlsein *et al* (1995) characterized the recent African isolates of the rinderpest virus in terms of their pathogenicity, pathology and antigen distribution in cattle. Their work, however, did not address the kudu-derived African lineage II isolate. Wamwayi *et al* (2002) showed experimentally that the kudu-derived African lineage II rinderpest virus isolate is unusually mild in cattle but highly pathogenic in buffalo. Their study, however, focused mainly on transmission dynamics and not on detailed studies of the pathology of the virus in the experimental hosts.

Comparative studies on the kudu-derived African lineage II and cattle-derived African lineage I isolates of RPV in wildlife and cattle may yield a better understanding of the current role of wildlife in the epidemiology of rinderpest. This will contribute information applicable in the design of comprehensive prevention, control and eradication strategies. The main aim of the present study is to contribute towards better understanding of the cross-transmission, pathogenesis and pathology of rinderpest in warthogs, buffaloes and cattle infected with African lineages I and II isolates of RPV.

1.2. Objectives of the Study

The main objective of this study is to determine the pathology of rinderpest in warthogs (*Phacochoerus africanus*) buffaloes (*Syncerus caffer*) and indigeneous cattle (*Bos indicus*) experimentally infected with African lineages II and I isolates of RPV. The specific objectives are:

I. To determine and compare the gross and microscopic lesions caused by the wildlife-derived African lineage II isolate and a cattle-derived African lineage I isolate (RBK/WP/86/1) of RPV in experimentally infected African Cape buffaloes (*Syncerus caffer*), common warthogs (*Phacochoerus africanus*) and indigenous cattle (*Bos indicus*).

II. To determine and compare the clinical signs caused by the two isolates of RPV in experimentally infected African Cape buffaloes (*Syncerus caffer*), common warthogs (*Phacochoerus africanus*) and indigenous cattle (*Bos indicus*).

III. To determine the haematological parameters in common warthogs, buffaloes and indigenous cattle infected by the two isolates of RPV at different stages of the disease.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Definition

Rinderpest is a disease of ruminants and pigs, caused by a virus belonging to the genus Morbillivirus and the family Paramyxoviridae. The disease is characterized by fever, erosive stomatitis, lymphocytolysis and gastroenteritis. It varies in severity from mild outbreaks with low morbidity and low mortality rates in endemic areas to extremely severe outbreaks in non-endemic areas with high morbidity and high mortality rates approaching 100% (Rossiter, 1994). The disease affects both domesticated and wild species.

2.2 History of Rinderpest

Rinderpest appears to have originated in Asia from where it regularly invaded Europe with army oxen used for transport in the18th and 19th centuries (Cilli *et al* 1951, cited by Rossiter, 1994). By the end of the nineteenth century, the disease had been eradicated from most of Europe by using slaughter and quarantine methods. By this time, mules and then motorized transport had replaced oxen as the main source of military draught power thereby decreasing chances of re-introduction through military oxen. A series of outbreaks however recurred after the First World War. The last reported of these was in 1949 in Rome Zoo, which was caused by importation of animals from a high-risk area (Cilli *et al* 1951, cited by Rossiter, 1994). The disease has never been reported in North America. The only outbreaks in South America and Australia occurred in 1920 and 1923,

respectively, both due to importation of live infected animals (Roberts, 1921; Weston, 1924).

Various writers have described the history of rinderpest in Africa in details (Mettam, 1937; Huncheon, 1902; Littlewood, 1905). It is often thought to have began with the 1889-1897 pandemic but it is highly likely that the disease had been introduced before the pandemic into North Africa, especially Egypt, and into West Africa (Mettam, 1937). The origin of the virus that started the rinderpest pandemic is uncertain but three possible sources have been documented (Huncheon, 1902, Littlewood, 1905). The most frequently quoted source was the introduction of infected Indian or Arabian cattle to Massawa in Eritrea between 1887 and 1889. The disease then spread throughout Ethiopia and into the Nile Valley in Sudan in 1890. The second possibility is that the virus, which reputedly had been introduced into Egypt through British military campaigns in 1884 and 1885, moved slowly up the Nile. The third possibility is that the virus was introduced by a Germany military expedition that brought cattle from Aden and Bombay to the East African coast in late 1889.

The rinderpest pandemic spread through Kenya, Uganda and Tanzania between 1890 and 1892 from there it moved further west to Congo and South to the Zambezi River and beyond. By 1897 the disease had reached the Cape, Natal, Lesotho and Namibia in Southern Africa (Huncheon, 1902, Littlewood, 1905).

The mortality rate of the pandemic was over 90 % in some areas clearing practically all cattle. Wildlife were also affected with buffalo, eland and warthog dying most (Huncheon, 1902, Littlewood, 1905). Other wildlife species reportedly affected were giraffe (*Giraffa spp.*) and many small antelopes especially bushbuck (*Tragelaphus scriptus*) and reedbuck (*Redunca spp.*). Elephants (*Loxodonta africana*), hippos (*Hippopotomus ambhibius*), rhinos (*Diceros bicornis, Cerathotherium simum*), all classes of hartbeest (*Alcelaphus spp.)* and waterbucks (*Kobus ellipsiprymnus*) reportedly seemed unaffected (Huncheon 1902).

The disease was briefly eradicated from South Africa in 1899 through strong control campaigns that included the use of newly developed prophylactic inoculations. However, it was re-introduced from Namibia in 1901, but this was mild and easier to control (Turner, 1906). Southern Africa was reported free of the disease in 1905 (Turner, 1906).

By mid 1970s the number of rinderpest outbreaks in Africa as a whole had been reduced greatly due to successful vaccination campaigns using attenuated rinderpest virus vaccines (Rossiter, 1994). By 1976 the virus had been eradicated from vast areas of the continent and was restricted to two zones where access and animal movement control had always been difficult- namely, the Mali/Mauritania border and the internal Niger delta in West Africa, and Ethiopia and Sudan in Eastern Africa (Rossiter, 1994). The first Pan-African Rinderpest vaccination campaign, Joint project number 15 (JP 15) of the Organization of African Unity (OAU) implemented between 1962 and 1976, was instrumental in drastically reducing the incidence of rinderpest in Africa (Rossiter, 1994).

When the project ended in 1976 a sense of security had been created, yet governments had no funds to maintain the high levels of herd immunity that had been induced. This coincided with a period of recession of the world economy and some countries ceased vaccinations completely and some partially (Rossiter, 1994).

A second rinderpest pandemic started in 1979 (Rossiter, 1994). Although the origin of the pandemic was not clearly known, its underlying causes were clearly associated with the cessation of vaccination three years previously. This had led to an increase in the proportion of susceptible cattle leading to spread of the virus from its endemic strongholds. A most successful emergency campaign carried out by veterinary departments supported by international funds managed to contain the second pandemic. Tens of millions of dollars were used in the emergency after which renewed calls to eradicate rinderpest from Africa came and as a result the Pan African Rinderpest Campaign (PARC) was launched in 1986 (Rossiter, 1994). This European Community-funded project, advised and assisted by various other donors and executed through the Inter-African Bureau for Animal Resources of the Organization of African Unity (OAU/IBAR) aimed to help Africa eradicate rinderpest within a few years (Rossiter, 1994)

Rinderpest has not been reported in West Africa since 1985 and is considered eliminated there (Bessin, 1999). Recent outbreaks have occurred in a few eastern Africa countries where the disease is believed to persist. It has however not been reported from Uganda since 1994, and Ethiopia and Tanzania since 1997(Bessin, 1999). Kenya has had

outbreaks between 1993 and 1997, and in 2001, all involving wildlife (Kock *et al* 1999, Kock, 2002).

With the conclusion of PARC on 31st March 1999, the rinderpest eradication campaign was passed on to a new program, the Pan-African Program for the Control of Epizootics (PACE), funded by the European Commission (Bessin, 1999). Realizing the role that wildlife play in the epidemiology of rinderpest, the PACE program incorporated a wildlife component, the African Wildlife Veterinary Project (AWVP) concerned with surveillance of the disease in wildlife. PACE aims at strengthening the capacity of African countries to control rinderpest and other important epizootics. The Food and Agriculture Organization's Global Rinderpest Eradication Programme (FAO-GREP) aims at global eradication of rinderpest by the year 2010 (Klooster and Roeder, 2002).

2.3 Aetiology of Rinderpest

The causative virus is a member of the genus Morbillivirus in the family Paramyxoviridae. The virus is pleomorphic and has roughly spherical forms measuring 100-300 nm in diameter (Plowright *et al.*, 1962). The virus contains a single strand of ribonucleic acid (RNA) of about 15 kilo base pairs and is enveloped (Barret, 1987). The envelope contains the haemaglutinin (H) and fusion (F) proteins underlain by the matrix (M) protein. The H protein, also found in other morbilliviruses, binds to cell receptors and the F protein induces the formation of syncytia (Barret, 1987). The RNA is protected within the virion by a nucleocapsid (N) protein. Transcription in infected cells is mediated by the polymerase (L) and polymerase associated (P) proteins. Two non-

structural proteins (C and V) are found in virus-infected cells but not in purified virions (Barret, 1987).

The entire nucleotide sequence of rinderpest virus has been determined by recent molecular studies of the Kabete "O" strain of the virus (Baron and Barret, 1995). Analysis of data on nucleotide sequence from current and historic isolates of the virus reveals three phylogenetically distinct lineages occurring in different parts of the world, namely Asian, African I, and African II (Barret *et al*, 1998). This technology may allow the source of an outbreak to be determined or at least provide evidence on the origin of the virus (Barret *et al*, 1998).

The virus is readily inactivated by lipid solvents and is sensitive to light, ultraviolet radiation, heat and extremes of pH. It is destroyed by most chemical disinfectants and by glycerol, which should, therefore, not be used in transport medium for diagnostic samples (Plowright, 1968)

The virus grows in monolayer cell cultures inducing various cytopathic effects including rounding of cells which also become refractile, cytomegally, cytoplasmic stranding, formation of large and small syncytia, and eventually degeneration of the cell sheet (Plowright and Ferris, 1959). In culture it is capable of producing plaques and induces interferon production (Taylor and Perry, 1970).

2.4 Natural Host Range

Natural infections are restricted to the even-toed ungulates belonging to the order *Artiodactyla.* Strains of the virus vary widely in their host affinities and in their virulence for particular hosts. The virus may not attack all the susceptible species at risk and host preferences may change with time (Anderson *et al.*, 1996).

Disease is most commonly observed in domestic ungulates particularly cattle and some wild ungulates particularly buffaloes. The occurrence of rinderpest in sheep and goats is rare but the rinderpest like disease *peste des petits ruminants* (PPR) more commonly affects these two species (Anderson et al., 1996). The Asian domestic sway-backed pig suffers from and succumbs to rinderpest while European pigs experience inapparent infections when exposed experimentally (Anderson et al., 1996).

African buffalo, eland, kudu and warthog are highly susceptible and suffer a percute disease. In Africa, bongo, bushbuck, bush pig, dik-dik, duiker, giant forest hog, giraffe, sitatunga and wildbeeste suffer acute infections that usually end fatally. In Asia, banteng, blackbuck, gaur, nilgai and sambar suffer acute infections (Anderson *et al*, 1996).

2.5 Epidemiology of Rinderpest

Infected animals excrete virus in all secretions and excretions from 24-48 hours before the onset of clinical signs until either death or after the development of high titres of antibody 7 to 10 days later (Scott, 1964a; Plowright, 1968). Since the virus is fragile and highly susceptible to inactivation by physical and chemical means, it usually survives for

only a few hours outside the host. Transmission, therefore, requires close contact between infected and susceptible animals. Airborne spread other than for a few metres inside animal pens has never been proven to occur over long distances outdoors. Recovered animals are solidly immune for the rest of their life and never re-excrete the virus (Rossiter, 1999). Transfer by formites is rare and arthropod-borne transmission is unknown. The disease is therefore maintained and spread by the mixing of infected and susceptible stock. As several recent outbreaks in Africa and Asia have shown, virtually all outbreaks can be traced to the introduction of unvaccinated stock especially trade animals (Rossiter, 1999).

At present it is generally accepted that rinderpest virus is maintained in eastern Africa by a continuous cycle of infection in domestic cattle and free ranging buffalo. The virus can be maintained in cattle alone by constant intermingling of livestock through migration of pastoralists (Rossiter, 1992). It is known that infected wildlife transmit the disease amongst themselves and can carry it over significant distances to re-infect cattle not linked to the original source of the infection (Carmichael, 1938; Plowright, 1968; Woodford, 1984). Wildlife are however considered incapable of persistently maintaining the virus, going by empirical observations and serology (Rossiter, 1999). There is evidence that the longest time that the infection can last in wildlife before dying out naturally is only one or two years (Rossiter *et al.*, 1987; Anderson *et al.*, 1990). Although Africa's wild ungulates are affected severely by rinderpest they are unlikely to be the reason for the persistence of rinderpest in East Africa, rather they are sentinels (Rossiter, 1999).

Molecular typing (Chamberlain *et al.,* 1993; Barret *et al.*, 1998) indicates that the virus responsible for the recent outbreaks in wildlife in Kenya is closely related to the RGK/1 strain, which was isolated from a sick giraffe in northeastern Kenya over 30 years ago (Leiss and Plowright, 1964). Since then, viruses of similar genotype had not been isolated from East Africa until the recent outbreaks. The RGK/1 strain and the virus causing the recent outbreaks were classified together based on their phylogenetic similarity into African type II lineage (Chamberlain *et al.,* 1993; Barret *et al.*, 1998). Analysis of virus isolates from previous outbreaks that had occurred in border areas of Kenya, Sudan and Ethiopia for ten years prior to the recent outbreaks revealed that they were all closely related and of a different genotypic lineage, collectively grouped as African type I (Chamberlain *et al.,* 1993; Barret *et al.*, 1998). These molecular studies provided evidence that the recent outbreaks originated from an unknown focus different from the previously reputed endemic foci. Wildlife surveillance studies suggest that the focus of origin for the African type II RPV is likely to be in the border areas between northeastern Kenya and Southern Somalia (Barret *et al*, 1998).

Interspecies transmission of rinderpest in wildlife is poorly understood. In some wildlife outbreaks most of the susceptible wildlife species are involved either sequentially or simultaneously, whereas in other outbreaks only some or perhaps one species is affected (Rossiter, 1999). The reasons for different patterns of occurrence are not clear. They may include factors such as different contact rates between species at different times, the

susceptibility of various species to different strains of the virus and changes in behavior of infected animals, among others. (Plowright *et al.*, 1964; Rossiter, 1999).

Although warthogs are reputed to be highly susceptible to rinderpest, they have been observed intermingling with infected buffalo without being affected. It was therefore suggested that the African type II lineage virus circulating in eastern Africa is not particularly pathogenic to the warthogs (Rossiter, 1999).

The African lineage II RPV is unusually mild in cattle, causing disease that may not be clinically recognizable. The disease may therefore occur in cattle in an area and go unnoticed (Rossiter, 1999; Wamwayi *et al*, 2002)

2.6 Pathogenesis of Rinderpest

Inhalation of aerosols containing virus and ingestion of infected material are the major routes of infection in natural outbreaks (Hornby, 1926). Primary multiplication occurs in the palatine tonsils, pharyngeal lymph nodes and to a lesser extent in other regional lymph nodes (Plowright, 1964). Following primary multiplication there is a viraemia that enables the virus to infect and replicate in lymphoid tissues throughout the body. This results in a further increase in viraemia, which transports the virus to epithelial tissues, especially those of the alimentary tract. The virus-induced cytopathic effects produce the typical lesions of the disease (Plowright 1968).

Virulent strains have greater ability to infect lymphoid cells and may grow to higher titres in these cells than strains which induce mild disease (Rossiter and Wardley, 1985). During disease the virus is also found in non-lymphoid organs such as the lungs, liver and kidney (Leiss and Plowright, 1964). Immunofluorescence antibody staining techniques have shown that the antigen-bearing cells in these organs are usually associated with the mononuclear phagocyte system and perivascular connective tissue (Rossiter and Jesset, 1982 b).

Virulent strains of rinderpest virus are excreted from epithelial cells one or two days before the appearance of fever or lesions (Leiss and Plowright, 1964), but the amount of excreted virus increases dramatically as the lesions develop and only starts to decline when the immune response becomes detectable usually four to six days after the start of fever. The virus is usually undetectable in secretions and blood by 12-14 days after the start of fever. At the height of virus excretion, virus titers of up to 10^5 tissue culture infectious doses ($TCID_{50}$) per swab and up to 10^6 $TCID_{50}$ per gram respectively can be found in nasal secretions and faeces from cattle infected with virulent strains (Leiss and Plowright, 1964).

Viral antigens are produced in large quantities throughout the lymphoid tissues and affected epithelia (Rossiter *et al*, 1982b) and stimulate an effective antibody response which begins four to six days after onset of clinical disease in virulent infections, and six to ten days after infection with mild or avirulent strains (Plowright and Ferris, 1959). Mainly immunoglobulins M (IgM) and G (IgG) are produced. IgM are the early response

and are detected by virus neutralization test (VNT), Enzyme Linked Immunosorbent Assay (ELISA), immunoprecipitation, compliment fixation, and measles virus haemaglutination inhibition (Rosssiter *et al.*, 1981; Anderson *et al.*, 1996). IgG antibodies, which persist much longer, usually for life, are measured by VNT or ELISA (Rossiter *et al.*, 1981; Plowright 1984).

The severity of the cytopathology caused by the virus before the onset of antibody development influences the course of the disease. Virulent strains cause severe lesions before being restrained by the immune response, and such animals if sufficiently damaged will die despite the high titres of antibody and low or undetectable amounts of virus (Scott, 1959).

Immunoperoxidase staining techniques have shown that the necrotic changes in the lymphoid tissues, epithelia and other organs are associated with large quantities of RPV antigen (Gathumbi *et al.*, 1989). Cells bearing RPV antigen are not only widespread in lymphoid tissues, but are also prevalent in cells in loose connective tissues (Rossiter and Jesset, 1982 b).

The massive destruction of lymphocytes causes immunosuppression. In rabbits, cell-mediated and humoral immunity are suppressed through the loss of both T and B-lymphocytes (Kobune *et al.*, 1976) and this is probably also the case in cattle.

2.7 Clinical Signs of Rinderpest

The clinical signs of rinderpest have been extensively described (Plowright, 1968; Scott, 1964 a; Rossiter, 1994). The following description gives the basic signs in the sequence in which they occur in cattle. The signs described are most apparent in the classical disease caused by virulent virus.

Acute natural infections and those induced by parenteral inoculations of RPV have an incubation period of three to five days. Incubation periods following experimental contact infection are longer, from eight to 15 days (Leiss and Plowright, 1964).

The first sign of the disease is fever of 40-41.5°C which can last for up to 2 weeks or less, before returning to normal. Depression, inappetence, restlessness and reduced milk yield may occur during the first or second days of fever. The visible mucosae are congested and a serous oculonasal discharge, one of the characteristic signs of rinderpest, becomes apparent within this early stage. Between the second and fifth days of fever the mucosal lesions develop as pinpoint white foci on the gums and lips, which spread rapidly to involve the buccal mucosa and ventral and lateral aspects of the tongue. The foci enlarge and coalesce into plaques of caseous necrotic debris that desquamate easily, leaving circumscribed erosions. Similar lesions may be seen on the palate, posterior dorsum of the tongue, pharynx, and in the nares and vagina. Severe cases often drool foetid saliva, presumably because of discomfort in the mouth and pharynx when swallowing. There is a strong halitosis.

In cattle infected with strains of high virulence, diarrhoea, sometimes preceded by an absence of defaecation (usually with no signs of constipation), starts four to five days after the onset of pyrexia, usually one to two days after mouth lesions become visible. The faeces are usually thin and dark, but sometimes contain blood, mucus and shreds of necrotic epithelium. The hindquarters are soiled and tenesmus with eversion of the rectal mucosa is frequently observed. The diarrhoea causes dehydration, weakness and prostration, and severe cases manifest abdominal pain. Recumbent animals face their flanks in a typical 'milk fever' posture. The oculonasal discharge becomes increasingly purulent during the course of the disease and conjunctivitis causes photophobia. Corneal opacity is rare in cattle, but has been recorded in some species of wildlife such as giraffe, buffalo and lesser kudu (Shanthikumar *et al*., 1985; Kock *et al*., 1999)

Skin lesions occur rarely appearing as a maculo-papular rash on areas of soft skin such as the axillae and groin. Hair becomes matted in such areas and the rash eventually becomes pustular and breaks open (Joshi *et al*, 1977).

Respiration may be laboured in acute disease and pulmonary emphysema may occur terminally. Lymphadenopathy is not usually evident even on palpation. Milk may become watery or dry up. Pregnant animals frequently abort, sometimes weeks or months after clinical disease.

Mucosal lesions may heal rapidly within three to six days after their appearance, and this marks the convalescence period. Diarrhoea diminishes more slowly and may persist in a

less severe form for up to two weeks. Complete recovery from rinderpest takes one to two months or more, depending on the extent to which the animal has lost condition and on the quality of subsequent nursing.

Infected animals are immunosuppressed to variable degrees frequently leading to the re-activation of chronic or latent diseases, especially those caused by blood parasites such as *Anaplasma marginale* (Scott, 1964 a).

In endemic areas the disease is milder than described above (Plowright, 1963) Most of the signs are much reduced in severity and some may be absent. Experimental infections with such strains have shown that they may cause no deaths, no diarrhoea, or only transient mouth lesions in a proportion of infected *Bos taurus* cattle, and only transient pyrexia in *Bos indicus* cattle (Plowright, 1963; Wamwayi *et al*, 2002).

Clinical signs vary in sheep and goats but are usually mild or absent (Rossiter *et al*, 1982b). Asian pigs show severe disease similar to that in cattle whereas European pigs usually show no signs (Hudson and Wong, 1950; Scott *et al*, 1962). In endemic areas, camels generally do not show clinical signs although mouth lesions, diarrhoea and death have been reported (Haji, 1932, Scott and MacDonald, 1962)

Wild ungulates exhibit a range of clinical signs, associated with erosive stomatitis and gastroenteritis, that cause death in African buffalo, eland, giraffe and warthog (*Phacochoerus aethiopicus*) (Percival, 1918; Thomas and Ried, 1944) or mild and non-

specific signs in impala (Scott *et al*., 1960). Corneal opacity and skin lesions have often been described for rinderpest in wildlife such as giraffe (*Giraffa spp*.), Grant's gazelle (*Gazelle granti*), African buffalo (*Syncerus caffer*) and eland (*Taurotragus oryx*) and lesser kudu (*Tragelaphus imberbis*) (Plowright, 1982; Shanthikumar *et al*., 1985;Kock *et al* 1999).

In recent outbreaks in Kenya attributed to the lineage II rinderpest virus, buffaloes (*Syncerus caffer*), lesser kudu (*Tragelaphus imberbis*) and eland (*Taurotragus oryx*) were most affected (Kock *et al*., 1999, Barret *et al* , 1998). Buffaloes had severe disease with the clinical signs described above for wild ungulates and their mortality was estimated at 25-60%. In addition, peripheral lymph nodes were enlarged in chronic cases and severe eye lesions including keratoconjunctivitis, corneal ulcers, uveitis, iritis and cataract were observed in buffaloes recovering from the infection. Very high mortality (above 80%) was reported in lesser kudu in which the most consistent clinical sign was blindness and marked tear staining. Swollen knee and hock joints were often seen. Affected eland hung back from the herd, were depressed, inappetent, with marked tearing, corneal opacity and diarrhoea in a few cases.

2.8 Pathology of Rinderpest

2.8.1 Clinical pathology

A proportion of infected cattle show slight lymphocytosis before the onset of pyrexia. This is followed by marked lymphopenia, caused by lymphoid necrosis, which in most cases lasts throughout the acute clinical stage of the disease (Robey and Hale, 1946).

During convalescence, lymphocyte levels slowly return to normal over a period of days or weeks. Neutrophils remain relatively unaltered although juvenile forms are common during the terminal stages of the fatal infection. A degree of neutropenia that parallels the lymphopenia may occur. Eosinophils may disappear from the blood during the early stages of clinical disease returning to normal levels two to three weeks later. In severe cases excessive loss of water causes haemoconcentration (Baldrey, 1906).

Wild ungulates show changes in blood similar to those described for cattle (Kock *et al.*, 1999). Leucopenia, lymphopenia and neutropenia are reported from early cases in buffaloes, progressing to leucocytosis. Lymphopenia or mild to moderate lymphocytosis, and high packed cell volume of up to 75 % can be recorded in lesser kudu, the latter indicating extreme haemoconcentration.

Serum aspartate transaminase and blood urea nitrogen levels increase during severe cases of the disease (Heuschele and Barber, 1966; Battacharya and Chakraborty, 1979). Serum chloride levels fall markedly in terminal illness, and other electrolytes may decrease in absolute terms although this can be masked by haemoconcentration. Blood clotting may be impaired in severely affected animals. Serum protein levels may be lowered especially in fatally infected animals (French, 1936; Dhir *et al.*, 1987).

2.8.2 Gross pathology

Rossitter (1994) has extensively reviewed the gross pathology of rinderpest. The lesions are a direct result of virus-induced cytopathology although complications do arise due to

re-activation of latent pathogens, especially protozoa (Holmes, 1904). Descriptions of the disease in cattle by Maurer *et al.*, (1956) and in wildlife by Kock *et al.* (1999) are quite elaborate. Anderson *et al.*, (1996) have reviewed the gross pathology in pigs.

2.8.2.1 Gross pathology in cattle and wild ruminants

The appearance at necropsy is similar for most species that die of typical severe rinderpest. The carcass is dehydrated, sometimes emaciated, and usually soiled with fluid faeces. The eyes are sunken and often encrusted with mucopurulent discharge and cheeks may show signs of epiphora.

Erosions with or without necrotic material may be found throughout the mouth but predilection sites are the gums, lips, buccal papillae, dorsal and ventral aspects of the tongue and the soft palate. The erosions often extend into the pharynx, anterior oesophagus, rumen (especially the pillars), the reticulum and the omasum. Necrotic areas some of which may penetrate the leaves of the omasum are sometimes present.

The folds of the omasum are congested and oedematous and often show necrosis, erosions and haemorrhage along the edges. The fundus of the abomasum may have small discrete erosions that increase in size towards the pylorus, where whole areas of mucosa may become desquamated. The early necrotic lesions are pale greyish, whereas the erosions are often red as a result of congestion of the underlying lamina propria. Haemorrhage may occur from the raw surfaces. The abomasum is almost invariably severely affected. Congestion, oedema and erosions may occur on the margins of

mucosal folds of the anterior duodenum and terminal ileum. The Peyer's patches, being lymphoid tissue, are severely affected and are swollen, dark-red to almost black as a result of haemorrhage, and may slough completely leaving deep crater-like ulcerated areas. Large erosions are commonly found on the ileo-caecal valve. In the large intestine, marked oedema and congestion accompanied by petechiae or larger haemorrhages occur, particularly along the crests of longitudinal folds of the mucosa. This can be very striking in the colon and rectum, meriting the earlier description of 'zebra striping'. In acute cases the gut has desquamated necrotic epithelium with blood and fibrin exuding from exposed lamina propria.

The urinary bladder and gall bladder are frequently congested and haemorrhagic with occasional erosions. The vaginal mucosa may be congested and have small erosions.

The mucosa of the upper respiratory tract is congested and often covered with mucopurulent exudate. Petechial haemorrhages and necrotic, erosive lesions may occur in the nares, extending to the larynx. The tracheal mucosa is frequently congested. Congestion and emphysema may also occur in the lungs, while secondary bronchopneumonia develops in chronic cases.

Skin lesions are rare in cattle but are common in domestic buffaloes. Exudative dermatitis, characterized by macular to pustular lesions, develops and may be complicated by secondary bacterial infections such as *Dermatophilus congolensis*.

Superficial and visceral lymph nodes show few changes that may include congestion, oedema and focal petechial haemorrhages. The lymph nodes of animals that die after a prolonged clinical course may be shrunken and may show greying radial streaks in the cortex, presumably due to haemorrhage. The spleen and haemolymphnodes are normal or slightly enlarged.

In recent outbreaks in wildlife in Kenya involving lineage II RPV (Kock *et al.*, 1999), most of the lesions described above were observed. In addition, synovitis and tendosynovitis around the radial and tarsal joints, as well as enlargement of liver and gall bladder and hyperemia of the kidneys were observed in lesser kudu. Splenic enlargement and congestion, as well as liver and gall bladder enlargement were recorded in eland.

2.8.2.2 Gross pathology in pigs

Anderson *et al.*, (1996) have extensively reviewed the pathology of rinderpest in pigs in the FAO Manual for Diagnosis of Rinderpest. According to these authors, rinderpest virus kills Asian domestic pigs but not European-type pigs. Fatal infections also occur in African wild pigs such as the warthog, bush pig and giant forest hog. The gross lesions in fatal infections as described by the authors are as follows.

The carcass is in poor condition, soiled with fluid faeces and foul smelling. Lesions in the alimentary tract include stomatitis and gastroenteritis. The stomatitis ranges in severity from cyanosis at the back of the tongue and in the pharynx to extensive diphtheresis involving all the oral surfaces. The gastritis varies from mild hyperemia in the pyloric

region to diffuse, deep congestion with ulcerative necrosis of the epithelium. The ulcers are often covered with diphtheritic pseudomembranes. Lesions in the small intestine range from small necrotic patches to occasional haemorrhagic enteritis extending from the duodenum to the rectum. Lesions are usually prominent in the caecum and include congestion, ulceration and diphtheria. In pigs that die late in the disease, the necrotic ulcers in the caecum may be the only lesions. The colonic mucosa has irregular blotches of congestion in the entire length. The liver is not affected. The lesions in the gall bladder, however, range from mild vascular arborescence to diffuse congestion of the mucosa.

Gross changes in the respiratory tract are common and consist of cyanosis of the larynx, haemorrhagic streaks in the upper trachea, pulmonary congestion and patches of secondary bronchopneumonia but opinions vary about the frequency of pulmonary emphysema. Varying extents of congestion occur in the kidney and the mucosa of urinary bladder.

Lymphoid organs exhibit a variety of necrotic lesions that are particularly conspicuous in the gut associated lymphoid tissues. The spleen is usually grossly normal although it may occasionally be swollen.

The gross lesions in the cardiovascular system include changes in the heart and blood. The heart, at most, shows pale, dry areas in the myocardium. Sub-endorcardial and sub-epicardial haemorrhages have not been described. The blood is dark and clots promptly.

Changes in the skin are common, ranging from discrete areas of congestion and cyanosis on the abdomen and legs to extensive purple blotching and subcutaneous ecchymosis. Eczematous eruptions may occur around the anus and on the perineum.

2.8.3 Histopathology

The histopathology of rinderpest was first described in infected rabbits (Brotherstone, 1951). Widespread necrosis of lymphocytes is apparent throughout the lymphoid tissues, together with syncytia and intracytoplasmic and less frequently, intranuclear inclusion bodies. The histopathology in cattle is similar (Maurer *et al.*, 1956) with lytic destruction of lymphoid tissues that is most pronounced in the germinal centres, which also may contain increasing numbers of macrophages. In acute cases, lymph nodes are virtually devoid of cells, leaving a reticular stroma containing eosinophilic material.

The early epithelial lesions in the stratified squamous epithelium of the digestive tract are associated with hydropic degeneration, the formation of syncytia and eosinophilic intracytoplasmic inclusions in the *stratum spinosum*. Immunohistochemical techniques have confirmed that the inclusion bodies are aggregates of viral antigens (Gathumbi *et al.*, 1989). Infected epithelial cells become necrotic and slough off leaving clearly demarcated erosions. Wohlsein *et al.*, (1995) characterized various African isolates of the rinderpest virus and showed that although their histopathologic lesions were similar, the milder strains caused less severe lesions that were less widely distributed in the tissues.

Ultrastructurally, infected cells contain large arrays of tubular nucleocapsid material, which are the intranuclear, and intracytoplasmic inclusion bodies seen by light microscopy (Breese and DeBoer, 1963).

2.9 Diagnosis

The diagnosis of rinderpest has been described in detail (Scott *et al.*, 1986; Anderson *et al.*, 1996). Presumptive diagnosis is based on clinical signs and gross pathology in endemic areas. In areas where the disease is not prevalent, especially those depending on livestock exports, laboratory confirmation is essential as soon as possible. Although histopathology is not sufficiently specific to confirm a diagnosis of rinderpest, demonstration of pertinent lesions in gastrointestinal and lymphoid tissues is supportive.

Detection of viral antigen using rabbit hyperimmune serum against RPV can be done by a number of tests. The most commonly used assay is the agar-gel immunodiffusion (AGID) test. Counter-immunoelectrophoresis is quicker and more sensitive than AGID (Forman *et al.*, 1983). Immunofluorescence and immunoperoxidase staining are very sensitive (Babu *et al.*, 1984; Rossiter and Jesset, 1982b).

Virus isolation is done using cell-cultures of bovine kidney or vero cells. Cattle inoculation using known immune and susceptible cattle can be done in isolation facilities if cell cultures are unavailable.

Serum antibodies increase four-fold or more in recovered cases. The virus neutralization tests (VNT) and enzyme linked immunosorbent assays (ELISA) and several other alternative techniques are available for detecting rinderpest virus antibodies (Rossiter and Jesset, 1982 a; Joshi *et al.*, 1984; Anderson *et al.*, 1996).

Nucleic acid probes have been developed for RPV but their use in hybridization has not yet offered a clear advantage over other methods (Rossiter, 1992). The polymerase chain reaction (PCR) is the newest technique used for rinderpest diagnosis (Barret *et al*, 1998). It has greatly improved the speed, accuracy and sensitivity of the diagnosis. The advantage of the PCR technique, apart from its sensitivity and specificity, is that the DNA product amplified from the sample can be sequenced. In this way the genome of the virus can be compared with viruses isolated previously from the same region and from other areas where the disease is endemic. This may allow the source of the outbreak to be identified and provide indications as to the likely origin of the virus (Barret *et al*, 1998).

2.9.1 Differential diagnosis of Rinderpest

All conditions that cause stomatitis and /or enteritis in domestic stock may be clinically confused with rinderpest. In cattle these include mucosal disease (MD), malignant catarrhal fever (MCF), bovine papular stomatitis (BPS), Jembrana disease, and foot-and-mouth disease (FMD). In small ruminants, peste des petits ruminants (PPR) and Nairobi sheep disease can resemble rinderpest. In pigs, infections with *campylobacter spp.*, *Serpulina (Treponema) hyodysenteriae* and *salmonella* serovars are possible differentials (Rossiter, 1994).

In practice, only MD in cattle and PPR in small ruminants present a problem (Brown and Scott, 1957; Scott. 1964b). The clinical signs and gross pathology in cattle with MD can be indistinguishable from rinderpest and diagnosis requires laboratory confirmation. However MD usually affects very few animals in a herd, whereas morbidity rates in rinderpest are much higher. Agar-gel immunodiffusion tests applied to tissue suspensions can rapidly differentiate the two diseases. Immunohistochemical techniques can be used on frozen sections of messenteric lymph nodes or on formalin- fixed tissues to distinguish between MD and rinderpest. Failing this, virus isolation and identification must be attempted with follow-up studies to detect rising antibody titers.

Differentiation of PPR and rinderpest is more difficult. PPR virus cross reacts with RPV and is difficult to differentiate with hyperimmune polyclonal sera. Monoclonal antibodies and cDNA probes are used in the differentiation (Rossiter, 1994). PPR should always be treated as a differential diagnosis whenever rinderpest like signs are observed in small ruminants (Rossiter, 1994).

2.9.2 Use of immunohistochemistry in diagnosis.

The gross and microscopic features of a lesion are the basis of histopathological diagnosis. A definitive diagnosis is often not possible from a haematoxylin and eosin (HE) stained section. In such a situation one or more conventional 'special' stains may be used to arrive at the diagnosis. In some instances, however, even special stains do not give a definitive diagnosis. Immunohistochemical techniques can be used to identify

specific substances such as viral antigens, which cannot be characterized by special stains. Such techniques have therefore become a useful tool for arriving at definitive diagnoses (Boorsma, 1982).

Immunohistochemical techniques are of two broad categories, namely; immunofluorescence and immunoenzyme methods. In both methods, a specific tissue component is made visible by applying a labeled antibody raised against it. In the immunofluorescence technique the antibody is labeled with a fluorescent dye and the tissue is viewed in a fluorescent microscope. In the immunoenzyme technique the antibody is labeled with an enzyme that yields a coloured reaction product that can be seen in the bright field microscope (Boorsma, 1982; Haaijman, 1983).

Compared to immunofluorescence, the immunoenzyme technique has various advantages. It requires no expensive equipment, it gives permanently stained preparations, and details of cellular morphology are retained. It can also be applied to formalin fixed, paraffin embedded tissues, allowing retrospective studies of archived material (Christensen and Strange, 1987).

Horseradish peroxidase (HRP) is the best of all the enzymes that have been used as labels for antibodies in immunohistochemistry (Boorsma, 1982). The enzyme can be visualized histochemically with hydrogen peroxide and 3,3',5,5'-diaminobenzidine tetrahydrochloride (DAB), which yields a dark-brown, amorphous precipitate at the site of the enzyme reaction. Methods that use HRP (immunoperoxidase methods) have been

used widely (Gathumbi *et al.*, 1989; Ward and Kaeberle, 1984; Johnson and Engstrom, 1986; Ellis *et al.*, 1988; Wohlsein *et al.*, 1995; Wamwayi *et al.*, 1995b).

There are three variants of the immunoperoxidase method depending on how the enzyme is attached (conjugated) to the antibody. Boorsma (1982) has reviewed the various conjugation methods as briefly described.

The first method is whereby the enzyme is directly attached to the primary antibody or to an antibody directed against the primary antibody. The linkage between enzyme and antibody may be a covalent bond achieved by chemical agents mostly gluteraldehyde and periodate. Alternatively, an enzyme-anti-enzyme immunoglobulin complex and a bridging antibody may be used. The enzyme-anti-enzyme complex is referred to as peroxidase-anti-peroxidase complex (PAP). The PAP procedure is economical and simple and high quality PAP preparations are commercially available at a working dilution of 1:300. The sensitivity of the technique is sometimes 20-200 times higher than that of other immunocytochemical methods. The PAP method also has the advantage of yielding very low background staining compared to most of the enzyme-antibody conjugation procedures (Vandesande, 1983).

The second variant of the immunoperoxidase method is one whereby the enzyme is linked to *Staphylococcal* protein A. Protein A has affinity for the Fc region of a wide variety of IgGs from several species. Thus it can be reacted with specific antibody or with the secondary antibody. The linkage between enzyme and protein A is a covalent bond

achieved by chemical agents mostly periodate. The drawback of this method is that protein A recognizes, besides the specific antibodies, IgGs which are endogenously located in the tissue under investigation.

The third variant of the immunoperoxidase method makes use of the interaction between biotin and avidin. This avidin-biotin system depends on the extremely strong interaction between biotin (a vitamin from the B series) and avidin (a protein from egg white). One of these is attached to the specific antibody or the developing antibody and the other one is attached to the enzyme mostly HRP. Usually biotin is attached to the antibody and avidin is covalently coupled to the enzyme using gluteraldehyde and periodate. Alternatively the biotin is attached to both the antibody and the enzyme and the avidin is used as a bridge between these biotinylated proteins. This is called bridged avidin-biotin method. The most sensitive variant of this method is the pre-formation of avidin-biotinylated enzyme complex (ABC). The ABC will be formed when avidin is incubated at room temperature for 15 min with biotinylated HRP in a molar ratio of about 4:1. ABC is applied to the tissue immediately following the incubation with biotinylated antibody. A potential problem from the application of the biotin-avidin system arises from the fact that many tissues contain endogenous biotin, which is a widely distributed vitamin. This problem is overcome by incubating the tissue with excess avidin followed by excess biotin before the regular immunological incubations. This treatment suppresses endogenous biotin activity

The PAP and the biotin-avidin system are the preferred immunoenzyme methods (Vandesande, 1983; Boorsma, 1982).

2.10 Control of the disease

In countries where the disease is not endemic, confirmed outbreaks are controlled by slaughter and disposal of all affected and in-contact animals as well as by imposition of rigid quarantine and animal-movement controls (Rossiter, 1994).

In endemic areas annual mass vaccination of all calves up to two years of age was previously practiced as well as serosurveillance of unvaccinated animals such as small ruminants and wildlife. This approach enabled the elimination of the disease from Asia and many countries in Africa by 1995. Currently, mild disease is suspected to be endemic only in the Somali ecosystem comprising of areas of Kenya, Ethiopia and Somalia occupied by the Somali ethnic community and their herds of livestock. The rest of the world is moving towards the final eradication of rinderpest. The mass annual vaccination of cattle against rinderpest has not been practiced since the end of the Pan African Rinderpest Campaign (PARC) Programme in 1999 and the control of rinderpest now depends on active disease searching with subsequent elimination of confirmed foci through targeted vaccination (Klooster and Roeder, 2002).

The most widely used vaccine is the cell culture-attenuated variant of the Kabete 'O' strain of RPV prepared in a lyophilized form. After reconstitution in normal saline, subcutaneous inoculation of at least 100 $TCID_{50}$ of virus induces high levels of

neutralizing antibody and life-long immunity is conferred to virtually all the susceptible animals. The vaccine is safe for use in pregnant cattle and calves but is inactivated by circulating maternal antibodies. Branding, ear tagging or ear punching help to identify vaccinated animals (Rossiter, 1992).

When a field diagnosis is made in high-risk areas, an immediate quarantine should be imposed even before laboratory confirmation of rinderpest. Ring vaccination is done immediately in all areas surrounding the initial outbreak (Rossiter, 1992).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Experimental facility

All the animal experiments in this study were carried out at Muguga in a quarantine facility of the Kenya Agricultural Research Institute (KARI) in the Veterinary Research Centre (VRC). The facility had five animal paddocks, four animal pens, a shower room and two dressing rooms. The area was accessible only through a compulsory disinfection road trough. Personnel entered the animal areas in protective clothing and showered with warm water and soap before changing back into their home clothes to leave.

The buffalo paddocks were double-fenced with an inner chain-link and an outer stone wall about three metres apart and three metres high. The warthogs were housed in hay-padded pens opening to an out-door area with a concrete floor and a four metre high chain-link fence with a one metre overhang.

3.2 Experimental animals

Six (6) one-year-old buffaloes (*Syncerus caffer*), seven (7) boran cattle aged 2-3 years, twelve (12) zebu cattle aged 1-2 years (*Bos indicus*) and thirteen (13) warthogs (*Phacochoerus africanus*) aged 4-5 months were used in the experiment. The buffaloes were obtained from Lake Nakuru National Park (LNNP), the cattle from the Kenya Agricultural Research Institute's Beef Research Centre in Lanet, Nakuru District and the warthogs from the Mbagathi area near Nairobi National Park (NNP).

3.3 Rinderpest virus

The African Lineage I (RBK/WP/86/1) and II (kudu/Bov/BK/V2) isolates of RPV were cultivated at the VRC from frozen stocks. The RBK/WP/86/1 African lineage I isolate of RPV was recovered from sick cattle in an outbreak of the disease in West Pokot in 1986 (Wafula and Kariuki, 1987). It had been passaged in primary bovine kidney (BK) cell cultures up to 4 times and maintained in frozen and lyophilized form at the VRC. The kudu/Bov/BK/V2 African lineage 2 isolate of RPV was recovered from a sick kudu in Tsavo West National Park during a rinderpest outbreak in wildlife in 1995 (Wamwayi *et al.*, 1995 b). It had been maintained by passage in primary bovine kidney (BK) cell cultures at the VRC and stored in a wet preparation at –70° C. The virulent Kabete 'O' strain (Asian lineage) was reconstituted from the lyophilized form and used after dilution to a titre of $10^{5.0}$ $TCID_{50}$.

3.3.1 Virus propagation

The RBK/WP/86/1 virus was cultivated in BK monolayer cells as follows: Each vial of freeze-dried virus was reconstituted with 1ml Glasgow Minimum Essential Medium (MEM) (Sigma®). Two reconstituted vials were pooled and then diluted 1:5 in MEM (2ml in 8ml of diluent). Two 50ml flasks with monolayer cells at 70% growth cover were infected with 3ml of the diluted virus suspension. The remaining aliquot of 4ml was stored at –70°C. The infected flasks were incubated at 37°C for 1 hour with agitating at 15 minutes intervals. Maintenance medium was then added and incubation continued for several days with daily examination. The virus was harvested at about 70% cytopathogenic effects (CPE) by freeze thawing followed by centrifugation. The

kudu/Bov/BK/V2 isolate was propagated in vero cells using virus stock that had been previously grown on BK cells and stored at –70 °C in wet form. The same procedure as for RBK/WP/86/1 strain was used. The harvested virus stocks were titrated in microtitre plates and the titres were calculated using the Spearman-Kaeber method (Lennette, 1969).

3.4 Chemical immobilization and acclimatization of buffalo

Six (6) buffaloes of about one year old were captured from Lake Nakuru National Park (LNNP) and tested using the microtitre virus neutralization test (VNT) as described by Rossiter and Jesset (1982) to confirm that they had no rinderpest antibodies.

The buffaloes were immobilized chemically using the Palmer Cap-Chur® system and 3.5-4.9 mg of etorphine mixed with 30mg of xylazine. They were revived with 15 mg of diprenorphine and 2mg of atipamezole (Bengis, 1993). The buffaloes were given short, medium and long acting tranquilizers so as to acclimatize to the captive situation without undue stress. The short-acting tranquilizer, haloperidol was given intravenously at a dose rate of 20 mg per animal. Zuclopenthixiol acetate, which is medium acting, was given intramuscularly at a dose rate of 100 mg per animal. The long acting tranquilizer perphenazine enanthate was also given intramuscularly at a dose rate of 20 mg per animal (Ebedes, 1993).

The captured buffaloes were treated for ectoparasites using a pour-on preparation of Permethrin (Coopers®) and for worms using 2ml of Ivermectin (Ivomec®) injected

subcutaneously. They were also given prophylactic treatment with 20,000 mg of long-acting Oxytetracycline (Adamycin LA®) intramuscularly. They were ear-tagged for identification.

After capture and treatment, the buffaloes were acclimatized in holding pens at LNNP for 4 days before being transported to KARI/VRC, Muguga. At Muguga the buffaloes were held in the pens for at least one month to acclimatize further before the experiment. They were fed on hay supplemented with calf pellets and commercial mineral licks. Water was provided *ad libitum*.

3.4.1 Chemical restraint of captive buffalo

Two alternative drug combinations were used for immobilizing the wild buffaloes in captivity as follows: first, a combination of 3mg Etorphine Hydrochloride (M99®), Norvatis south Africa (Pty) Ltd) and 20 mg Xylazine (Chanazine®) and the second, a combination of 2 mg Etorphine Hydrochloride and 25 mg Xylazine. Three alternative drug combinations were used to reverse the buffaloes as follows: first, a combination of 12 mg Diprenorphine Hydrochloride (M5050®, Norvatis South Africa (Pty) Ltd, Animal Health Sector) and 2 mg Atipamezole Hydrochloride (Antisedan®, Norvatis South Africa (Pty) Ltd); second, a combination of 6 mg Diprenorphine Hydrochloride and 2 mg Atipamezole Hydrochloride, and third, 12 mg Diprenorphine Hydrochloride alone.

3.5 Chemical immobilization and acclimatization of warthogs

Thirteen (13) warthogs aged 4-5 months were captured from the Mbagathi area using capture nets and tested using the microtitre VNT (Rossiter and Jesset, 1982) to confirm absence of rinderpest antibodies.

The warthogs were given short, medium and long acting tranquilizers for transportation and acclimatization to captivity with minimal stress. Each warthog was tranquilized with 30mg of Azaperone, 50mg Perphenazine enanthate and 50 mg Zuclopenthixiol acetate given intramuscularly immediately after capture (Ebedes, 1993). Captured warthogs were ear tagged for identification and blood taken from each for serological tests and haematology. They were treated with 0.5 ml Ivermectin (Ivomec®) given subcutaneously and 1000mg of long acting Oxytetracycline (Adamycin LA®) given intramuscularly. They were then crated and transported to KARI/VRC, Muguga where they were kept indoors in pens with access to an outdoor area.

The warthogs were kept for at least one month in order to adapt to captive conditions before starting the experiment. They were fed on enriched porridge for the first few days and subsequently on pig starter meal, kales, carrots, green grass and green maize. Water was provided *ad libitum*.

3.5.1 Chemical restraint of captive warthogs

Four alternative drug combinations were used in the immobilization of the wild warthogs in captive situation as follows: first, a combination of 1mg Etorphine Hydrochloride with

40mg Azaperone for immobilization and 3mg Diprenorphine Hydrochloride for reversal; second, a combination of 1 mg Etorphine Hydrochloride with 30mg Azaperone for immobilization and 3 mg Diprenorphine Hydrochloride for reversal; third, a combination of 1 mg Etorphine Hydrochloride and 12.5 mg Xylazine for immobilization and 3mg Etorphine Hydrochloride and 250mg of Tolazoline (2-Benyl-2-imidazoline) Hydrochloride for reversal and fourth, a combination of 200mg Ketamine Hydrochloride (Ketaset®) with 5 mg Xylazine for immobilization and 2mg Atipamezole for reversal. The drugs were administered using the Daninject blowgun system.

3.6 Experimental infection of warthogs, buffalo and cattle

The various groups of animals experimentally infected with African lineages I and II isolates of RPV and the control groups are summarized in table 3.1.

3.6.1.Direct inoculation of warthogs with kudu/Bov/BK/V2 isolate of RPV and horizontal transmission to other warthogs and cattle

Four (4) out of the 13 warthogs with ear-tag numbers TW10, TW13, TWY and TW33 were randomly selected (Group I) and inoculated subcutaneously with 1 ml of a virus suspension containing $10^{4.8}TCID_{50}$ of the kudu/Bov/BK/V2 isolate of rinderpest virus in MEM. They were kept with two healthy warthogs (TW1and TW2) and four yearling zebu cattle (HK66, HK65, HK68 and HK54) in the same pen in which they intermingled and shared water and feed (Fig 3.1). Two negative control warthogs (Group II) were inoculated subcutaneously with 1ml of placebo comprising MEM containing no virus and kept with two uninfected zebu cattle in a separate unit. All the cattle in groups II and I

were examined daily for any clinical signs starting with group II to avoid transmitting the disease mechanically from the infected group to the control group. The warthogs were also observed daily and immobilized for closer examination and sampling every other day from day 3 to day 4-9 for inoculated warthogs and day 22 for the in-contact and control warthogs.

3.6.2. Direct inoculation of warthogs with the virulent Kabete 'O' strain of RPV

Two other warthogs (Group III) with ear-tag numbers TWX1 and TWA were inoculated subcutaneously with 1 ml of virus suspension containing $10^{5.0}$ $TCID_{50}$ of the virulent Kabete 'O' train of rinderpest virus in MEM. The warthogs were observed daily for any clinical signs and immobilized for physical examination and sampling every other day from day 3 till termination of the trial.

3.6.3 Direct inoculation of cattle with RBK/WP/86/1 and horizontal transmission to cattle and buffaloes

Four Boran cattle 2-3 years old were inoculated subcutaneously with $10^{4.8}TCDI_{50}$ of the RBK/WP/86/1 isolate of RPV. Two of the four inoculated cattle (558K, 566K) were kept in contact with 3 healthy Boran cattle (552K, 568K, 551K) and 4 healthy buffaloes (EC/B20, EC/B10, EC/B8, EC/B21) (Fig 3.2). The two were euthanised at the late stage of the disease on day 11 and 12. The other two inoculated cattle (569K, 550K) were kept separate in an indoor facility and were euthanised in the early stage of the disease on day 8 for necropsy. Two negative control buffaloes EC17 and EC14 were kept in a separate paddock. All the boran cattle were examined daily and samples of blood were obtained.

The buffaloes were immobilized on alternate days from day 4 to day 16 for examination and sampling.

Table 3.1. A summary of the various groups of experimental animals in the study of the pathology of rinderpest in common warthog, buffalo and cattle experimentally infected with lineages I and II isolates of rinderpest virus.

GROUP	ANIMAL SPECIES	EARTAG NUMBER	TREATMENT
1	4 warthogs	TW10, TW13, TWY, TW33	Kudu/Bov/BK/V2
	2 warthogs	TW1, TW2	Non-infected
	4 zebu	HK 66, HK65, HK68, HK54	Non-infected
2	2 warthogs	TW11, TWX *	Negative control
	2 zebu	HK 67, HK 62	Negative control
3	2 warthogs	TWX1, TWA	Virulent Kabete "O"
4	2 Boran cattle	569 K, 550K	RBK/WP/86/1
5	2 Boran cattle	558K, 566K	RBK/WP/86/1
	3 Boran cattle	552K, 568K, 551K	Non-infected
	4 Buffaloes	EC/B20, EC/B10, EC/B 8, EC/B21	Non-infected
6	2 Buffaloes	EC/B17, EC/B14	Negative control
7	4 zebu cattle	HK45, HK10, HK49, HK69	Kudu/Bov/BK/V2
	2 zebu cattle	HK32, HK43	Non-infected
	4 warthogs	TW6, TW7, TW8, TW9	Non-infected

* Warthog TWX was latter move from group 2 to group 3 and given tag number TWX1

Total numbers of different animal species used in the study were as follows:

Warthogs = 13
Zebu cattle = 12 (5 heifers and 7 steers)
Boran = 7 (4 heifers and 3 steers)
Buffaloes = 6

Figure 3.1 A zebu heifer sharing feed with warthogs that had been inoculated with kudu/Bov/bk/V2.

Figure 3.2 Buffalo sharing feed with cattle that had been inoculated with RBK/WP/86/1

3.6.4. Direct inoculation of cattle with Kudu/Bov/BK/V2 and horizontal transmission to cattle and warthogs

Four zebu cattle HK45, HK10, HK49 and HK69 aged 1-2 years old were inoculated subcutaneously with a virus suspension containing $10^{4.8}$ $TCID_{50}$ of the kudu derived African lineage II strain of rinderpest virus in MEM. They were kept with two healthy zebu cattle (HK32 and HK43) and four healthy common warthogs (TW6, TW7, TW8 and TW9) in the same pen in which they intermingled and drank and ate from the same containers. All the cattle and warthogs were examined daily over a period ranging from 5-15 days. The warthogs were immobilized for physical examination and sampling every other day from day 9 to day 15.

3.7 Sample collection

3.7.1 Sample collection from buffaloes

Following infection, samples were collected from all infected, non-infected and negative control animals. The samples included blood and ocular secretions. Sampling was done three times a week, on Mondays, Wednesdays and Fridays. The buffaloes were chemically immobilized using a combination of Etorphine and Xylazine administered using the Telinject® remote delivery system (Rohr and Mckenzie, 1993) as described in 3.4.1. Complete physical examination was done on immobilized animals. Rectal temperatures, respiratory and heart rates were recorded. Any lesions on the skin or the mucus membranes of the mouth, rectum, vagina and eyes were recorded.

Whole blood samples were collected from each animal in three 10 ml EDTA vacutainers while clotted blood for serum was collected from each animal in two plain vacutainers. The blood was collected from the jugular vein following the procedure recommended by Schalm (1986). Blood in EDTA tubes was mixed thoroughly by gently inverting the tube continuously for 15 seconds immediately after collection. It was kept in a cool box and transported to the laboratory for analysis within three hours. Thin blood smears were made from fresh blood on cleaned grease-free slides following the standard procedure described elsewhere (Schalm, 1986; Cheesbrough, 2000).

3.7.2 Sample collection from warthogs

Samples of blood and ocular secretions were taken from all inoculated warthogs and the uninfected controls using the same procedure as for buffaloes. Sampling was done three times a week, on Mondays, Wednesdays and Fridays.

3.7.3 Sample collection from cattle

The cattle were restrained manually in a crush for clinical examination and the sampling procedure was identical to that of buffaloes and warthogs.

3.8. Blood processing and analysis

Whole blood samples from 13 warthogs and 12 cattle from experimental and control groups were processed using a microhaematocrit centrifuge and a ZN-Coulter Counter® model of haematology analyzer to determine the packed cell volume (PCV), haemoglobin

concentration and total cell counts. Differential leucocyte counts were determined using thin blood smears stained with Giemsa stain (Schalm, 1986, Cheesbrough, 2000).

3.9 Post mortem procedures

Parenterally inoculated cattle and warthogs were euthanised for necropsy in both early (day 5-8) and late (day 9-12) stages of the disease. Euthanasia was done using (10-30ml) of 20% pentobarbitone sodium given intravenously (Bywater, 1982). Necropsy was done using the standard procedure described by Olafson (1954). Tissues samples were preserved in 10% neutral buffered formalin (Culling, 1963). Samples were taken in such a way as to include the abnormal and the normal looking parts in the same piece. At least 5 samples of about 2 cm thickness were taken from each organ. The organs that were sampled included the various parts of the digestive system (the muzzle, snout, dental pad, tongue, lips, palate, oesophagus, fore-stomachs, abomasum, duodenum, ileum, caecum, colon, rectum), the lymphoid organs (lymph nodes and spleen), brain, skin, muscles, eyes, liver, kidney, urinary bladder, heart, lungs and trachea and glandular organs (adrenal glands, salivary glands, pancreas, thyroid gland).

3.11 Histopathology

Tissue samples from a total of eight warthogs and nine cattle divided into five experimental and two control groups were processed for histopathological study using the standard procedure described by Culling (1963). The tissues were trimmed into 3-5mm pieces that were processed and embedded in paraffin wax.

Tissues were processed using an automatic tissue processor. The tissues were first dehydrated in alcohol baths of gradually increasing concentration namely, 50%, 70%, 96%, and 100%. They were then cleared in xylene for 3 hours to displace the alcohol before impregnating them with paraplast. Impregnation with paraplast was done in glass jars heated to 56-60^{0}C. Impregnated tissues were transferred into a final molten wax bath in a clearly labeled mould of paper-boat, which was then cooled to form a block. Small blocks of embedded tissue were trimmed and attached to blocks of wood using heat (blocking). Each blocked tissue was clearly labeled with the laboratory identification number and stored in a box for later sectioning.

Tissue sections were prepared at a thickness of 5 microns using a rotary microtome. Sections were floated on a warm water bath to straighten out. Each section was then transferred to an egg albumin coated microscope slide by dipping the slide in the water bath, and pulling it out in such a way as to allow the section to adhere to the albumin coated side. The slides were clearly labeled using a diamond pen. They were then dried on an electrically warmed rack at 60 ^{o}C for 30 minutes to adhere the sections on to glass slides.

The sections were de-waxed using xylene for 1-2 minutes, then hydrated by decreasing baths of alcohol for 2 minutes (1 minute in 100% and 1 minute in 90%). They were then stained with haematoxylin for 3-4 minutes and blued in tap water of pH 8 for about one minute. The sections were differentiated by washing in acid alcohol for a few seconds and then transferring them to the washing tray until blue again. Sections differentiated

and 'blued' were stained in 1% eosin for 3-4 minutes. The sections were then transferred to the washing tray for 3-4 minutes to differentiate the eosin. After removal from the eosin bath, the sections were dehydrated by agitation in 90% alcohol for 10-15 seconds. This was followed by agitation in a first bath of absolute alcohol for 10-15 seconds and then in a second bath of absolute alcohol for 30 seconds. Dehydrated sections were cleared in two baths of xylene each for 15 seconds and preserved permanently using DPX mountant.

Cover slips of appropriate size for the sections were wiped with a soft glass cloth before use. The slide with the section was removed from xylene and wiped at its back and around the section. This wiping removed excess xylene without drying the section. One to two drops of DPX was placed along the middle of the section and the cover slip lowered slowly over the DPX in such a way as to allow the mountant to spread under the cover slip without forming air bubbles. The cover slip was then guided to the desired position using a dissecting needle and the slide left to dry. Mounted slides were stored in labeled slide boxes.

Each slide was examined under a light microscope at x10 objective to note general features before being examined in details at x 40 objective. Any feature warranting examination at high power (x100) for clarification was examined accordingly under oil immersion (Culling1963).

CHAPTER 4

4. RESULTS

SECTION 1

4.1 Clinical manifestation of Rinderpest in cattle, buffaloes and warthogs experimentally infected with African lineages I and II isolates of RPV.

4.1.1 Clinical signs in cattle

The clinical findings in 21 cattle from the various experimental groups and one negative control group are described below and summarised in Table 4.1 and Figures 4.1-4.3.

4.1.1.1 Clinical signs in zebu cattle inoculated with the Kudu/Bov/BK/V2 isolate of RPV

Four yearling zebu cattle, HK45, HK10, HK49 and HK69 that were inoculated parenterally with the kudu/Bov/BK/V2 isolate developed very mild clinical signs. All had good appetites and remained bright throughout the experiment. Very mild oral, ocular and nasal lesions were noticed in some animals only after careful examination. The clinical signs at the early (day 5-6) and late (day8-9) stages of disease were as follows.

One (HK45) of the two parenterally inoculated zebu cattle that were euthanised for necropsy at the early stages of the disease developed a fever of 40.2 °C on day 5 when it was euthanised. The other (HK10) developed fever of 39.8 °C on day 6 accompanied with excessive salivation, congestion on the under surface of the tongue, a few shallow focal

erosions of about 2-3mm diameter on the upper surface of the tongue, and a clear bilateral nasal discharge. It was euthanised for necropsy on the same day.

The third zebu (HK49) was euthanised for necropsy at the late stages of the disease, on day 8. Initially the animal presented a transient clear nasal discharge on day 3 post inoculation. This reappeared on day 7 accompanied with shallow focal erosions of about 2mm in diameter on the upper gum, a few focal areas of congestion on the lower gum and a sweaty muzzle (Fig. 4.4 a). The erosions on the upper gum resolved overnight but the rest of the lesions remained until day 8. The animal had a transient fever of 40.0 °C on day 5 followed by excessive salivation and mild congestion of the conjunctivae from day 6 to day 8.

The remaining zebu (HK69) was also euthanised for necropsy at the late stages of the disease, on day 9. It presented a clear bilateral nasal discharge on day 4, becoming copious on day 6 with excessive salivation, a sweaty muzzle and congestion of the under surface of the tongue. Small shallow focal erosions of about 2-3mm appeared on the upper surface of the base of the tongue on day 7, resolving by day 9. Moderate congestion of the conjunctivae occurred on day 8 resolving partially by day 9.

4.1.1.2 Clinical signs in zebu cattle kept in contact with other zebu cattle that had been inoculated with Kudu/Bov/BK/V2.

Both zebu cattle HK32 and HK43 that were in contact with zebu cattle inoculated with the Kudu/Bov/KK/V2 developed very mild signs of disease with no fever.

One of the two in-contact cattle (HK32) developed three foci of necrosis of about 2-3mm diameter on the mucosa of the left nostril on day 6, followed by a unilateral mucopurulent nasal discharge from the right nostril on day 7, both lesions resolving by day 8. Shallow focal and coalescent erosions of about 2-10mm diameter appeared on the upper gum on day 8 accompanied by congestion of the tongue and a clear bilateral nasal discharge (Fig 4.4b). By day 9, the erosions resolved but the buccal mucosa was congested and one papilla at the right commissure of the lip was congested. On day 10, the animal developed a sweaty muzzle, copious mucopurulent nasal discharges, congested ocular mucosa and excessive lacrimation. All clinical signs resolved by day 11.

The other in-contact zebu cattle (HK 43) had a congested tongue on day 7 followed, on day 8, by a few very shallow focal erosions of about 2-3mm diameter on the muzzle and a few focal areas of congestion of similar size on the lips, both lesions resolving by day 10. Shallow erosions appeared at the base of the tongue on day 9 followed on day 10, by bilateral mucopurulent nasal discharges, congested buccal mucosa and a sweaty muzzle. All the lesions resolved by day 11 after contact.

4.1.1.3 Clinical signs in zebu cattle kept in contact with warthogs inoculated with Kudu/Bov/BK/V2.

Four zebu cattle, HK65, HK66, HK68 and HK54, which were in contact with four warthogs inoculated with Kudu/Bov/BK/V2 and two non-inoculated warthogs, showed very mild signs of disease as follows

One out of four in-contact zebu cattle (HK 65) had mild congestion of the buccal mucosa and the under surface of the tongue from day 9 to day 10 and a clear nasal discharge on day 15, resolving by day 16. One in-contact zebu steer (HK 66) had mild congestion of the under surface of the tongue from day 9 to day 10 and a sweaty muzzle on day 16. Another in-contact zebu steer (HK 68) developed mild congestion of the eyes, the under surface of the tongue and the lower lips and a clear bilateral nasal discharge, all persisting from day 9 to day 12. A shallow necrotic plaque of 5-10mm diameter developed on its upper left gum on day 12, resolving overnight with recurrence of slight congestion of the inner surface of the lower lips and a sweaty muzzle from day 16 to day 17.

Another in-contact zebu steer (HK 54) developed mild congestion of the under surface of the tongue and the buccal mucosa on 7 with the latter lesion persisting to day 11. The animal developed a small superficial necrotic plaque of about 5mm on the lower left gum on day 8 followed by excessive salivation on day 9, both resolving by day 10 (Fig.4.4c). It developed a slight clear nasal discharge on day 11 followed, on day 13, by intermittent coughing, moderate ocular congestion and a unilateral mucopurulent nasal discharge. On day 16 and 17 it had watery diarrhoea with reduced appetite and a serous ocular discharge persisting with the intermittent coughing to day 21.

4.1.1.4 Clinical signs in Boran cattle inoculated with RBK/WP/86/1 isolate of RPV.

The four Boran cattle, 569K, 550K, 558K and 566K that were parenterally inoculated with RBK/WP/86/1 developed very mild signs of the disease, maintaining good appetites and essentially normal external appearances. Very mild oral, ocular and nasal lesions were noticeable upon careful examination accompanied by slight fever in some of the

animals. The clinical signs in the early (day 7) and late (day12-13) stages of the disease were as follows.

Both parenterally inoculated Boran heifers that were kept separately were euthanised on day 7, in the early stages of the disease. One (550K) of these developed moderate congestion of ocular and buccal mucosae as well as oral papillae from day 4 to day 5 after inoculation (Fig.4.4d). On day 6, it presented a mild peak fever of 39.8 °C, bloodstained stool, and a shallow necrotic plaque of 3-5 mm in diameter on the lower left gum. The other heifer (569K) developed congested ocular mucosae on day 4 followed on days 5 and 6, by excessive salivation, clear nasal discharges and congestion of the oral mucosa. On day 6, it developed a peak fever of 39.7 °C, a clear ocular discharge, and a shallow necrotic plaque of about 20mm by 10 mm in diameter on the lower lip, in addition to the signs seen the previous day. The necrotic plaque and fever resolved by day 7.

The two parenterally inoculated Boran cattle that were kept together with three and four non-infected cattle and buffaloes, respectively, were euthanised for necropsy in the late stages of the disease. One (566K) of the two developed a sweaty muzzle on day 4-post inoculation followed on day 5, by excessive salivation, bilateral nasal discharge and a congested buccal mucosa. It developed a peak fever of 40.3 °C on day 7, followed on day 9, by a few shallow erosions of about 3-5mm on the gum and under the tongue as well as a few congested oral papillae. These lesions persisted to day 13 when the animal was euthanised for necropsy. The other animal (558K) had slight congestion of ocular and

oral mucosae on day 5 and a bilateral mucopurulent nasal discharge on day 6 that resolved overnight. It developed a small shallow necrotic plaque of 3-5mm on the lower gum on day 9, which was partially resolved by day 12 when it was euthanised for necropsy. The animal developed no fever.

4.1.1.5 Clinical signs in Boran cattle kept in contact with Boran cattle inoculated with RBK/WP/86/1

All three in-contact cattle 551K, 552K and 568 K developed very mild signs of disease from day 12 to 14 post contact that resolved rapidly. On day 12, one heifer (552K) developed a few erosions of about 3-5mm diameter on the dental pad, a few congested oral papillae, and small shallow erosions on the upper surface of the tongue. All the lesions had resolved by day 13 after contact. One in-contact Boran steer (551K) developed bilateral serous nasal discharges on days 6 and 13 followed on day 14 by a fever of 39.9 °C. The other in-contact Boran steer (568K) developed excessive salivation on day 5, mucopurulent nasal discharges on day 10 and a few small and shallow necrotic lesions of about 5 mm diameter on the gum and tongue on day 13. The latter lesions had resolved fully by the end of the day.

4.1.1.6 Clinical observations in the uninfected control cattle

The two negative control zebu cattle HK 62 and HK 67 that were kept together with two negative control warthogs showed no signs of disease throughout the duration of these experiments.

Table 4.1. A summary of clinical signs in five groups of cattle infected with African lineages I and II isolates of RPV and in one uninfected control group.

Clinical sign	Frequency						
	I-WP	Icc-WP	I-kudu	Icw-kudu	Icc-kudu	C	**Total**
Fever	3/4	1/3	2/4	0/4	0/2	0/2	**6/19**
Congested ocular mucosa	4/4	0/3	2/4	2/4	1/2	0/2	**9/19**
Congested oral mucosa	4/4	0/3	3/4	4/4	2/2	0/2	**12/19**
Reddened oral papillae	2/4	1/3	0/4	0/4	1/2	0/2	**4/19**
Erosions on oral mucosa	4/4	2/3	3/4	2/4	2/2	0/2	**11/19**
Excessive salivation	2/4	0/3	3/4	1/4	0/2	0/2	**6/19**
Nasal discharge	3/4	2/3	3/4	4/4	2/2	0/2	**13/19**
Lacrimation	1/4	0/3	0/4	1/4	1/2	0/2	**3/19**
Diarrhoea	0/4	0/3	0/4	1/4	0/2	0/2	**1/19**
Sweaty muzzle	0/4	0/3	2/4	3/4	2/2	0/2	**7/19**
Bloody stool	1/4	0/3	0/4	0/4	0/2	0/2	**1/19**
Total	**24/44**	**6/33**	**18/44**	**18/44**	**11/22**	**0/22**	

Key

I-WP-parenterally inoculated with RBK/WP/86/1; **Icc-WP**-in contact with other cattle that were parenterally inoculated with RBK/WP/86/1; **I-kudu**-parenterally inoculated with kudu-RPV; **Icw-kudu** in contact with warthogs that were parenterally inoculated with kudu-RPV; **Icc-kudu**-in contact with cattle that were parenterally inoculated with kudu-RPV; **C**- uninfected controls treated with placebo.

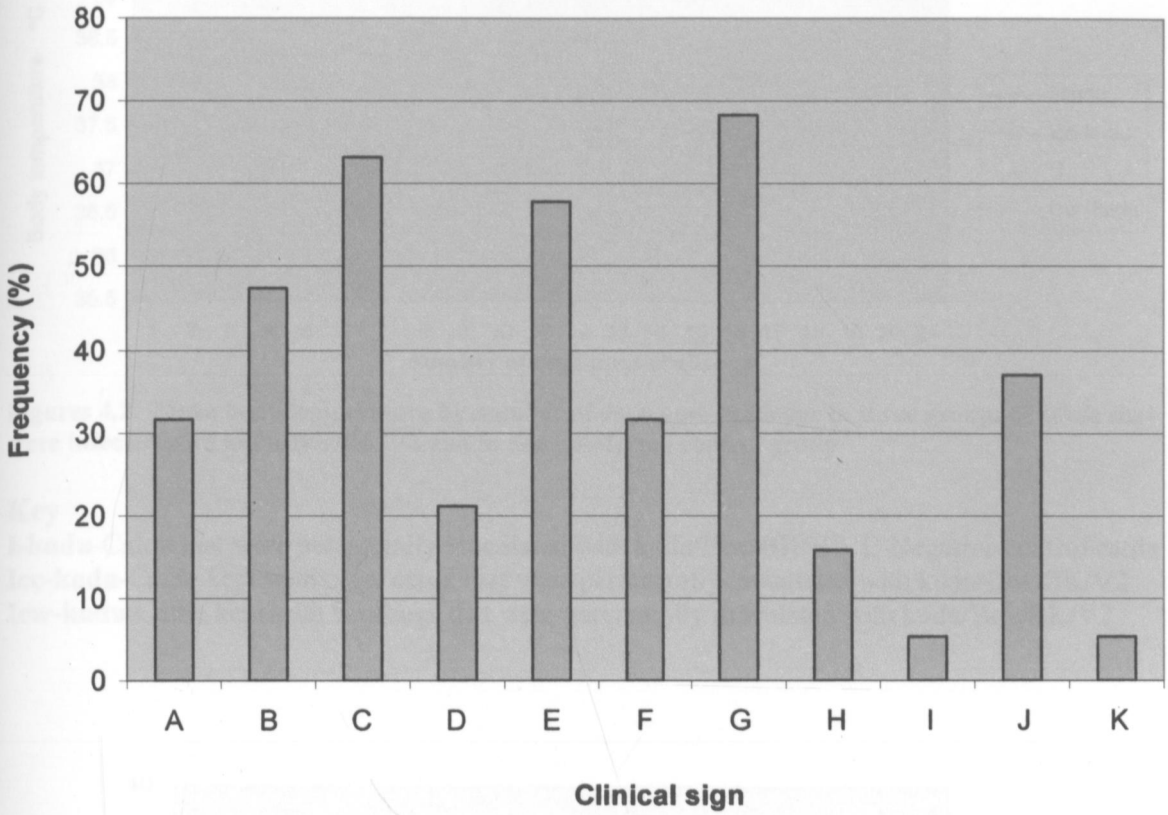
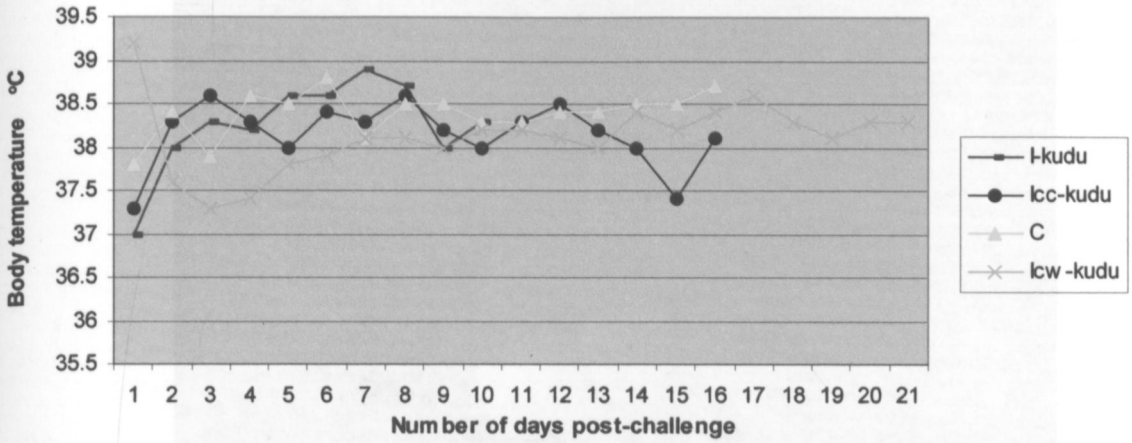


Figure 4.1. Overall frequency of clinical signs in cattle that were infected with RBK/WP/86/1 and kudu/Bov/BK/V2 isolates of RPV by direct inoculation and by contact transmission

Key

A-Fever; **B**-ocular congestion; **C**-Congestion of oral mucosa; **D**- Reddened oral papillae; **E**-Erosion of oral mucosa; **F**- Excessive salivation; **G**-nasal discharge; **H**-ocular discharge; **I**-Diarrhoea; **J**-Sweaty muzzle; **K**-bloody stool



Figures 4.2. Mean body temperature by number of days post-challenge in three groups of cattle that were infected with kudu/Bov/BK/V2 and in one uninfected control group

Key

I-kudu-Cattle that were parenterally inoculated with kudu/Bov/BK/V2, **C**-Negative control cattle
Icc-kudu-Cattle kept with other cattle that were parenterally inoculated with kudu/Bov/BK/V2
Icw-kudu-Cattle kept with warthogs that were parenterally inoculated with kudu/Bov/BK/V2

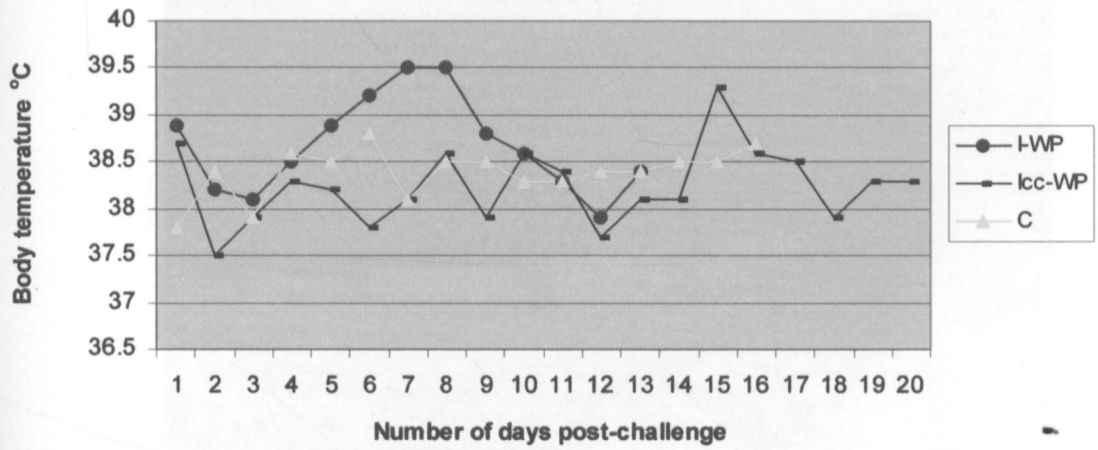


Figure 4.3. Mean body temperature by number of days post-challenge in two groups of cattle that were infected with RBK/WP/86/1 and in one uninfected control group

Key

I-WP-Cattle that were parenterally inoculated with RBK/WP/86/1, **C**-Negative control cattle
Icc-WP-Cattle kept with other cattle that were inoculated with RBK/WP/86/1

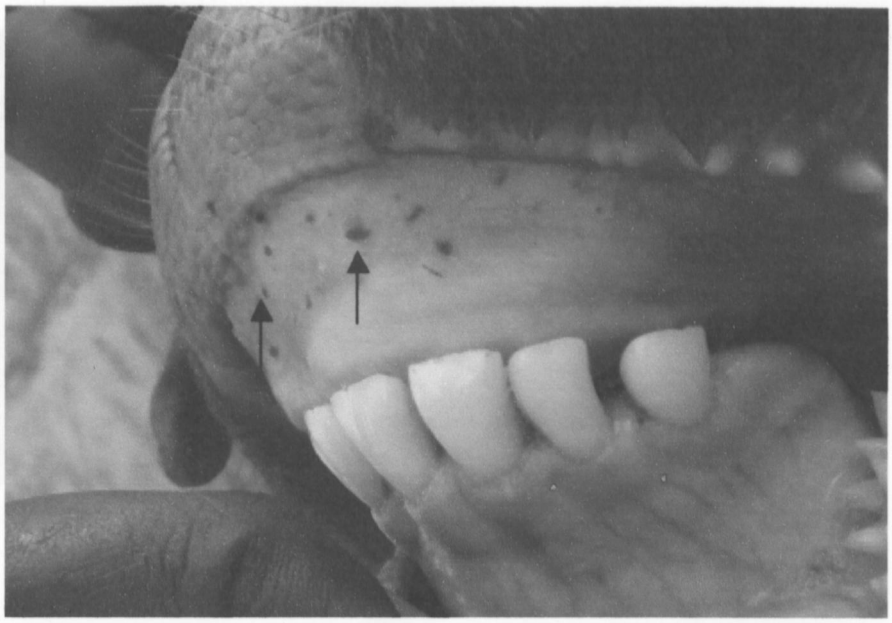


Figure 4.4 a. Shallow focal erosions (arrows) on the upper gum of a steer (HK49) seven days after it was inoculated parenterally with kudu/Bov/BK/V2 isolate of RPV

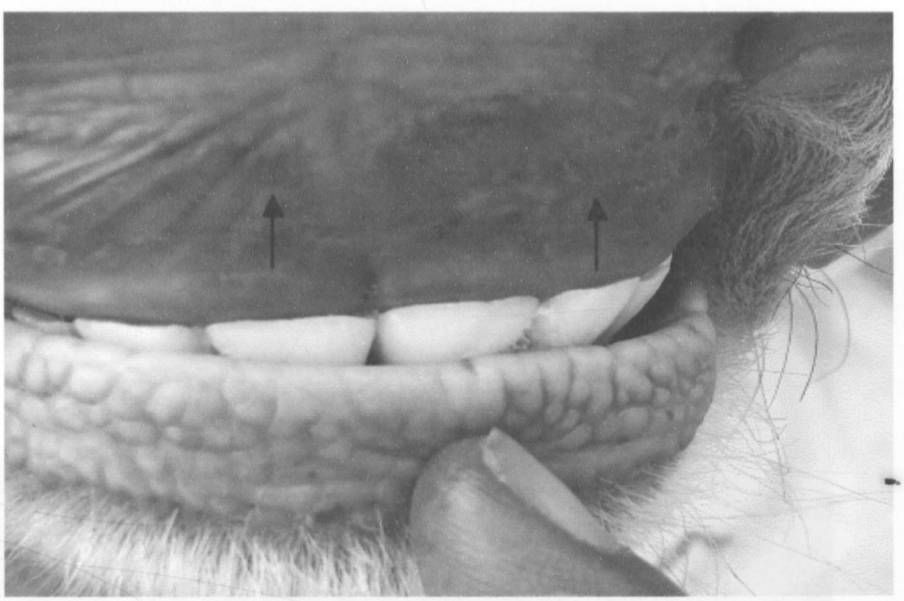


Figure 4.4 b. Focal coalescent erosions (arrows) on the upper gum of a zebu heifer (HK 32) eight days after being kept in contact with cattle that had been inoculated parenterally with kudu/Bov/BK/V2 isolate of RPV

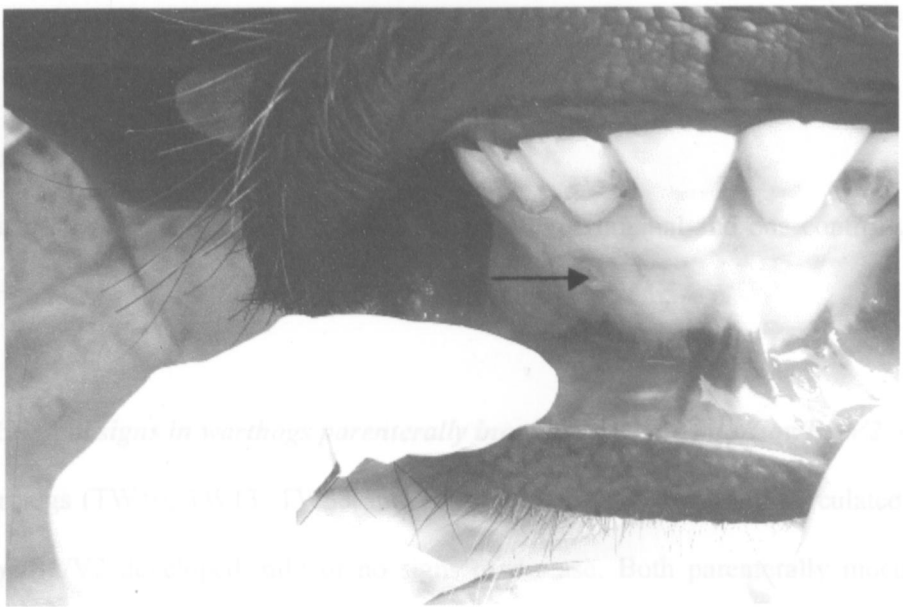


Figure 4.4c. A small necrotic plaque (arrow) on the lower left gum of a zebu steer (HK54) eight days after being kept in contact with warthogs that had been inoculated parenterally with kudu/Bov/BK/V2 isolate of RPV

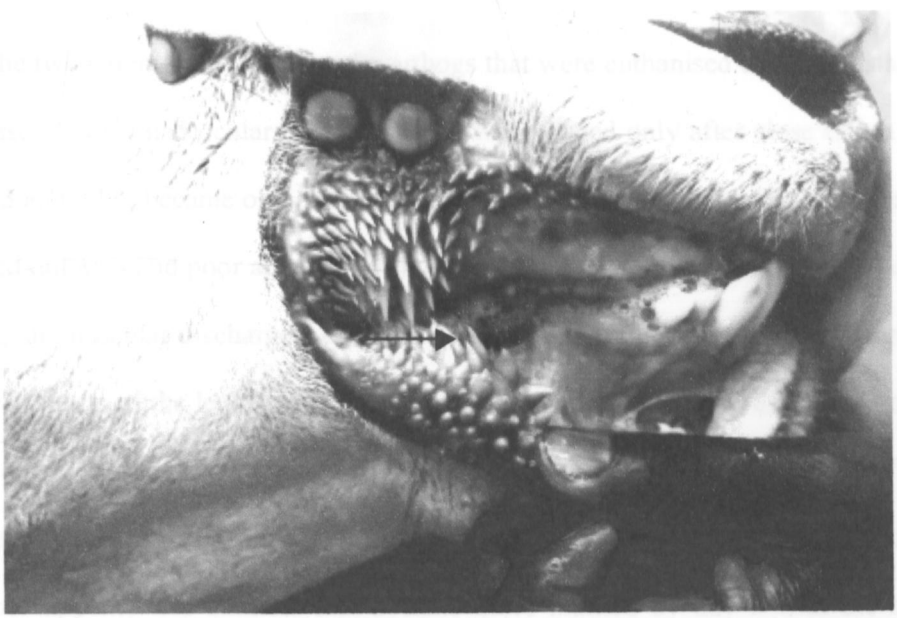


Figure 4.4 d. Congested oral papillae (arrow) of a Boran heifer (550K) five days after being inoculated parenterally with RBK/WP/86/1 isolate of RPV

4.1.2 Clinical signs in warthogs

The clinical signs in 13 warthogs divided into four experimental and one control group are described below and summarized in Table 4.2 and Figures 4.5-4.6.

4.1.2.1 Clinical signs in warthogs parenterally inoculated with Kudu/Bov/Bk/V2

Four warthogs (TW10, TW13, TW33 and TWY) that were parenterally inoculated with Kudu/Bov/Bk/V2 developed mild or no signs of disease. Both parenterally inoculated warthogs that were euthanised at the early stages of the disease (TW10 and TW13) showed no clinical signs of disease but one of them (TW10) was found dead on day 4 post-inoculation while the other was euthanised on day 6 post-inoculation.

One of the two parenterally inoculated warthogs that were euthanised at the late stages of the disease (TW33) had ocular congestion that was noticed only after close examination on days 3 and 5 but became obvious on day 6, persisting to day 8. On day 7, the warthog developed dullness and poor appetite followed, on day 8, by uneasiness, slight arching of the back, slight ocular discharges, congested lower gum, moderate cyanosis of the belly and inner aspects of the legs, and frequent drinking. The warthog was euthanised on day 8. The other warthog that was euthanised at the late stage of the disease (TWY) developed congested ocular mucosae on days 5 and 6 post-inoculation followed, on day 7, by poor appetite and dullness that became more marked by day 8 with the animal staying isolated from the rest. On day 9, it developed moderate cyanosis of the medial aspects of the legs and belly, congestion of the tongue and dental pad, and superficial

erosion and congestion on the inner aspects of the lower lip and gum (Fig.4.7a). The warthog was euthanised on day 9.

4.1.2.2 Clinical signs in two warthogs kept in contact with the four warthogs inoculated with Kudu/Bov/BK/V2

Both warthogs TW1 and TWX that were in contact with those that had been parenterally inoculated with Kudu/Bov/BK/V2 showed marked clinical signs of disease. In-contact warthog TW1 had congestion of the oral and ocular mucosae, cyanosis of the abdomen and inner aspects of the legs, and a white ocular discharge on day 9. Ocular congestion persisted to day 22. On day 11, the animal developed vesicles of about 2-5 mm on the rear aspects of both thighs (Fig.4.7b). These persisted to day 21 and turned pustular. On day 15, it developed shallow erosions of about 10-20 mm in diameter on the dental pad and the lower lips, and a fever of 39.8 °C. By day 17, the animal's body condition had deteriorated markedly and its appetite was greatly reduced. On day 21, in addition to the above signs, the animal had diarrhoea, widespread coalescent erosions on the tongue, and cyanosis of the dental pad. The left hind leg was swollen and congested around the fetlock joint and the skin was peeling off around the coronet. The preputial opening was constricted, with the skin appearing shrivelled and cyanotic, and urine accumulating in the prepuce to form a swelling of about 5-6 cm diameter. On day 22, in addition to the previous signs, there were erosions and cyanosis of the interdigital skin, cracks at the areas of attachment of the dewclaws, peeling of the skin around the coronet on three legs and superficial erosions of the skin on various areas of the body especially the shoulders. The animal was euthanised on day 22 for necropsy.

In-contact warthog TW2 had very severe ocular congestion on day 7 with swelling and protrusion of the third eyelid, resolving by day 9 after contact. The animal developed congestion of the oral mucosa and cyanosis of the medial aspects of the legs and abdomen on day 9. This was followed on day 13 by bilateral mucopurulent ocular discharges and congestion of the buccal mucosa and under surface of the tongue. Congestion of buccal and lingual mucosae persisted to day 15 when a focal area of erosion about 3-5mm diameter appeared between the lower gum and inner surface of the lower lip. On day 21, the animal had numerous shallow coalescent erosions of various sizes on the tongue (Fig.4.7c). Similar erosions were also present on the dental pad accompanied by cyanosis. The warthog recovered fully from clinical signs by day 28 after contact.

4.1.2.3 Clinical signs in four warthogs that were kept in contact with four zebu cattle inoculated with Kudu/Bov/BK/V2

The four warthogs TW6, TW7, TW8 and TW9 that were kept in contact with zebu cattle inoculated with kudu/Bov/BK/V2 showed extremely mild signs of disease comprising of mild to moderate ocular congestion and lacrimation, which were transient.

In-contact warthog TW6 developed moderate ocular congestion on day 11, persisting to day 14 and accompanied by light ocular discharges on day 13. In-contact warthog TW7 developed several spots of reddening of about 2-3mm diameter under the tongue on day 9. These had resolved by day11 and were followed by mild ocular congestion with light

ocular discharges on day 12. In-contact warthog TW8 had a transient mild ocular congestion on day 13. In-contact warthog TW9 had moderate ocular congestion on days 11 to 13 accompanied by light serous ocular discharges on days11 and 12. Body temperatures remained normal for all the warthogs except warthog TW7 that had a high fever of above 40 °C coinciding with marked capture related excitement.

4.1.2.4 Clinical signs in two warthogs inoculated with the virulent Kabete 'O' strain of RPV

Warthogs TWA and TWX1 that were inoculated with the virulent Kabete 'O' strain of rinderpest virus developed signs of severe terminal disease by day 4. Warthog TWA developed a mucopurulent discharge from the left eye that persisted from day one to day 3. This was followed by a light clear nasal discharge on day 2. On day 4 it had extreme weakness, congestion of ocular mucosae, severe congestion and swelling of the third eyelid and bloody stool coated with thick mucus. The animal was found dead in the morning on day 5. Warthog TWX1 had a light clear discharge from both eyes on day 3 followed, on day 5, by extreme weakness, diarrhoea, sunken eyes, a light clear nasal discharge and a low body temperature of 36.4°C (Fig. 4.7d). The animal died during chemical immobilization on day 5, vomiting violently following administration of the antidote.

4.1.2.5 Clinical signs of uninfected control warthogs

Control warthog TW11 showed no signs of disease. It was euthanised for necropsy on day 15 after inoculation with placebo. Control warthog TWX had a transient mild congestion on the lower gum and inside the lower lip on day 9.

4.1.3 Clinical signs in four buffaloes kept in contact with two Boran cattle inoculated with RBK/WP/86/1

The four buffaloes EC/B20, EC/B10, EC/B8 and EC/B21 that were kept in contact with two Boran cattle inoculated with RBK/WP/86/1 showed very mild signs of disease. Buffalo EC/B20 had a reddened rectal mucosa before contact with inoculated cattle. This was present on day 12 after contact in addition to a rough hair coat, dullness, small superficial erosions on both lips, a few white pinpoint lesions under the tongue and reddened conjunctivae, the latter persisting to day 16. Buffalo EC/B21 had a slightly reddened rectal mucosa and a few white pinpoint foci on both lips on day 10 post-contact followed on day 16 by a few superficial erosions on the upper and lower gums. Buffalo EC/10 had a dry muzzle on day 10 post-contact but no other signs were evident during the experiment whereas buffalo EC/8 remained normal throughout.

4.1.4. Magnitude of clinical response in cattle, warthogs and buffalo experimentally infected with different isolates of Rinderpest virus

The clinical responses of zebu and Boran cattle, warthogs and buffaloes to different isolates of rinderpest virus by mode of infection are summarized in Table 4.3.

Table 4.2 Summary of clinical signs in four groups of warthogs that were infected with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV and in one uninfected control group.

Clinical sign	Frequency					
	I-kudu	Icw-kudu	Icc-kudu	I-rbok	C	**All**
Fever	0/4	1/2	0/4	0/2	0/2	**1/14**
Congested ocular mucosa	2/4	2/2	4/4	1/2	0/2	**9/14**
Congested oral mucosa	2/4	2/2	0/4	0/2	1/2	**5/14**
Haemorrhage of oral mucosa	1/4	0/2	0/4	0/2	0/2	**1/14**
Erosions on oral mucosa	1/4	2/2	0/4	0/2	0/2	**3/14**
Nasal discharge	0/4	0/2	0/4	2/2	0/2	**2/14**
Ocular discharge	1/4	2/2	4/4	2/2	0/2	**9/14**
Bloody stool	0/4	0/2	0/4	1/2	0/2	**1/14**
Diarrhoea	0/4	1/2	0/4	1/2	0/2	**2/14**
Cyanosis of lighter parts of skin	2/4	2/2	0/4	0/2	0/2	**4/14**
Foot lesions	0/4	1/2	0/4	0/2	0/2	**1/14**
Skin lesions	0/4	1/2	0/4	0/2	0/2	**1/14**
Poor demeanour	2/4	2/2	0/4	2/2	0/2	**6/14**
All	**11/52**	**16/26**	**8/52**	**9/26**	**1/26**	

Key
I-kudu-parenterally inoculated with kudu-RPV; **icw-kudu**-in contact with other warthogs that were parenterally inoculated with kudu-RPV; **icc-kudu**-in contact with cattle that were parenterally inoculated with kudu-RPV; **I-rbok**-parenterally inoculated with the virulent Kabete 'O' strain of RPV; **C**- uninfected controls

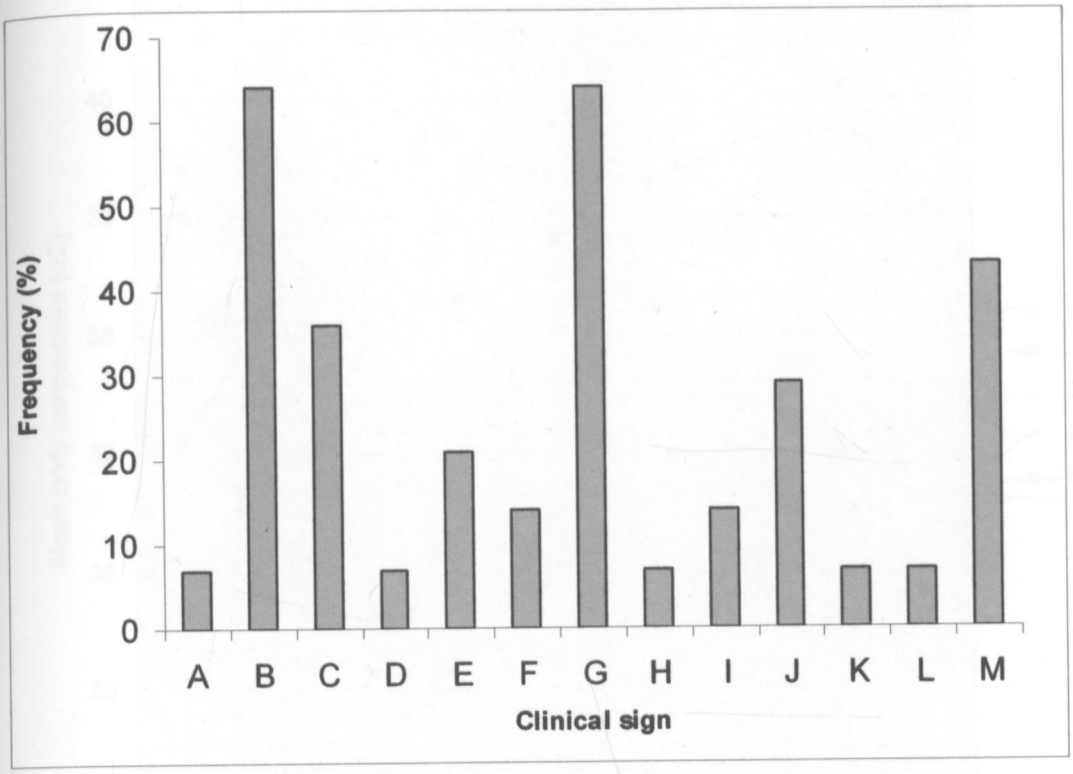


Figure 4.5. Overall frequency of clinical signs in warthogs that were infected with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV either by direct inoculation or by contact transmission

Key:
A-Fever; **B**-Ocular congestion; **C**-Congestion of oral mucosa; **D**-Haemorrhage of oral mucosa; **E**-Erosions on oral mucosa; **F**-Nasal discharge; **G**-ocular discharge; **H**-bloody stool; **I**-diarrhoea; **J**-Cyanosis of the skin; **K**-foot lesions; **L**-skin lesions; **M**-poor demeanour

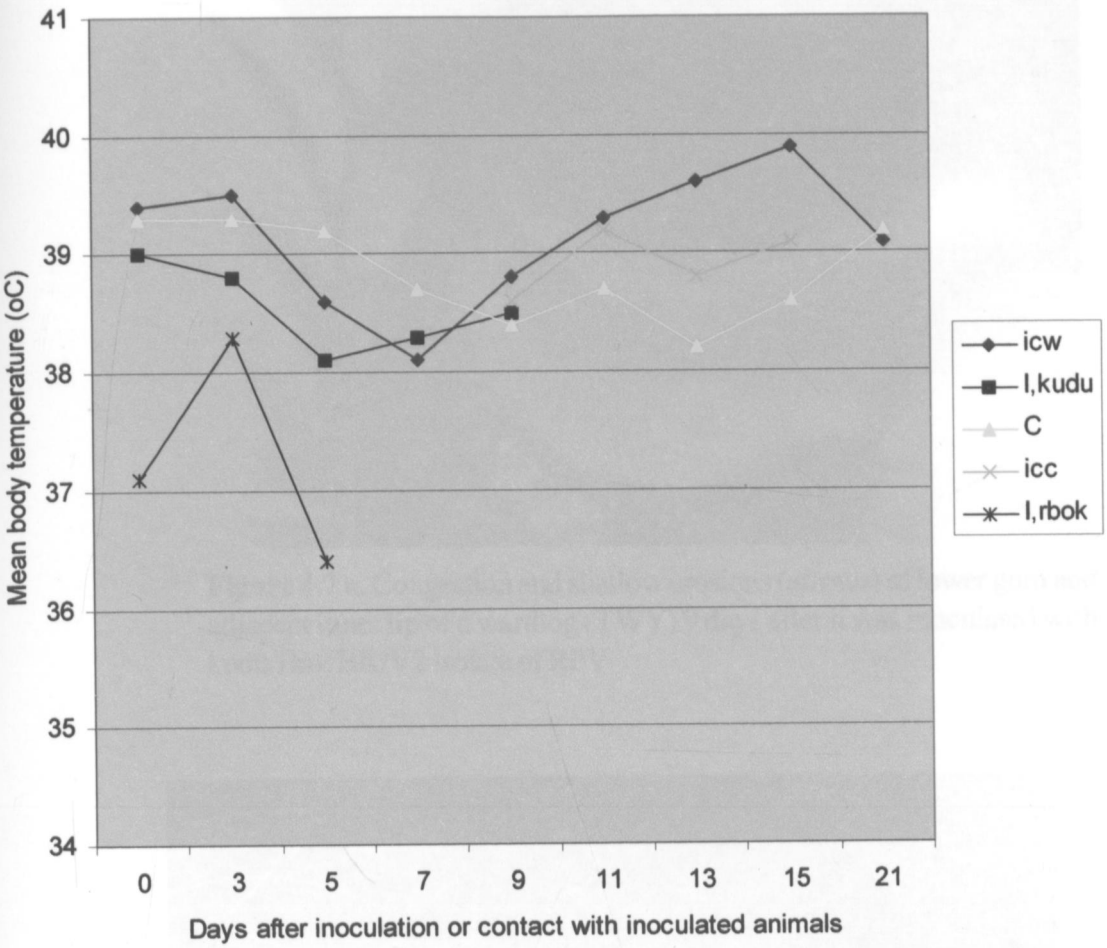


Figure 4.6. Mean body temperature by number of days after inoculation or contact in warthogs that were infected with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV and in the control group

Key

icw-Warthogs kept in contact with other warthogs that had been inoculated with kudu/Bov/BK/V2

I, kudu-Warthogs parenterally inoculated with kudu/Bov/BK/V2

C-Uninfected controls

icc-Warthogs kept in contact with cattle that were inoculated with kudu/Bov/BK/V2

I,rbok-Warthogs parenterally inoculated with the virulent Kabete 'O' strain of RPV

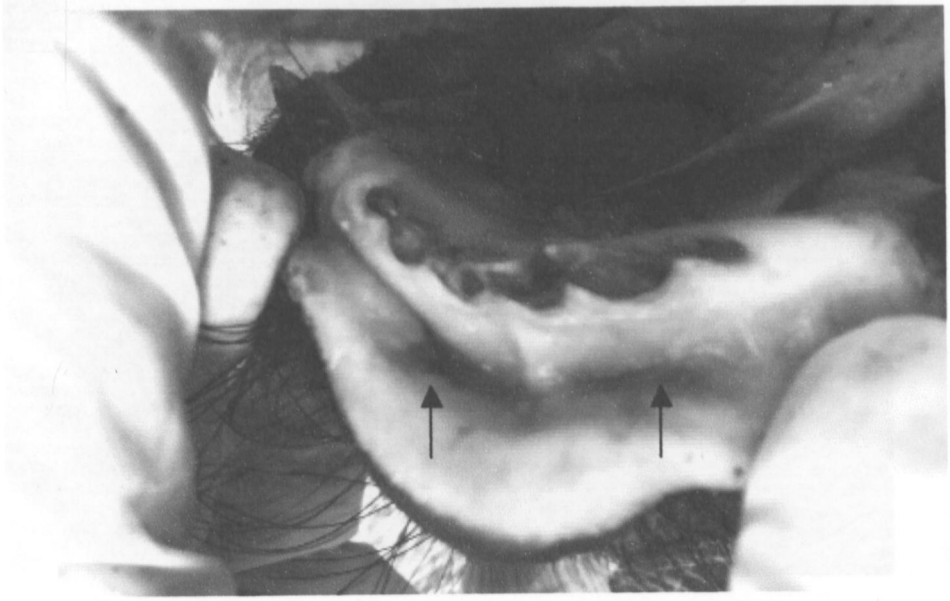


Figure 4.7 a. Congestion and shallow erosions (arrows) of lower gum and adjacent inner lip of a warthog (TWY) 9 days after it was inoculated with kudu/Bov/BK/V2 isolate of RPV

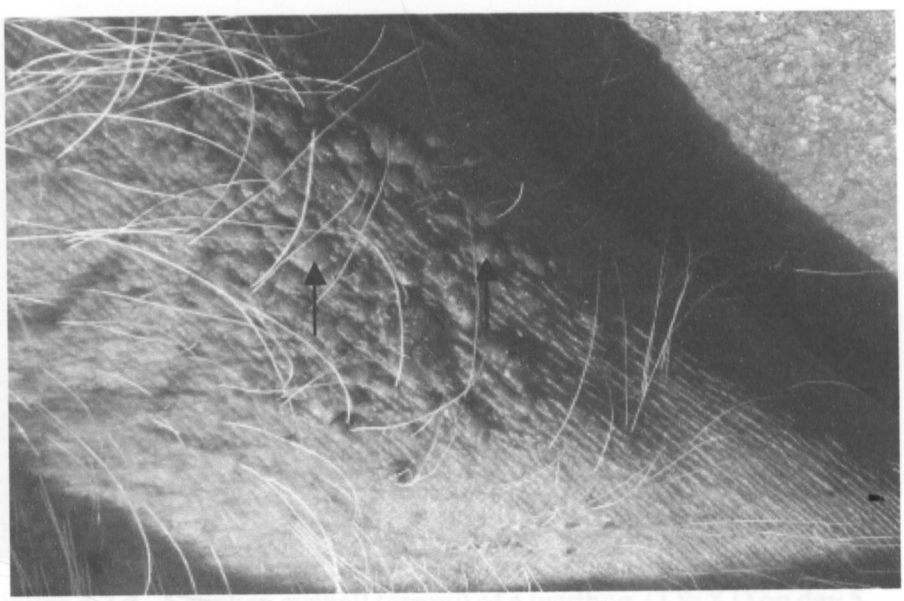


Figure 4.7 b. Vesicular cutaneous eruptions (arrows) on the hind legs of a warthog (TW1) on day 11 after being kept in contact with other warthogs that had been inoculated with kudu/Bov/BK/V2 isolate of RPV

Figure 4.7 c. Extensive shallow coalescent erosions (arrows) on the surface of the tongue of a warthog (TW2) on day 21 after being in contact with warthogs that had been inoculated with kudu/Bov/BK/V2 isolate of RPV.

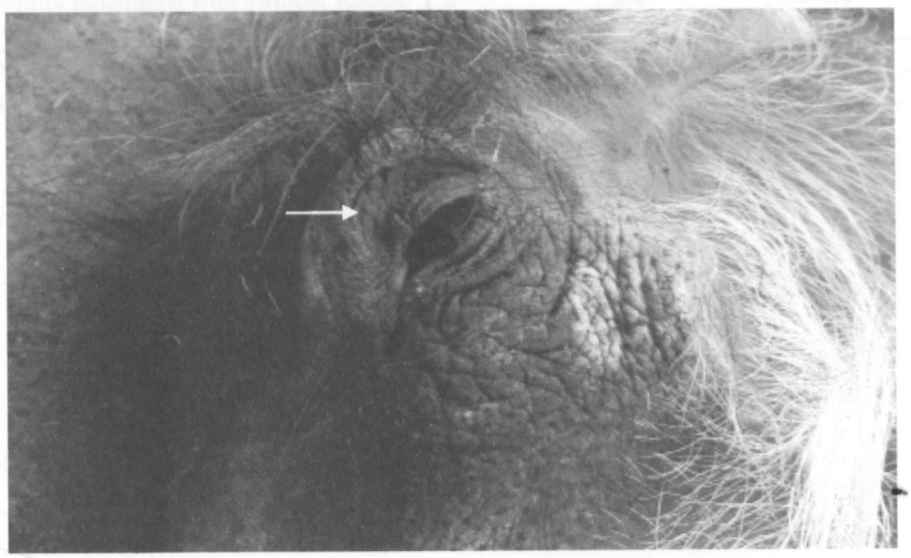


Figure 4.7 d. A sunken eyeball (arrow) of a warthog (TWX1) on day 5 after being inoculated parenterally with the virulent Kabete 'O' strain of RPV

Table 4.3. A summary of clinical responses in cattle, warthogs and buffaloes that were infected with RBK/WP/86/1, kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV either by direct inoculation or by contact transmission

Group	Number of animals	Proportion of reactors	Overall clinical reaction
Cattle parenterally inoculated with RBK/WP/86/1 (African lineage 1)	4	4/4	Mild
Cattle in contact with others inoculated with RBK/WP/86/1 (African lineage 1)	3	3/3	Mild
Buffalo in contact with cattle parenterally inoculated with RBK/WP/86/1 (African lineage 1)	4	3/4	Mild
Cattle parenterally inoculated with kudu/Bov/BK/V2 (African lineage 2)	4	4/4	Mild
Cattle in contact with other cattle that were parenterally inoculated with kudu/Bov/BK/V2 (African lineage 2)	2	2/2	Mild
Cattle in contact with warthogs parenterally inoculated with kudu/Bov/BK/V2 (African lineage 2)	4	4/4	Mild
Warthogs parenterally inoculated with kudu/Bov/BK/V2 (African lineage 2)	4	2/4	Mild
Warthogs in contact with other warthogs that were parenterally inoculated with kudu/Bov/BK/V2 (African lineage 2)	2	2/2	Moderately severe
Warthogs in contact with cattle that were parenterally inoculated with kudu/Bov/BK/V2 (African lineage 2)	4	4/4	Mild
Warthogs parenterally inoculated with virulent Kabete 'O' strain of RPV (Asian type)	2	2/2	Severe
Uninfected control cattle	2	0	None
Control warthogs	2	0	None

SECTION 2

4.2 Haematological Values

Total red and white blood cell counts, differential white blood cell counts, packed cell volume and haemoglobin concentration values in zebu cattle and warthogs infected with kudu/Bov/BK/V2 or the virulent Kabete 'O' isolates of RPV by different modes is described below and summarized in Tables 4.4-4.5.

4.2.1. Haematological values of cattle inoculated with Kudu/Bov/BK/V2

The mean total red blood cell count (rbc) in zebu cattle inoculated with Kudu/Bov/BK/V2 decreased steadily between day 0 and day 7 post-inoculation but remained within the normal range over the experimental period (Fig.4.8). The packed cell volume (pcv) (%) and haemoglobin concentration (Hb)(g/dl) for the group remained normal. A high mean white blood cell count (wbc) and a high mean differential lymphocyte count was recorded for the group on days 6 and 7 respectively (Figs.4.9, 4.10). The trends in other blood parameters in individual animals were as described below.

One of the two parenterally inoculated zebu heifers that were euthanised at the early stages of the disease (HK 10) developed a transient leucopaenia of 7000 cells /µl on day 5 post-inoculation, resolving by day 6. The other heifer (HK45) had a high differential lymphocyte count of 80 % on day 5 post-inoculation. Other blood values remained normal in both cattle.

One of the two parenterally inoculated zebu steers that were euthanised at the late stages of the disease (HK 49) had a high leucocyte count of 29,800 cells /μl on day 6 post-inoculation, which dropped sharply to a low normal count of 8,400 cells /μl on day 7. The other (HK 69) had a slightly high leucocyte count of 16 000 cells /μl on day 6, which dropped by day 7 to a leucopaenia of 7,800 cells /μl.

4.2.2. Haematological values in zebu cattle kept in contact with other zebu cattle that were inoculated with Kudu/Bov/BK/V2

The mean rbc count for the group was normal over the period except on day 7 post-contact when a slightly low value of 4.8 x 10^6 cells /μl was recorded (Fig. 4.11). The pcv and Hb values for the group remained within the normal ranges. The mean wbc count for the group remained within the normal range over the period except on day 6 when a high value of 20, 000 cells /μl was recorded (Fig. 4.12). High mean differential lymphocyte counts of 83.5 % and 77% were recorded for the group on days 10 and 14 respectively (Fig. 4.13). Trends in blood values in individual animals were as follows.

One of the two in-contact zebu cattle (steer HK 43) had a high total leucocyte count of 15,700 cells /μl on day 10 accompanied by a high differential lymphocyte count of 82%. The leucocyte count returned to normal by day11 but the differential lymphocyte count remained high until day 15. The other (heifer HK32) had a very high differential lymphocyte count of 85% on day 10 with a normal total leucocyte count, followed by a leucopaenia of 7,900 cells /μl on day 11 with a normal lymphocyte count of 53%.

Table 4.4 Mean haematological values in groups of cattle that were infected with kudu/Bov/Bk/V2 either by direct inoculation or by contact transmission.

Block	Summary									
	Frequency (n)	Hb(g/dl)	Pcv (%)	Rbc x 10^6	Wbc x 10^3	Seg (%)	Lym (%)	Mon (%)	Eos (%)	Bas %)
I-kudu	15.0	11.3±1.8	32.0±4.0	6.2±2.2	11.4±5.8	29.9±11	64.6±13.6	0.5±1.1	4.5±5.7	0.6±1.6
icw	56.0	10.3±1.3	30.3±3.4	8.8±0.8	16.1±4.1	28.3±6.4	69.6±6.9	0.3±0.6	1.8±1.9	0.0±0.0
icc	22.0	10.6±1.3	32.1±3.1	7.5±1.4	11.7±2.2	27.4±8.9	68.7±9.5	0.7±1.6	3.0±2.5	0.0±0.0
C	19.0	11.2±1.5	32.4±3.3	8.8±0.9	17.5±3.2	27.8±4.9	70.0±5.5	0.1±0.0	2.1±2.2	0.0±0.0
All	112	10.6±1.5	31.3±3.5	8.2±1.5	14.8±4.4	28.2±7.5	68.8±8.6	0.3±0.9	2.5±3.0	0.1±0.6

Key:
I-kudu –Cattle inoculated with kudu/Bov/BK/V2 **icw**-Cattle in contact with warthogs that were inoculated with kudu/Bov/BK/V2 **C**-Uninfected control cattle **icc**- Cattle in contact with other cattle that were inoculated with kudu/Bov/BK/V2 **Count**-number of samples **Hb**- Haemoglobin concentration **pcv**- Packed cell volume **Rbc**-Red blood cell count **Wbc**- White blood cell count **Seg**- Differential neutrophil count **Lym**- Differential lymphocyte count **Mon**- Differential monocyte count **Eos**- Differential eosinophil count **Bas**- Differential basophil count

Table 4.5 Mean haematological values in groups of warthogs that were infected with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV either by direct inoculation or by contact transmission.

Block	Summary									
	Frequency (n)	Hb (g/dl)	Pcv (%)	Rbc x 10^6	Wbc x10^3	Seg (%)	Lym(%)	Mon (%)	Eos (%)	Bas (%)
I-kudu	14.0	12.4± 1.1	36.6±3.6	7.1±1.5	9.7±2.6	59.9±12.1	39.3±11.5	0.3±0.6	0.6±1.0	0.0±0.0
icw	16.0	11.9±1.2	35.5±3.0	7.3±1.5	10.6±2.2	56.1±14.6	42.9±15.2	0.3±0.7	0.8±1.4	0.0±0.0
icc	20.0	12.5±1.4	36.8±3.6	6.6±0.6	10.0±2.2	42.4±13.1	55.6±13.7	1.7±2.9	0.3±0.8	0.0±0.0
C	15.0	12.5±1.3	36.3±3.2	7.2±1.2	11.6±1.8	47.3±11.2	48.4±17.8	0.1±0.3	1.1±2.0	0.0±0.0
I-rbok	5.0	14.0±2.7	40.8±6.1	10.0±3.1	10.9±3.5	46.6±18.4	52.2±18.5	0.0±0.0	1.2±1.6	0.0±0.0
All	70.0	12.5±1.5	36.6±3.7	7.2±1.6	10.5±2.4	50.4±14.6	47.6±15.8	0.6±1.7	0.7±1.4	0.0±0.0

Key:
I-kudu -inoculated with kudu/Bov/BK/V2 **icw**-in contact with warthogs that were inoculated with kudu/Bov/BK/V2 **C**-uninfected control group **icc**- in contact with cattle that were inoculated with kudu/Bov/BK/V2, **I-rbok**-inoculated with the virulent Kabete 'O' strain of RPV **Count**-number of samples **Hb**- Haemoglobin concentration **pcv**- Packed cell volume **Rbc**-Red blood cell count **Wbc**- White blood cell count **Seg**- Differential neutrophil count **Lym**- Differential lymphocyte count **Mon**- Differential monocyte count **Eos**- Differential eosinophil count **Bas**- Differential basophil count

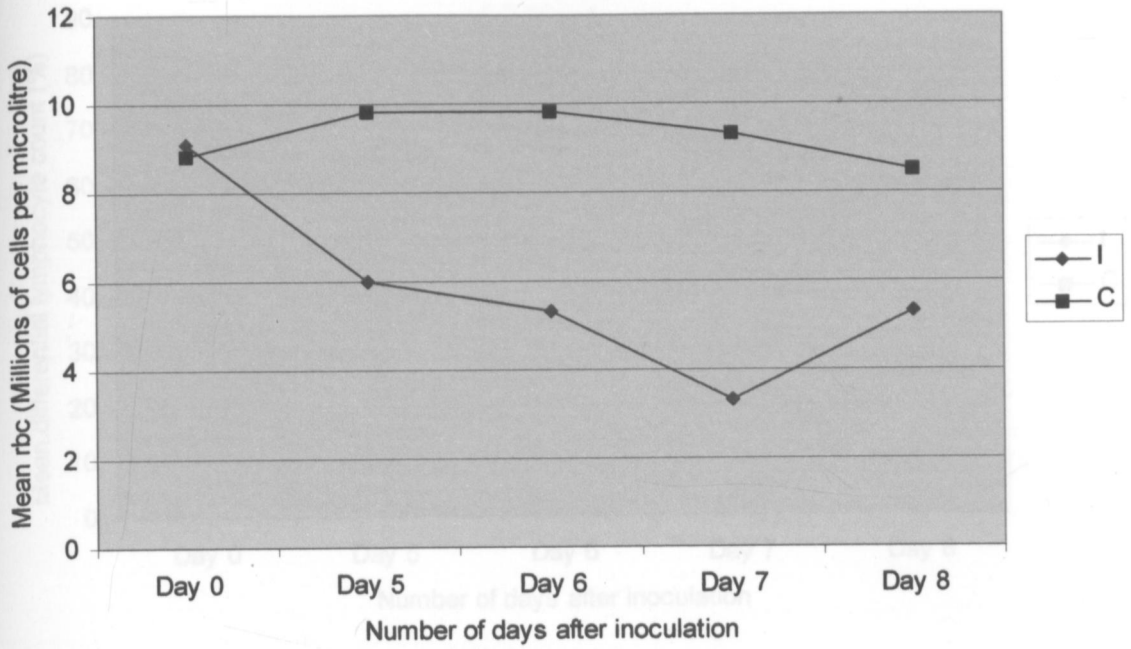


Figure 4.8. Mean rbc by number of days post inoculation in a group of cattle that were inoculated with kudu/Bov/BK/V2 and in the uninfected control group.

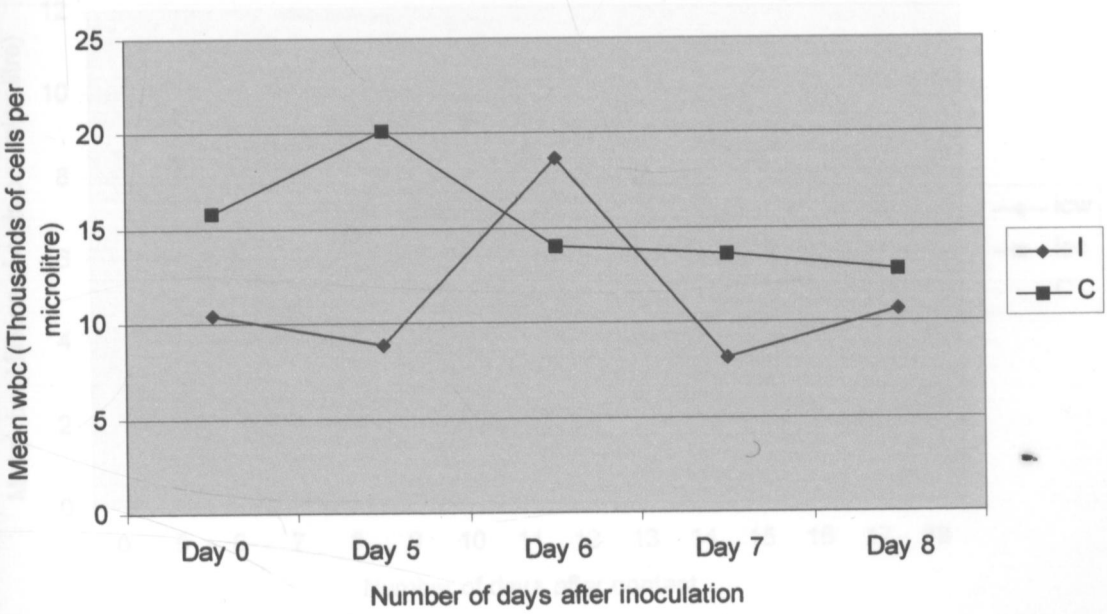


Figure 4.9. Mean rbc by number of days post inoculation in a group of cattle that were inoculated with kudu/Bov/BK/V2 and in the uninfected control group.

Key

I-cattle inoculated with kudu/Bov/BK/v2 **C**- Uninfected control cattle

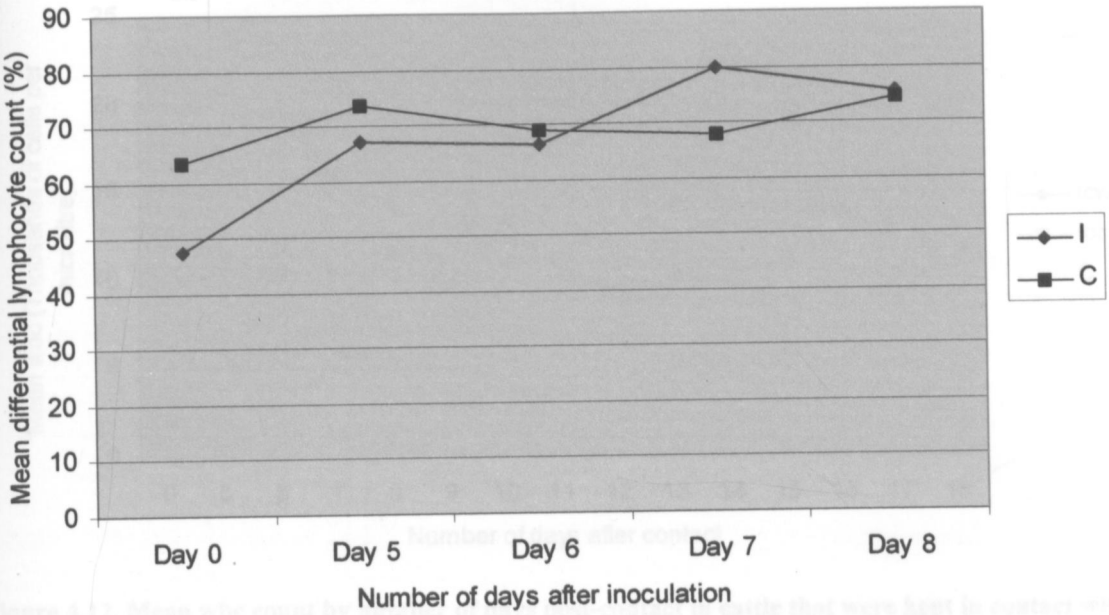


Figure 4.10. Mean differential lymphocyte count by number of days post inoculation in a group of cattle that were inoculated with kudu/Bov/BK/V2 and in the uninfected control group.

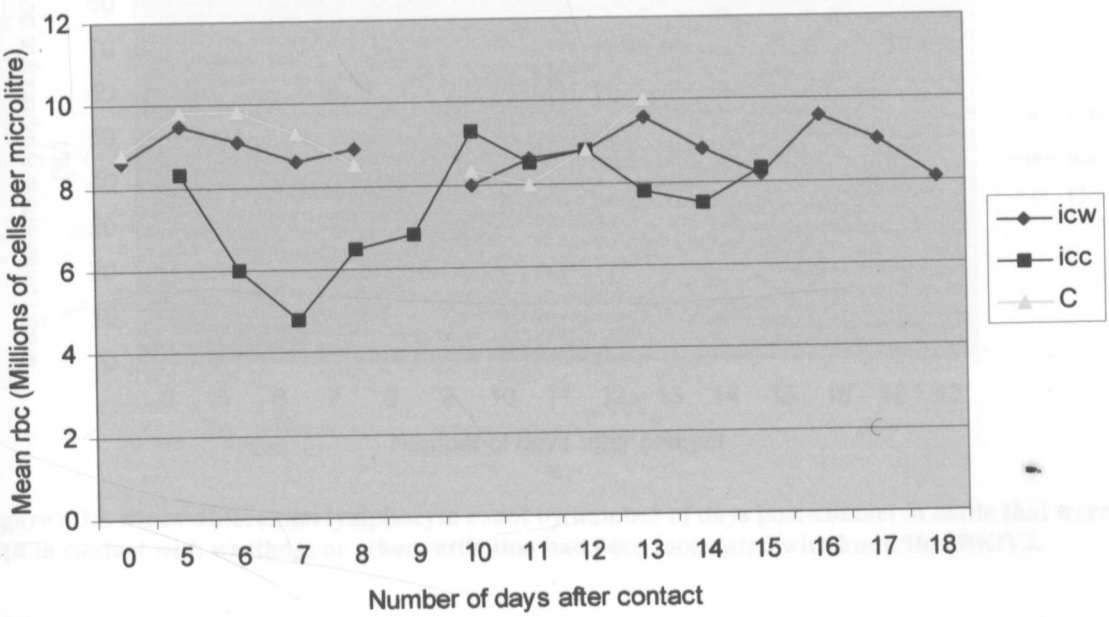


Figure 4.11. Mean rbc count by number of days post contact in cattle that were kept in contact with warthogs or other cattle that had been inoculated with kudu/Bov/BK/V2.

Key

C-Uninfected control cattle **icw**-Cattle kept with warthogs that were inoculated with kudu/Bov/BK/V2 **icc**-Cattle that were kept with other cattle that were inoculated with kudu/Bov/BK/V2 **I**-cattle parenterally inoculated with kudu/Bov/BK/V2

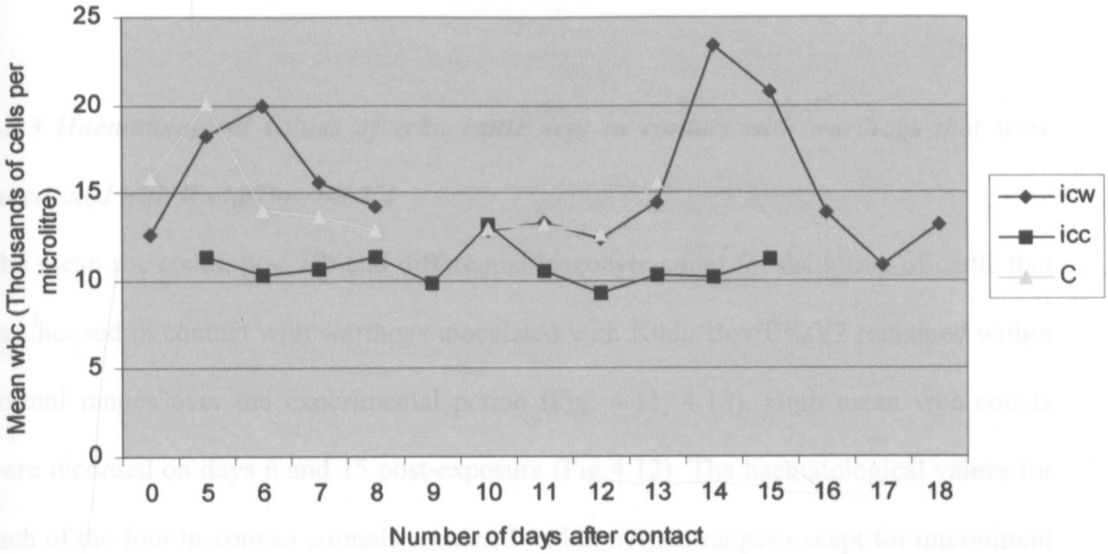


Figure 4.12. Mean wbc count by number of days post-contact in cattle that were kept in contact with warthogs or other cattle that had been inoculated with kudu/Bov/BK/V2.

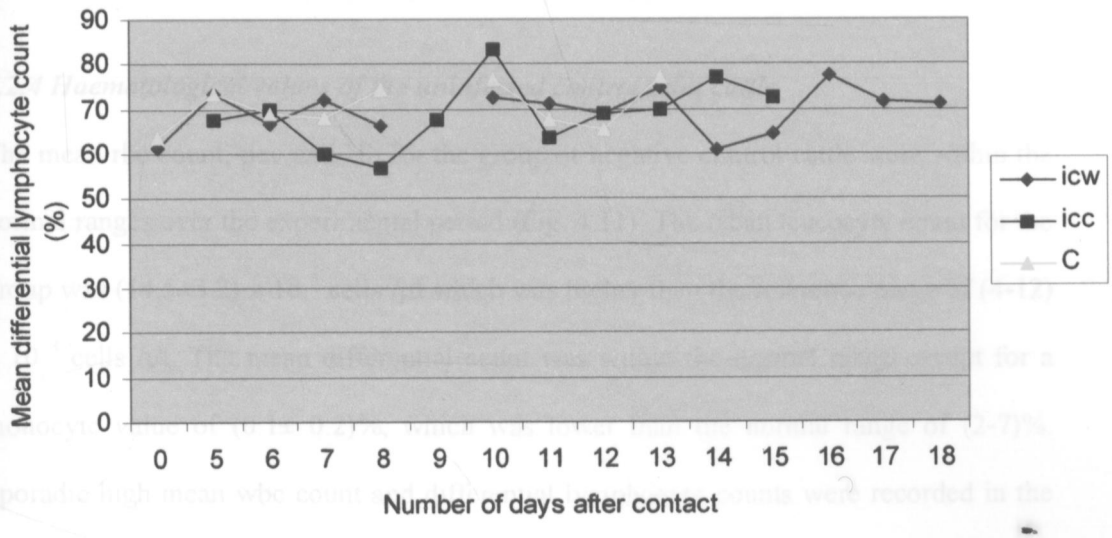


Figure 4.13. Mean differential lymphocyte count by number of days post-contact in cattle that were kept in contact with warthogs or other cattle that had been inoculated with kudu/Bov/BK/V2.

Key

C-Uninfected control cattle **icw**-Cattle kept with warthogs that were inoculated with kudu/Bov/BK/V2 **icc**-Cattle that were kept with other cattle that were inoculated with kudu/Bov/BK/V2

4.2.3 Haematological values of zebu cattle kept in contact with warthogs that were inoculated with Kudu/Bov/BK/V2

The mean rbc count, pcv, Hb and differential leucocyte count for the group of cattle that was housed in contact with warthogs inoculated with Kudu/Bov/BK/V2 remained within normal ranges over the experimental period (Fig. 4.11, 4.13). High mean wbc counts were recorded on days 6 and 15 post-exposure (Fig 4.12). The haematological values for each of the four in-contact animals remained within normal ranges except for intermittent sporadic leucocytosis accompanied with balanced differential leucocyte counts or occasional high differential lymphocyte counts.

4.2.4 Haematological values of the uninfected control zebu cattle

The mean rbc count, pcv and Hb for the group of negative control cattle were within the normal ranges over the experimental period (Fig. 4.11). The mean leucocyte count for the group was (14.5±3.2) x 10^3 cells /µl which was higher than the reference range of (4-12) x 10^3 cells /µl. The mean differential count was within the normal range except for a monocyte value of (0.1± 0.2)%, which was lower than the normal range of (2-7)%. Sporadic high mean wbc count and differential lymphocyte counts were recorded in the control group over the period (Figs.4.12, 4.13).

4.2.5 Haematological values of warthogs that were inoculated with Kudu/Bov/BK/V2

The mean rbc count for the group remained normal over the experimental period whereas mean wbc count rose to a high value of 14.5 x 10^6 cells /µl on day 6, dropping to leucopaenic levels of 7 x 10^6 cells /µl and 7.8 x 10^6 cells /µl on days 7 and 8 respectively (Figs. 4.14, 4.15). Mean differential lymphocyte count for the group dropped to a low of 23% on day 6 post-inoculation but was within the normal range the rest of the time (Fig 4.16). Haemoglobin concentration and pcv for each of the four inoculated warthogs remained normal during the experiment. Trends in blood values in individual warthogs were as follows during the early and late stages of the disease.

The wbc count of one of the two warthogs that were euthanised in the early stage of the disease (TW10) dropped from a normal of 10.4 x 10^3 cells /µl on day 0 to a leucopaenic level of 7.7 x 10^3 cells /µl on day 3, whereas its differential leucocyte count and other blood parameters remained normal over the period. The warthog was found dead on day 4. The other warthog that was euthanised in the early stage of the disease (TW13) had normal blood values except high differential neutrophil counts of 75% and 77% on days 0 and 7 respectively. It was euthanised on day 6 for necropsy.

The wbc count of one of the two warthogs euthanised in the late stages of the disease (TW33) dropped from 8.2 x 10^3 cells /µl on day 0 to leucopaenic levels of 7.4 x 10^3 cells /µl and 5.6 x 10^3 cells /µl on days 3 and 7 respectively. The wbc count rose again to 7.8 x 10^3 cells /µl accompanied by the neutrophilia of 70% on day 8 when the animal was euthanised for necropsy. The other warthog (TWY) had a high differential neutrophil

count of 70% with a normal wbc count of 11.5 x 10^3 cells /µl on day 5, the latter dropping to 8.3 x 10^3 cells /µl on day 7 and further to a leucopaenic level of 6.0 x 10^3 cells /µl on day 9 when it was euthanised for necropsy.

4.2.6 Haematological values of warthogs housed in contact with other warthogs that were infected with Kudu/Bov/BK/V2

The mean rbc count for the group was normal over the period (Fig. 4.17). A high mean wbc count of 15.4 x 10^3 cells /µl was recorded for the group on day 22 (Fig. 4.18). A low mean differential lymphocyte count of 29% and a correspondingly high differential neutrophil count were recorded for the group on day 15 (Fig. 4.19). The trends in blood values in individual animals were as follows.

The wbc counts in one of the two in-contact warthogs (TW1) dropped from a normal of 9.6 x 10^3 cells /µl on day 13 to a marginally low of 8.2 x 10^3 cells /µl on day 15, rising again on day 22 to a very high count of 14.6 x10^3 cells /µl accompanied by a high differential neutrophil count of 63%. The rbc count, Hb and pcv for the warthog remained within the normal ranges over the period. High differential neutrophil counts of 67% and 70 % were recorded for this warthog on days 0 and 11, respectively, and a high differential lymphocyte count of 68 % was recorded on day 5.

The other in-contact warthog had a drop in wbc from a normal of 12.6 x10^3 cells /µl on day 3 to a marginally low count of 8.0 x10^3 cells /µl on day 5, rising on day 22 to a very high count of 16.3 10^3 accompanied with a high differential neutrophil count of 65%. A

high differential neutrophil count of 71% was recorded for the warthog on day 0 and a high differential lymphocyte count of 70% on day 11 with normal wbc count in both cases.

4.2.7 Haematological values of warthogs housed in contact with zebu cattle that were inoculated with Kudu/Bov/BK/V2

The mean rbc count and wbc count for the group that was kept with cattle inoculated with kudu/Bov/BK/V2 were normal over the period. A slightly high mean differential lymphocyte count of 69% was recorded for the group on day 9. The rbc, pcv and Hb of individual warthogs were normal over the period. The trends in other blood values in individual warthogs were as follows.

One of the four in-contact warthogs (TW7) had a drop in wbc from 8.4 $x10^3$ cells /µl on day 9 to leucopaenic levels of 6.1 $x10^3$ cells /µl and 6.3 $x10^3$ cells /µl on days 11 and 13 respectively, accompanied with a high differential lymphocyte count of 69% on the latter day. The haemogram was normal by day 15 with a total leucocyte count of 9.0 $x10^3$ cells /µl. One other in-contact warthog (TW9) had a very low total leucocyte count of 5.4 $x10^3$ cells /µl on day 9 accompanied by a high differential lymphocyte count of 74%. A high differential lymphocyte count of 81% was recorded for the warthog on day 13 with a normal wbc count of 9.1 $x10^3$ cells /µl. One of the four warthogs (TW6) had normal blood values throughout the period while the other (TW8) had high differential lymphocyte count of 78% on day 9 and a low differential lymphocyte count of 26% on day 15.

4.2.8 Haematological values of warthogs inoculated with virulent Kabete 'O' strain of RPV

Mean rbc count and wbc for the group on days 0 and 3 when both animals were bled were normal (Fig.4.19, 4.20). However, one of the two warthogs (TWA) had a drop in wbc from 10.2 x 10^3 cells /µl on day 0 to a low of 7.4 x10^3 cells /µl with normal differential counts on day 3. It was found dead on day 5. The other warthog (TWX1) had high rbc, pcv and Hb of 15.5 x 10^6 cells /µl, 51%, 18.6 g/dl, respectively on day 5 accompanied by a low wbc count of 8 x 10^3 cells /µl and very high differential lymphocyte count of 85%. The overall mean rbc, pcv and Hb for the two warthogs over the entire period of the experiment, from day 0 to 5, were higher than those of any of the other groups indicating haemoconcentration.

4.2.9 Summary of haematological response in all animals

The haematological response to rinderpest virus challenge in cattle and warthogs infected by various modes with different isolates of RPV is summarized in Table 4.6.

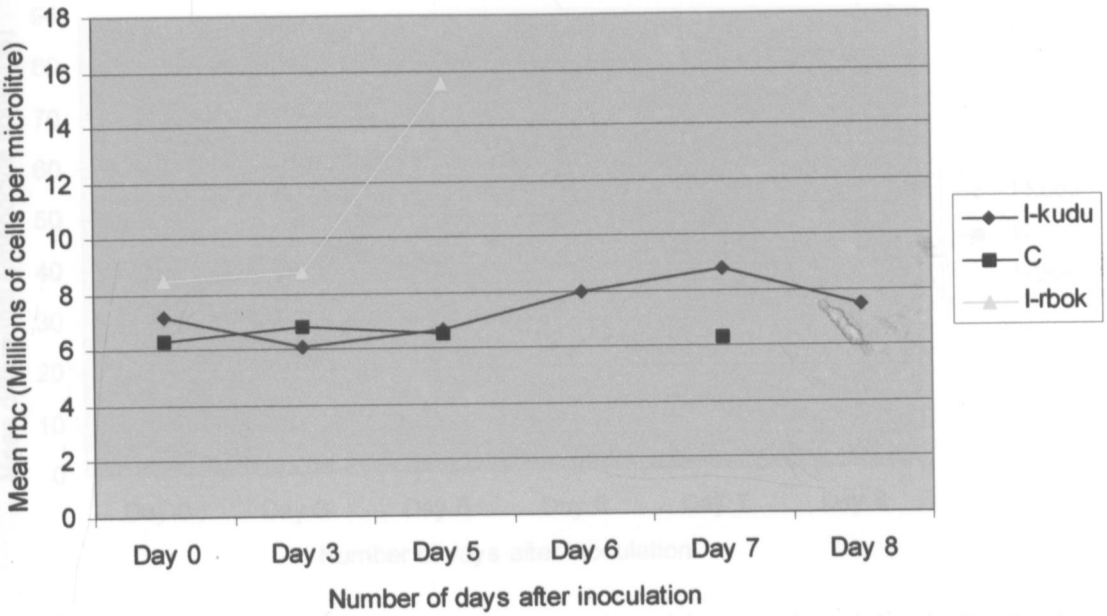


Figure 4.14. Mean rbc by number of days post inoculation in groups of warthogs that were inoculated with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV.

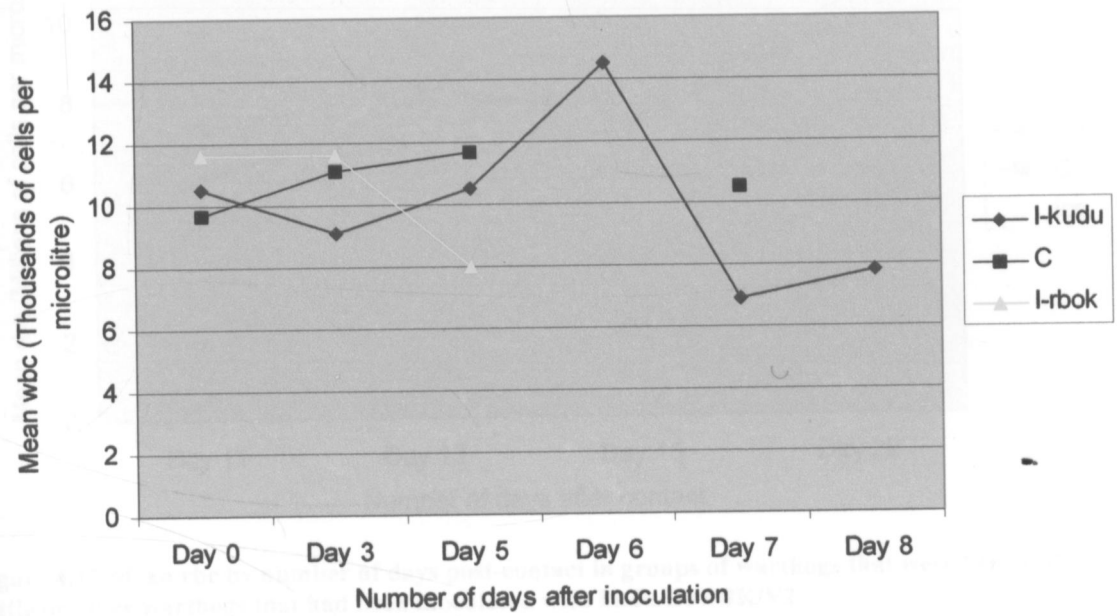


Figure 4.15. Mean wbc by number of days post inoculation in groups of warthogs that were inoculated with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV.

Key

I-kudu-Warthogs inoculated with kudu/Bov/BK/V2 **I-rbok**-Warthogs inoculated with the virulent Kabete 'O' strain of RPV **C**-Uninfected control warthogs

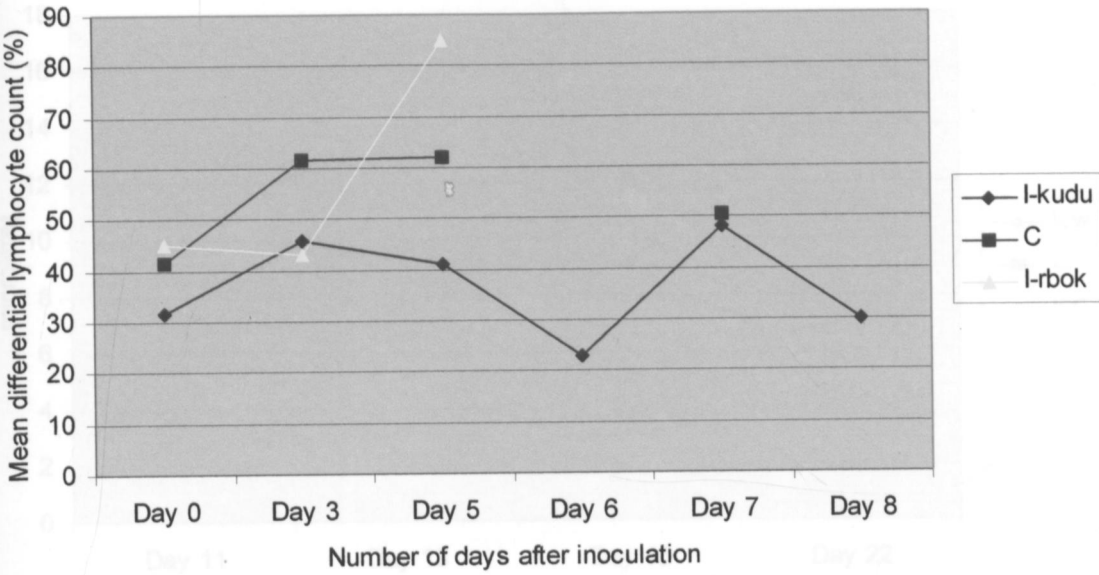


Figure 4.16. Mean differential lymphocyte count by number of days post inoculation in groups of warthogs that were inoculated with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV.

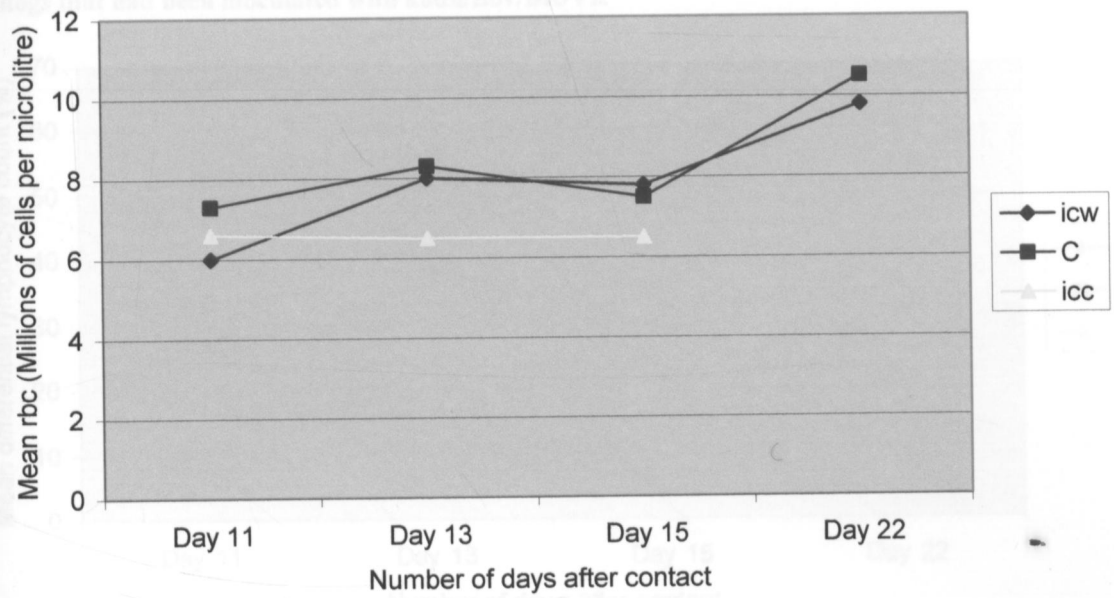


Figure 4.17 Mean rbc by number of days post-contact in groups of warthogs that were kept with cattle or other warthogs that had been inoculated with kudu/Bov/BK/V2.

Key

I-kudu-Warthogs inoculated with kudu/Bov/BK/V2 **I-rbok**-Warthogs inoculated with the virulent Kabete 'O' strain of RPV **icw**-warthogs kept in contact with other warthogs that were inoculated with kudu/Bov/BK/V2 **icc**-warthogs kept with cattle that were inoculated with kudu/Bov/BK/V2 **C**-Negative control warthogs

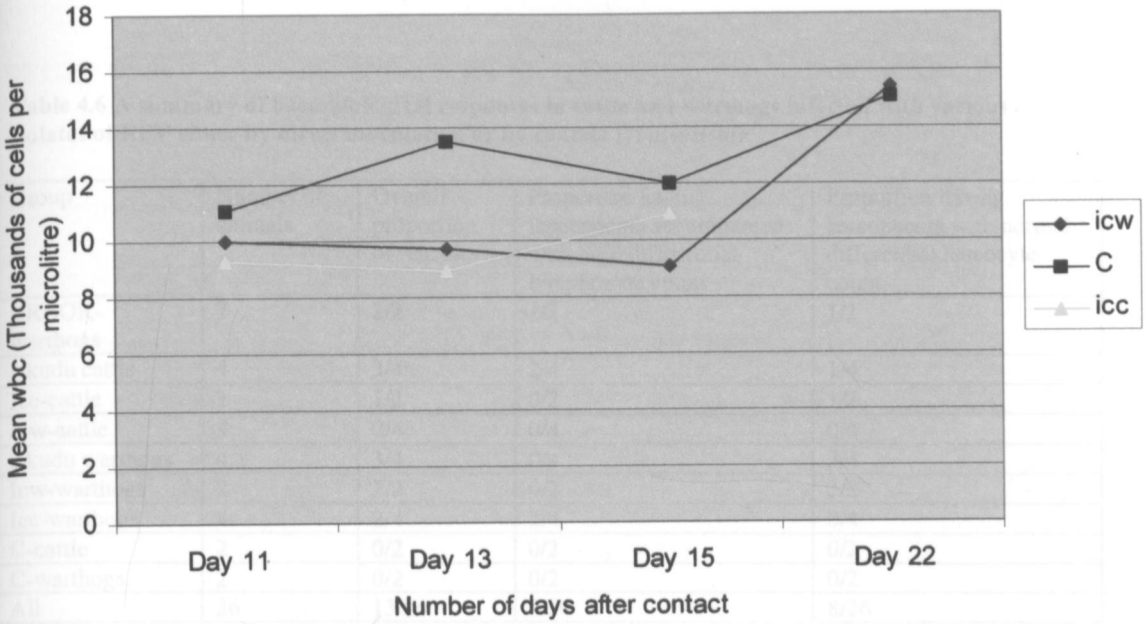


Figure 4.18. Mean wbc by number of days post-contact in warthogs that were kept with cattle or other warthogs that had been inoculated with kudu/Bov/BK/V2.

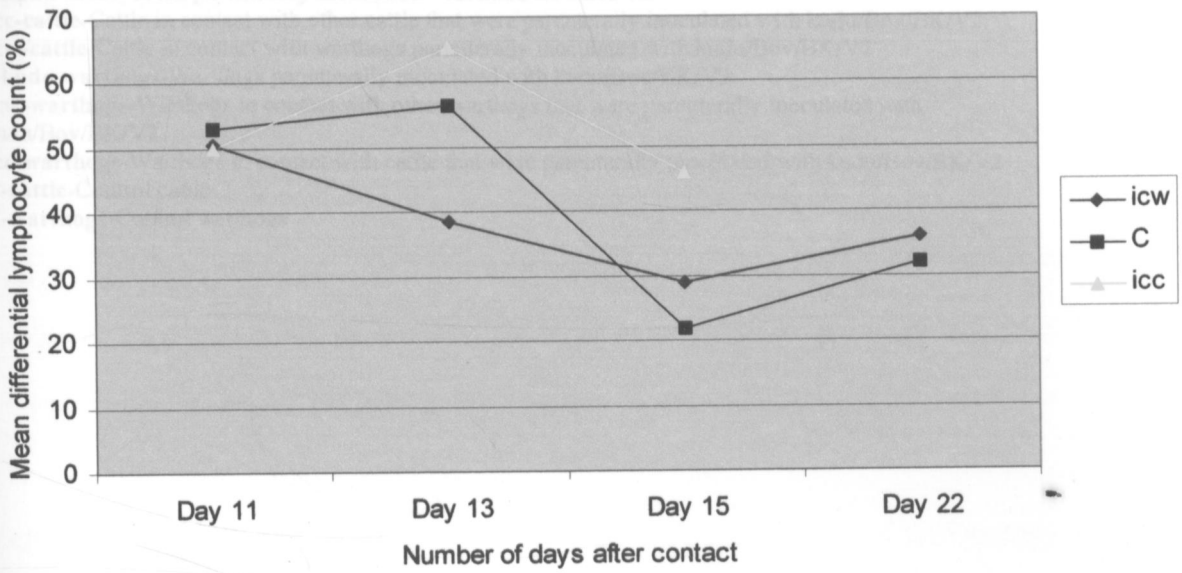


Figure 4.19. Mean differential lymphocyte count by number of days post-contact in groups of warthogs that were kept with cattle or other warthogs that were inoculated with kudu/Bov/BK/V2.

Key

icw-Warthogs kept with other warthogs that had been inoculated with kudu/Bov/BK/V2
icc- Warthogs kept with cattle that had been inoculated with kudu/Bov/BK/V2 **C-** uninfected control warthogs.

Table 4.6 A summary of haematological responses in cattle and warthogs infected with various isolates of RPV either by direct inoculation or by contact transmission

Group	Number of animals	Overall proportion of reactors	Proportion having leucopaenia accompanied with high differential lymphocyte count	Proportion having leucopaenia with normal differential leucocyte count
I-RBOK-warthogs	2	2/2	1/2	1/2
I-kudu cattle	4	3/4	2/4	1/4
Icc-cattle	2	1/2	0/2	1/2
Icw-cattle	4	0/4	0/4	0/4
I-kudu warthogs	4	3/4	0/4	3/4
Icw-warthogs	2	2/2	0/2	2/2
Icc-warthogs	4	2/4	2/4	0/4
C-cattle	2	0/2	0/2	0/2
C-warthogs	2	0/2	0/2	0/2
All	26	13/26	5/26	8/26

Key
I-RBOK-warthogs-Warthogs parenterally inoculated with virulent Kabete 'O' strain of RPV
I-kudu cattle-Cattle parenterally inoculated with kudu/Bov/BK/V2
Icc-cattle-Cattle in contact with other cattle that were parenterally inoculated with kudu/Bov/BK/V2
Icw-cattle-Cattle in contact with warthogs parenterally inoculated with kudu/Bov/BK/V2
I-kudu warthogs-Warthogs parenterally inoculated with kudu/Bov/BK/V2
Icw-warthogs-Warthogs in contact with other warthogs that were parenterally inoculated with kudu/Bov/BK/V2
Icc-warthogs-Warthogs in contact with cattle that were parenterally inoculated with kudu/Bov/BK/V2
C-cattle-Control cattle
C-warthogs-Control warthogs

SECTION 3

4.3 Gross postmortem lesions

A total of nine (9) cattle and thirteen (13) warthogs from experimental as well as control groups were euthanised and examined during post mortem in the early and late stages of the disease. The pathological lesions induced by the different isolates in cattle and warthogs are summarized below.

4.3.1 Gross lesions in cattle

The gross lesions in four zebu cattle parenterally inoculated with kudu/Bov/BK/V2, four Boran cattle parenterally inoculated with RBK/WP/86/1, and one negative control zebu, were as follows.

4.3.1.1 Gross lesions in zebu cattle inoculated with Kudu/Bov/BK/V2

Two out of four zebu cattle that had been inoculated with kudu/Bov/BK/V2 were euthanised on days 5 and 6 representing the early stages of the disease. The other two were euthanised on days 8 and 9 represented the late phase of the disease.

Zebu heifer HK45 that was euthanised on day 5 had diffuse congestion of the intestinal mucosa that was mild in the caecum, colon and rectum, moderate in the jejunum and ileum, and marked in a small strip of the ileo-caecal valve (Figs. 4.22a and 4.22b). Zebu heifer HK10 that was euthanised on day 6 for necropsy had two small superficial erosions of about 2-3mm on the upper surface of the tongue. The intestinal mucosa had diffuse

congestion that was mild in the jejunum, moderate in the caecum and proximal colon and marked in the ileum with slightly enlarged Peyer's patches in the latter (Fig. 4.22c).

Zebu steer HK49 that was euthanised on day 8 had a few superficial erosions of about 2-5mm on the base of the tongue. The intestinal mucosa had diffuse congestion that was marked in the jejunum, ileum and ileo-caeco-colic junction, and moderate in the caecum, proximal colon and rectum. The mucosal surface of the gall bladder and urinary bladder were markedly congested. Mesenteric, prescapular and mandibular lymph nodes were moderately enlarged and oedematous, and the sub-mandibular lymph nodes had ecchymotic haemorrhages.

Zebu steer HK69 that was euthanised on day 9 had mild diffuse congestion of the mucosa of the jejunum and distal end of the ileum with slightly prominent Peyer's patches in the latter. The mucosa of the colon had several slightly raised hyperemic foci of about 2-5mm. The lymph nodes adjacent to the caeco-colic junction were congested and oedematous (Fig.4.22d). The inner surface of the urinary bladder was slightly congested.

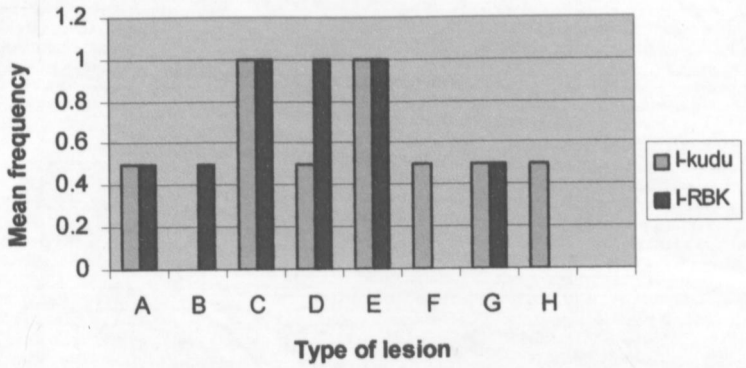


Figure 4.20.Mean frequency of various gross pathological lesions by strain of virus in cattle parenterally inoculated with kudu/Bov/BK/V2 or RBK/WP/86/1

Key
I-kudu- Cattle that were parenterally inoculated with kudu/Bov/BK/V2 **I-RBK**-Cattle that were parenterally inoculated with RBK/WP/86/1 **A**-Oral erosions **B**-Congestion of abomasum **C**-Congestion of small intestine **D**-Congestion of ileocaecal valve **E**-Congestion of large intestine **F**-Prominent Peyer's patches **G**-Congestion/oedema of lymph nodes **H**- Congestion of gall bladder or urinary bladder

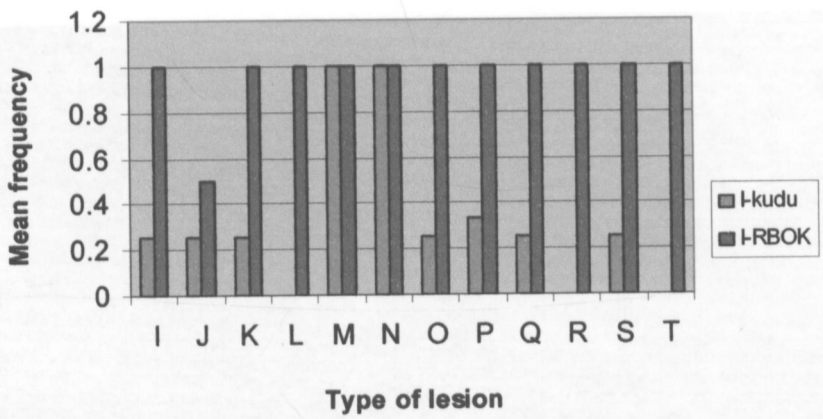


Figure 4.21.Mean frequency of various gross pathological lesions by strain of virus in warthogs parenterally inoculated with kudu/Bov/BK/V2 or the virulent Kabete 'O' strain of RPV

Key
I-kudu-Warthogs that were parenterally inoculated with kudu/Bov/BK/V2 **I-RBOK-** Warthogs that were parenterally inoculated with the virulent Kabete 'O' strain of RPV **I**- Congestion/erosions in mouth **J**-Congestion in conjunctiva **K**-Congestion /haemorrhage in pharynx **L**-Congestion./erosions in oesophagus **M**-Congestion haemorrhage/erosion/necrosis in abomasum **N**- Congestion haemorrhage /erosion/ necrosis in small intestine **O**- Congestion haemorrhage/erosion/necrosis in ileocaecal junction /valve. **P**- Congestion haemorrhage/erosion/necrosis in large intestine **Q** –congestion/ enlargement / oedema in lymph nodes **R**-Prominence/Congestion/haemorrhage in Peyer's patches **S**-Congestion/haemorrhage in urinary gladder and urinary tract **T**-Congestion/ haemorrhage in kidneys

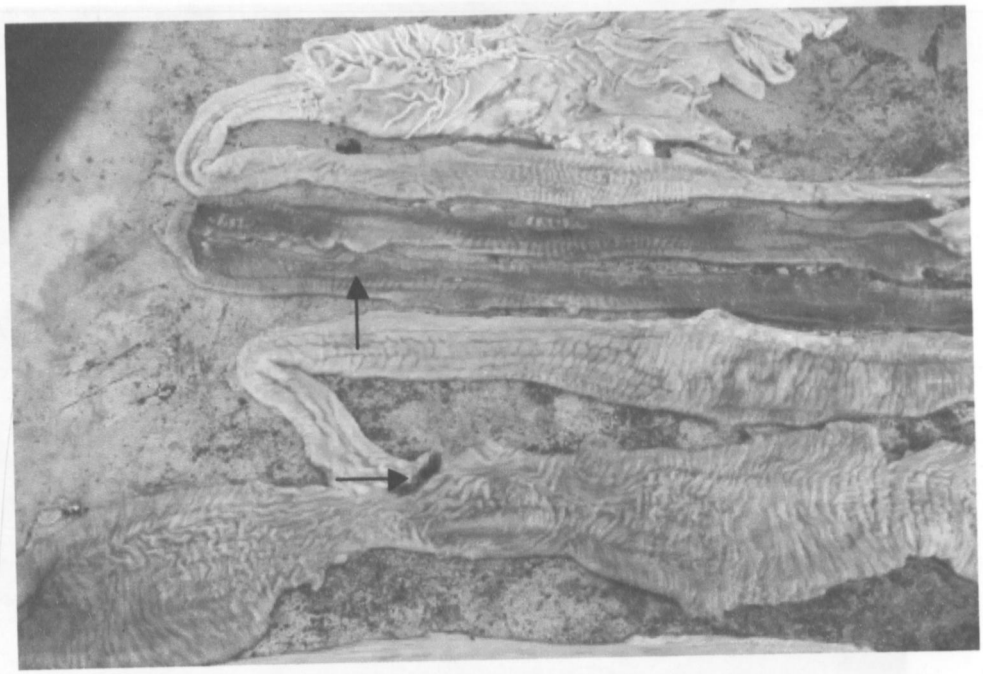


Figure 4.22a Congested sections of the small intestines and ileo-caecocolic junction (arrows) of a zebu heifer (HK45) that was inoculated with kudu/Bov/ BK/V2 and euthanised 5 days after inoculation.

Figure 4.22b. A normal ileo-caecocolic junction of a zebu heifer (HK62) that was treated with a placebo and kept separate from experimentally infected cattle

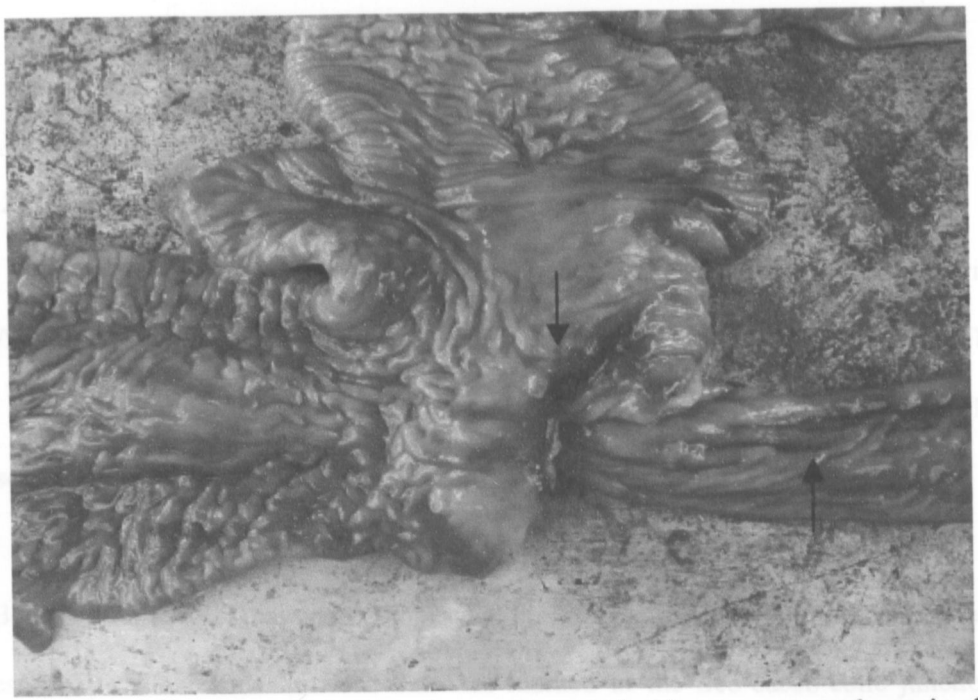


Figure 4.22 c. Congestion of the ileum, ileocaecal valve, caecum and proximal colon of a heifer (HK10) that was inoculated with kudu/Bov/BK/V2 and euthanised 6 days after inoculation

Figure 4.22 d. A congested colonic lymph node (arrows) of a steer (HK69) that was inoculated with kudu/Bov/BK/V2 and euthanised 9 days after inoculation

4.3.1.2 Gross lesions in Boran cattle inoculated with RBK/WP/86/1

Two (569K and 550K) out of four Boran cattle that had been inoculated with RBK/WP/86/1 were euthanised on day 8, representing the early stages of the disease. The other two (558K and 566K) were euthanised on days 11 and 12, respectively, representing the late stages of the disease.

One Boran heifer (550K) that was euthanised at the early stages of the disease had diffuse congestion of the intestinal mucosa that was marked in the ileo-caeco-colic junction, jejunum and ileum, but mild in the caecum, and accompanied with 'zebra striping' in some areas (Fig.4.22e). The rectum had slight petechial haemorrhages in a few areas. Messenteric lymph nodes were moderately enlarged and congested.

The other Boran heifer (569K) that was euthenised at the early stages of the disease had a few areas of congestion at the pyloric region of the abomasums. It had diffuse congestion of the intestinal mucosa that was marked in the ileo-caeco-colic valve and ileum and mild in the duodenum and caecum with mild zebra striping in the caecum (Fig.4.22f). Pre-scapular and mandibular lymph nodes were moderately enlarged.

One Boran steer (566K) that was euthanised at the late stages of the disease had a few small (2-5mm) superficial erosions on the gum. Its abomasum was slightly congested on the mucosal surface. It had diffuse congestion of the intestinal mucosa that was marked in

the ileo-caeco-colic valve and colon and mild in the jejunum. The colon had a moderate roundworm infestation.

One Boran heifer (558K) that was euthanised at the late stages of the disease had a few small superficial erosions on the tongue that had almost completely resolved. Its intestinal mucosa had diffuse congestion that was marked in the ileo-caeco-colic junction but mild in the duodenum, caecum and colon. The ileum had a small patch of mild congestion on the mucosal surface.

4.3.1.3 Gross post mortem findings in the uninfected control zebu heifer

Control zebu heifer HK62 had no gross lesions except mild congestion of the gall bladder and marked congestion of the urinary bladder.

Figure 4.22 e. Congestion with "zebra striping"(arrows) in the ileum of a Boran heifer (550K) that was inoculated with RBK/WP/86/1 and euthanised 7 days after inoculation

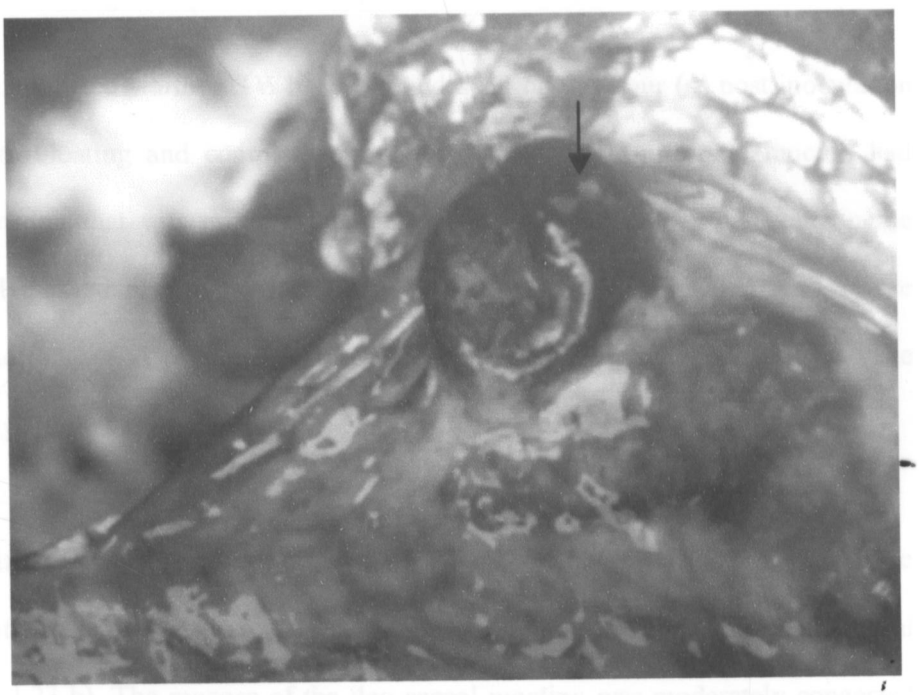


Figure 4.22 f. A Severely congested ileocaecal valve (arrow) of Boran heifer (569K) that was inoculated with RBK/WP/86/1 and euthanised 7 days after inoculation

4.3.2 Gross lesions in warthogs

The gross lesions in seven warthogs infected either by direct inoculation or by contact transmission with the kudu/Bov/BK/V2 isolate or the virulent Kabete 'O' strain of RPV, and one uninfected control are described below.

4.3.2.1 Gross lesions in warthogs inoculated with kudu/Bov/BK/V2

Two out of the four warthogs (TW10 and TW13) parenterally inoculated with kudu/Bov/BK/V2 and examined during post mortem on days 4 and 6 after inoculation, represented cases at the early stages of the disease. The other two infected warthogs (TW33 and TWY) that were examined during post mortem on days 8 and 9 represented cases at the late stages of the disease. The lesions were as follows.

Parenterally inoculated warthog TW10 was found dead on day four (4) post inoculation with moderate bloating and complete rigor mortis. The gastro-intestinal mucosa had diffuse congestion that was mild in the rectum, caecum and colon, moderate in the stomach, and marked in the proximal ileum where it was accompanied with severe diffuse haemorrhage and prominence of the associated mesenteric blood vessels. The affected ileal portion was necrotic and its contents were bloody (Fig.4.23a).

Parenterally inoculated warthog TW13 that was euthanised on day 6 had marked diffuse congestion of the entire small intestine, evident from both the serosal and mucosal surfaces (Fig.4.23 b). The mucosa of the ileo-caecal junction was moderately congested

and that of the colon mildly congested. The abomasum had a few moderately congested patches on its mucosal surface.

Parenterally inoculated warthog TW33 that was euthanised on day 8 was moderately cyanotic on the abdomen and medial aspects of the legs. The lower gum was moderately congested. The gastro-intestinal mucosa had diffuse congestion that was marked in the duodenum and ileum, and moderate in the abomasum, jejunum, ileo-caecal valve, caeco-colic junction, lower colon and rectum. The pharynx was moderately congested with moderate petechial haemorrhages on the epiglottis (Fig.4.23c). The prescapular, mandibular and messenteric lymph nodes were moderately congested.

Parenterally inoculated warthog TWY that was euthanised on day 9 had moderate cyanosis on the abdomen and medial aspects of the legs, and congestion of the conjunctivi and lower gum accompanied by shallow erosions in the latter. The gastrointestinal tract had diffuse congestion that was marked in the duodenum, jejunum and ileum and moderate in the abomasum, ceco-colic junction, colon and proximal part of the rectum. There was a worm cyst attached to the serosal surface of the stomach. The prescapular and mandibular lymph nodes were moderately enlarged and oedematous. The mucosa of the urethra was markedly congested and there was moderate petechial haemorrhage on the mucosal surface of the urinary bladder.

4.3.2.2 Gross lesions in the in-contact warthogs

One (TW1) of the two warthogs kept in contact with other warthogs parenterally inoculated with kudu/Bov/BK/V2 was euthanised on day 22 from the start of the experiment, while it was still clinically sick, and the other (TW2) was euthanised after recovery from clinical disease. The four warthogs (TW6, TW7, TW8 and TW9) kept in contact with cattle that were inoculated with kudu/Bov/BK/V2 were all euthanised for necropsy after recovery.

Warthog TW1 that was euthanised on day 22 was in poor body condition and had cutaneous eruptions affecting the rear aspects of the thighs and widespread shallow cutaneous erosions especially at the shoulders. The skin was peeling off along the coronet and cracking at the points of attachment of the dewclaws (Fig.4.23 e). The tissues around the left tarsal joint were moderately swollen. Both surfaces of the tongue had shallow extensively coalescent erosions. The dental pad had a focal erosion (about 20mm by 10mm) and the stomach had a few erosions of about 10-30mm. The duodenum had a few focal erosions of about 5mm and the entire small intestine, colon and rectum were moderately congested. The large intestines had very loose contents. The urinary bladder and urethra were moderately congested. The prepuce was shriveled and cyanotic, and its opening was constricted causing accumulation of urine around it to form a swelling of about 50mm diameter. There were a few roundworms and one tapeworm in the small intestine. Warthog TW2 had mild congestion of the small intestine and a few petechial haemorrhages on the mucosal surface of the urinary bladder. No other lesions were evident in this warthog.

Warthog TW6 had congestion of the intestinal mucosa that was moderate in the small intestine and mild in the caecum, rectum and colon. Its urinary bladder was mildly congested. Warthogs TW7, TW8 and TW9 had congestion of the mucosa of the small intestine that was mild and limited to the duodenum in both TW8 and TW9 but marked and affecting the duodenum and ileum in TW7. Warthog TW8 had a bladder worm on the omentum.

4.3.2.3 Gross lesions in warthogs inoculated with the virulent Kabete 'O' strain of RPV

Two warthogs TWA and TWX1 that were inoculated parenterally with the virulent Kabete 'O' strain of RPV were examined during post mortem on days 4 and 5, respectively.

Warthog TWA that was found dead on day 4 had sunken eyeballs (Fig 4.7d) and marked congestion of the conjunctiva, the base of the tongue, the pharynx and the oesophagus. The mucosa of the stomach had numerous ulcers of about 2-3mm diameter especially on the cardial gland area (Fig.4.23 f). The mucosal surface of the entire small intestine was severely congested and had ecchymotic and petechial haemorrhages as well as multiple foci of necrosis (2-10mm in diameter) with hyperemic borders. The Peyer's patches in the ileum were very prominent, congested and haemorrhagic (Fig.4.23 g). The caecum, rectum, and the entire length of the colon had numerous hyperemic foci of necrosis (about 2-10mm diameter)(Figs.4.23 h and 4.23 i). The ileocaecal valve had similar foci of necrosis accompanied by diffuse haemorrhage. The gall bladder was engorged with thick dark bile. Mesenteric, prescapular and mandibular lymph nodes were enlarged and

congested. The kidneys were congested and adrenal glands were enlarged. The lungs were markedly congested with profuse frothy exudates in the trachea and bronchi. The mucosal surfaces of the urinary bladder and reproductive tract were moderately congested.

Warthog TWX1 that was euthanised on day 5 had reddened nostrils and shallow erosions of 5-20mm in diameter on the dental pad and lower gum. The base of the tongue and pharynx were severely congested. The mucosa of the oesophagus had numerous tiny erosions of about 1-2mm. The entire mucosa of the stomach had severe congestion, extensive haemorrhage and numerous tiny foci of necrosis on the cardial gland region (Fig. 4.23j). The small intestine had severe congestion and extensive haemorrhage along its entire length with prominent and haemorrhagic Peyer's patches in the ileum. The mucosa of the caecum and ileo-caecal valve were severely congested and had numerous focal erosions of about 3-5mm diameter. The colon had multiple foci of necrosis (about 5mm in diameter) with hyperemic borders along the entire length. The rectum had multiple haemorrhagic foci of about 5-10mm diameter. The gall bladder was engorged with thick dark bile and the spleen had multiple foci of haemorrhage. The mesenteric, prescapular and mandibular lymph nodes were enlarged and markedly congested (Fig.4.24k). Both kidneys had numerous subcapsular petechial haemorrhages. The urinary bladder and mucosa of the reproductive tract were mildly congested.

4.3.2.4 Gross postmortem findings in the uninfected control warthog

The uninfected control warthog TW11 had a few fibrinous strands attached loosely to the serosal surface of the intestines and a bladder worm attached to the omentum of the

stomach wall. The mucosa of the small intestine had congestion along its entire length that was marked in the duodenum and mild to moderate in the rest (Fig.4.23 d). The small intestine was infested with a few roundworms. The colon had mildly reddened nodular lesions of about 5mm diameter.

4.3.3 Summary of gross post-mortem lesions and their frequency of occurrence in experimental rinderpest

The gross postmortem lesions induced in cattle by both kudu/Bov/Bk/V2 and RBK/WP/86/1 were mild. The lesions induced by kudu/Bov/Bk/V2 were mild in warthogs that were parenterally inoculated but moderately severe in the warthogs that were kept in contact with the parenterally inoculated ones. The virulent Kabete 'O' strain of RPV induced extremely severe lesions in warthogs. A summary of the gross lesions and their frequency of occurrence in the experimental animals are shown in table 4.7 and figures 4.20 and 4.21.

Table 4.7.A summary of gross pathological lesions in cattle and warthogs infected with various isolates of RPV either by direct inoculation or by contact transmission

Group	Number of animals examined at postmortem	Proportion of reactors	Overall gross reaction
Cattle parenterally inoculated with RBK/WP/86/1	4	4/4	Mild
Cattle parenterally inoculated with kudu/Bov/BK/V2	4	4/4	Mild
Warthogs parenterally inoculated with kudu/Bov/BK/V2	4	2/4	Mild
Warthogs kept with other warthogs that were parenterally inoculated with kudu/Bov/BK/V2	1	1/1	Moderate
Warthogs parenterally inoculated with the virulent Kabete 'O' strain	2	2/2	Severe
Control cattle	1	0	None
Control warthogs	1	0	None

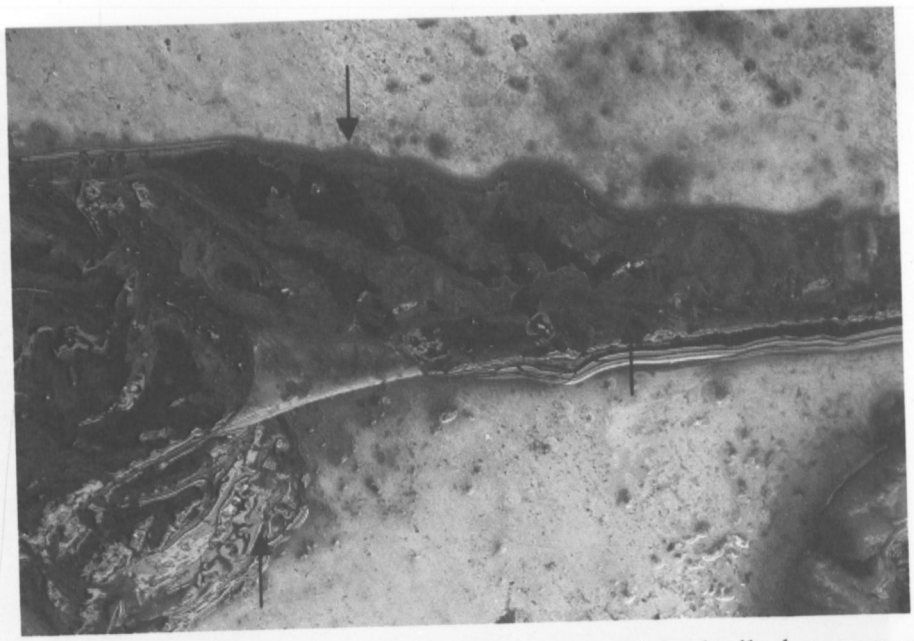


Figure 4.23 a. Severe diffuse haemorrhage (arrows) in the ileal mucosa of warthog TW10 that was inoculated with kudu/Bov/BK/V2 and found dead 4 days after inoculation

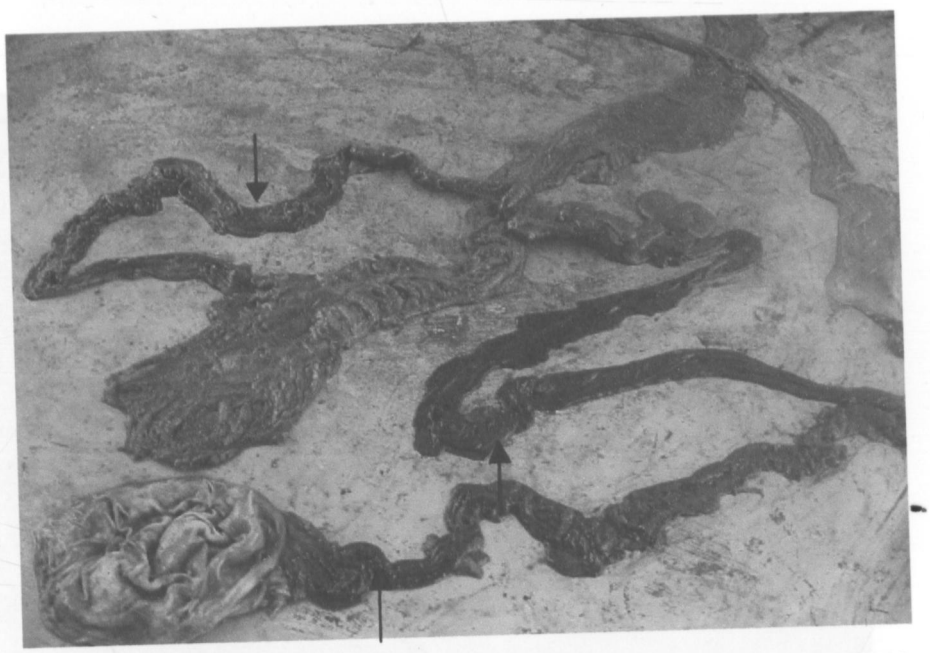


Figure 4.23 b Congested sections (arrows) of the gut of warthog TW13 that was inoculated with kudu/Bov/BK/V2 and euthanised 6 days after inoculation

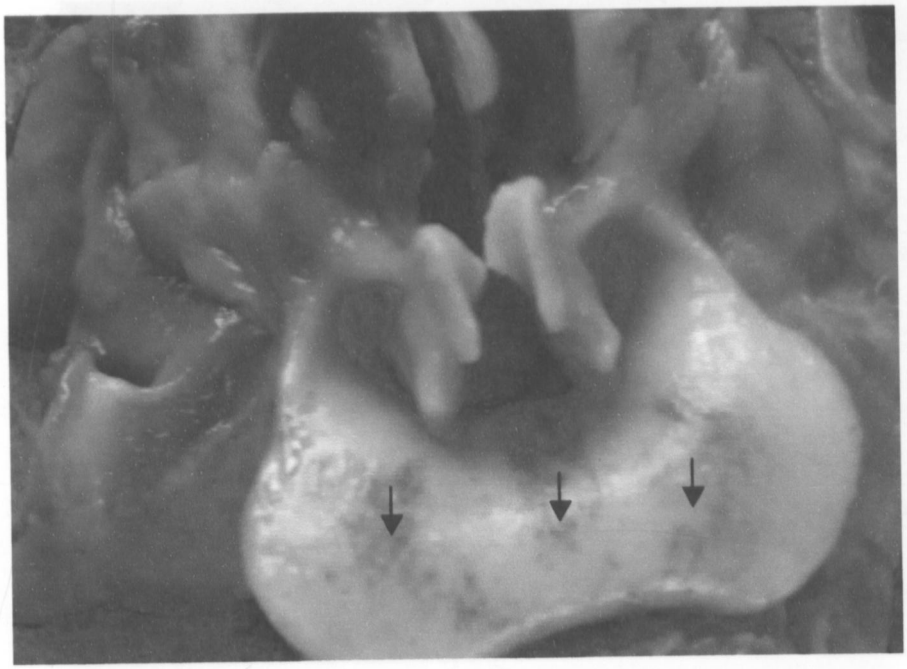


Figure 4.23 c. Petechial haemorrhage (arrows) in the epiglottis of a warthog (TW33) that was inoculated with kudu/Bov/BK/V2 and euthanised 8 days after inoculation.

Figure 4.23 d. Marked congestion of the duodenum (arrows) of a control warthog (TW11) that was treated with placebo and kept separate from those that were infected with RPV

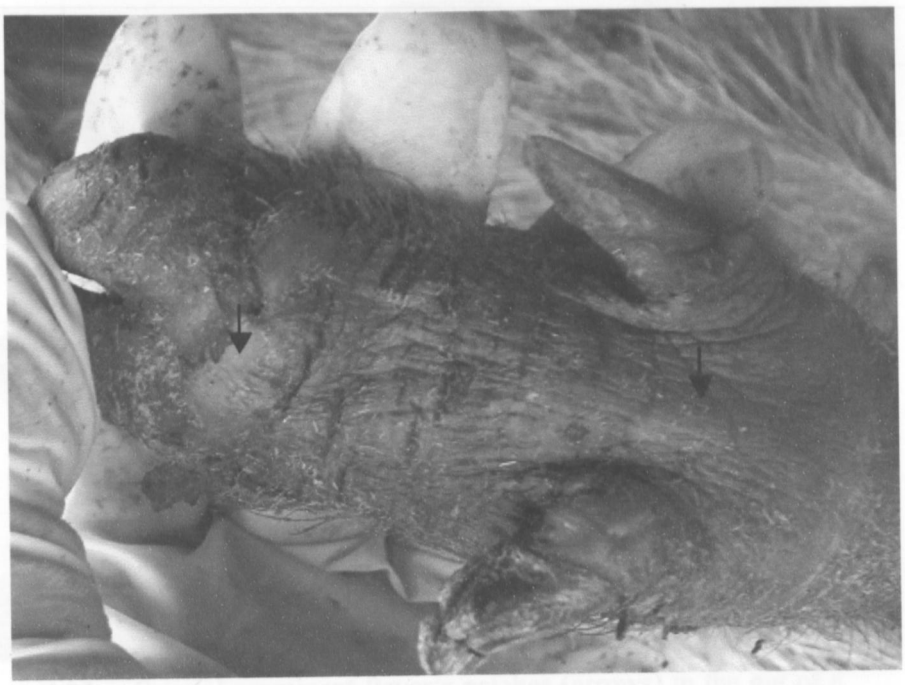


Figure 4.23 e. Swollen tarsus and peeling of skin around the coronet (arrows) of a warthog (TW1) that was euthanised 22 days after being kept in contact with warthogs that had been inoculated with kudu/Bov/BK/V2.

Figure 4.23 f. Numerous ulcers (arrows) in the stomach of warthog TWA that was inoculated with the virulent Kabete 'O' strain of RPV and found dead 5 days after inoculation

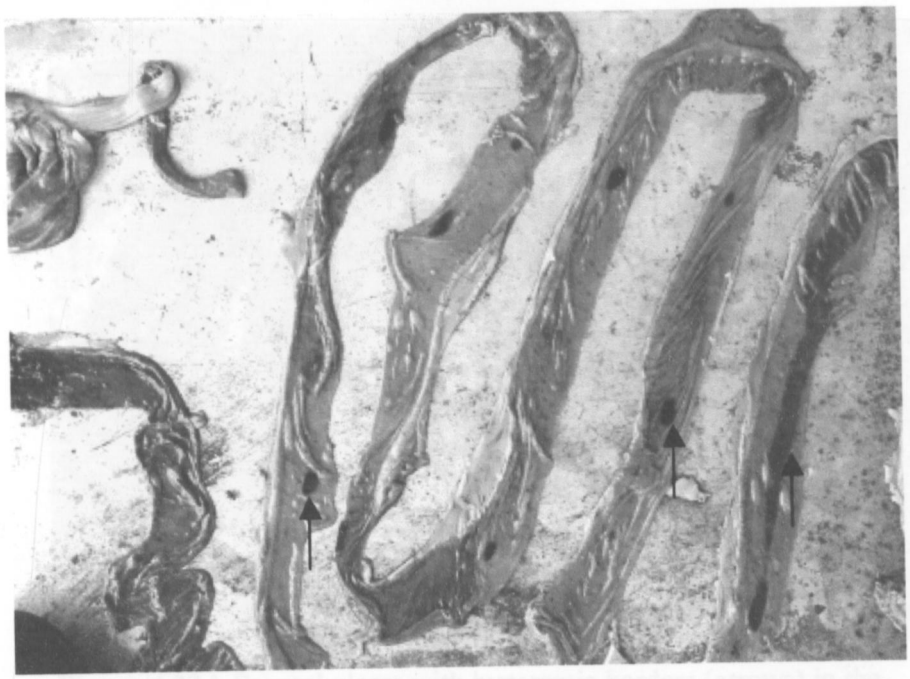


Figure 4.23 g. Several prominent Peyer's patches (arrows) in the gut of a warthog (TWA) that was inoculated with the virulent Kabete 'O' strain of RPV and found dead 5 days after inoculation

Figure 4.23 h. Numerous nodular haemorrhagic foci (arrows) in the colon of a warthog (TWA)that was inoculated with the virulent Kabete 'O' strain of RPV and found dead 5 days after inoculation

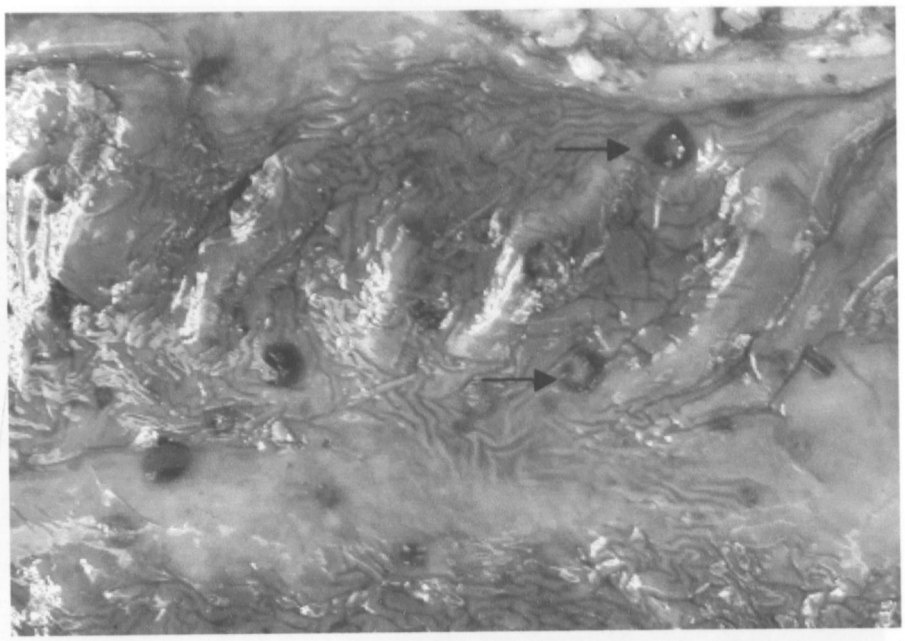


Figure 4.23 i. Several ulcers with hyperemic borders (arrows) in the caecum of warthog TWA that was inoculated with the virulent Kabete 'O' strain of RPV and found dead 5 days after inoculation

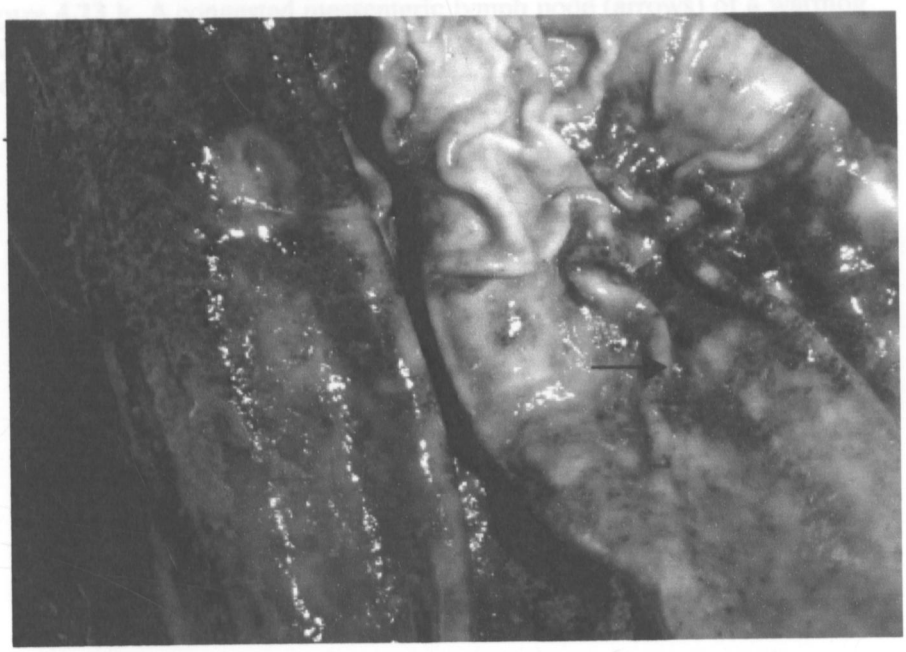


Figure 4.23 j. Severe congestion and haemorrhage (arrows) in the gastric mucosa of warthog TWX1 that was inoculated with the virulent Kabete 'O' strain of RPV and found dead 5 days after inoculation

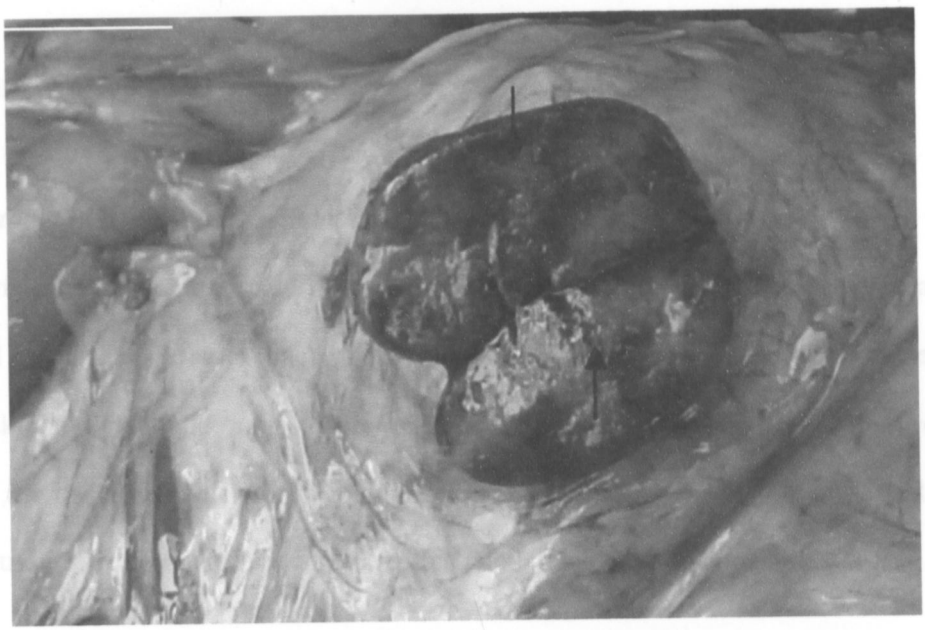


Figure 4.23 k. A congested messenteric lymph node (arrows) of a warthog (TWX1) that was inoculated with the virulent Kabete 'O' strain of RPV and died 5 days after inoculation, following chemical immobilization

SECTION 4

4.4 Histopathological Lesions

4.4.1 Histopathological lesions in cattle

Up to forty-nine different tissues from each of the cattle representing the digestive tract, lymphoid organs, respiratory tract, reproductive tract, major internal organs, skeletal muscle, skin and glandular organs, were examined and the microscopic lesions that were observed are described below and summarized in Figure 4.24. The lesions observed in different tissues from each of the cattle are illustrated in table 4.8 in appendix 1.

4.4.1.1 Histopathological lesions in zebu cattle inoculated with kudu/Bov/BK/V2

One of the two zebu heifers that were euthanised in the early stage of the disease (HK45) had congestion of the intestinal mucosa that was marked in the ileum, moderate in the caecum, and mild in the jejunum, duodenum and colon. No other lesions were observed in sections of other tissues collected from this animal. The other heifer (HK10) had congestion of the intestinal mucosa that was marked in the ileum, moderate in the caecum, and mild in the duodenum, jejunum, caecocolic junction and colon. A moderate infiltration with neutrophils was present in the intestinal submucosa, often near congested blood vessels, and was accompanied with plasma cells in some areas. Focal lymphocytolysis accompanied with infiltration of neutrophils was evident in the colon.

One of the two zebu steers that were euthanised in the late stage of the disease (HK49) had focal necrosis in the epithelium of the tongue associated with sloughing of the epithelium and a moderate to dense infiltration of the underlying lamina propria with neutrophils. These lesions were either superficial or deep, spanning the entire stratum spinosum to form a wide funnel shape in the latter case. The intestinal mucosa had congestion that was marked in the duodenum and ileum, moderate in the jejunum, and mild in the ileocaecal valve, caecocolic junction and colon. Extensive submucosal oedema was evident in the duodenum. Focal lymphocytolysis and lymphocellular degeneration were evident in the Peyer's patches associated with necrosis and sloughing of the overlying mucosal epithelium. The messenteric lymph nodes had focal and moderately extensive lymphocytolysis and lymphocellular degeneration accompanied by eosinophilic intracytoplasmic inclusions and mild congestion (Figs.4.25 a and 4.25 b). The other steer (HK69) euthanised in the late stage of the disease only had mild congestion in the jejunum and ileum.

4.4.1.2 Histopathological lesions in Boran cattle inoculated with RBK/WP/86/1

One of the two Boran heifers that were euthanised in the early stage of the disease (569K) had congestion of the intestines that was marked in the ileocaecal valve and rectum, moderate in the ileum and caecum, and mild in the duodenum. Plasma cells were abundant in the mucosa of the duodenum. There was moderate to marked congestion of prescapular and mandibular lymph nodes accompanied with moderate perivascular oedema in the latter. The other heifer (550K) had congestion of the intestines that was marked in the ileum, ileocaecal valve and caecocolic junction, and mild in the duodenum

and caecum. Marked congestion was also evident in the mesenteric lymph nodes. One of the two Boran cattle that were euthanised at the late stage of the disease (558K) had congestion of the intestines that was moderate in the duodenum, jejunum, ileum and caecocolic junction, and mild in the colon. There was perivascular neutrophilic infiltration in the submucosa of the caecocolic junction associated, in a few areas, with congestion. The prescapular lymph nodes had a few foci of oedema. The other animal (566K) had congestion of the gastrointestinal mucosa that was marked in the duodenum, moderate in the ileum, and mild in the abomasum, jejunum and caecum.

4.4.1.3 Histopathological lesions in the uninfected control zebu heifer

The uninfected control zebu heifer HK 62 had marked congestion of the gall bladder and moderate congestion of the urinary bladder. No other lesions were evident in this animal.

4.4.2 Histopathological lesions in warthogs

Up to forty-six different tissue samples from each warthog representing the digestive tract, lymphoid organs, respiratory tract, reproductive tract, major internal organs, skeletal muscle, skin and glandular organs, were examined and the microscopic lesions observed are described below and summarized in Figure 4.26. The lesions observed in different tissues from each of the warthogs are illustrated in table 4.9 in appendix 2.

4.4.2.1 Lesions in warthogs that were parenterally inoculated with kudu/Bov/BK/V2

One warthog that was found dead in the early stage of the disease (TW10) had severe hyperemia of the prescapular, mesenteric and mandibular lymph nodes accompanied by multifocal haemorrhage in the prescapular and messenteric ones. It had congestion of the

gastrointestinal tract that was marked in the stomach, ileum and caecocolic junction, moderate in the caecum, colon, rectum and ileocaecal valve, and mild in the duodenum and jejunum. Extensive autolysis was evident in some parts of the ileum. Congestion was marked in the lungs, moderate in the kidneys and mild in the epiglottis.

One warthog that was euthanised in the early stage of the disease (TW13) had single cell necrosis and a few minute foci of necrosis in the stratum spinosum of the gum epithelium. The mandibular, prescapular and mesenteric lymph nodes had moderate to marked congestion often accompanied by perivascular and subcapsular oedema, and multifocal haemorrhage. Multifocal lymphocytolysis and lymphocellular degeneration were present in the prescapular, mesenteric and mandibular lymph nodes accompanied by neutrophil infiltration, and formation of syncytia and eosinophilic intracytoplasmic inclusions. The spleen had moderate congestion and multifocal haemorrhage. The intestinal mucosa was congested markedly in the duodenum, ileum and colon, moderately in the jejunum, and mildly in the ileocaecal valve and rectum. These changes were accompanied by pulmonary and renal congestion.

One of the two warthogs that were euthanised in the late stage of the disease (TW33) had a few minute foci of necrosis in the stratum spinosum of the epithelium of the lips, hard palate, pharynx and oesophagus (Fig.4.27 a). Mandibular, prescapular and mesenteric lymph nodes had oedema within lymphoid tissue, and in the subcapsular and peritrabecular sinuses with widening of the sinuses. There was formation of syncytia and focal infiltration of neutrophils into lymphoid tissues in the prescapular and mandibular

lymph nodes. The intestinal mucosa had hyperemia that was marked in the duodenum, moderate in the jejunum, ileum, colon, ileocaecal valve and caeco-colic junction, and mild in the stomach and caecum. It had focal infiltration of neutrophils into the submucosa of the ileocaecal valve (Figs.4.27c and 4.27d).

The other warthog that was euthanised at the late stage of the disease (TWY) had a few minute foci of necrosis in the stratum spinosum of the epithelium of the snout, lips, cheeks, tongue, and hard palate. Single cell necrosis was observed in a few areas within the epithelium appearing as rounded cells with deeply eosinophilic cytoplasm and pyknotic or disintegrating nuclei. Ballooning degeneration preceded the single cell necrosis (Fig.4.27 b). The prescapular lymph nodes had marked congestion and oedema especially around the blood vessels, and in the subcapsular sinuses. The mandibular lymph node had extensive oedema. Multifocal lymphocytolysis and lymphocellular degeneration was present in the prescapular lymph node associated with formation of syncytia. Congestion in the gastrointestinal tract was marked in the ileum, moderate in the stomach, jejunum and caecocolic junction, and mild in the duodenum, caecum and colon.

4.4.2.2 Histopathological lesion in the in-contact warthog

One in-contact warthog (TW1) had focally coalescent and often extensive areas of necrosis in the epithelium of the lips, tongue and pharynx, spanning the entire stratum spinosum in many areas. The lesions were densely infiltrated with neutrophils and associated with areas of several syncytia (Figs.4.27e and 4.27f). Eosinophilic

intracytoplasmic inclusion bodies were occasionally seen in the stratified squamous epithelium. The lamina propria underlying areas of necrosis was similarly infiltrated with neutrophils. A few minute intraepithelial foci of necrosis were present in the oesophagus. The messenteric and prescapular lymph nodes had focal lymphocytolysis and lymphocellular degeneration. Mandibular lymph nodes had small foci of lymphocytolysis accompanied by infiltration with neutrophils. Multifocal haemorrhage within the parenchyma and focally extensive haemorrhage into the subcapsular sinus was seen in the messenteric lymph nodes. Syncytia formation was present in the prescapular and mandibular lymph nodes. The jejunum and rectum were mildly congested.

4.4.2.3 Histopathological lesions in two warthogs that were inoculated with the virulent Kabete 'O' strain of RPV

Severe lesions were observed especially in the lymphatic and gastrointestinal tissues of warthogs TWA and TWX1 that were parenterally inoculated with the virulent Kabete 'O' strain of RPV.

One (TWA) of the two warthogs that were parenterally inoculated with the virulent Kabete 'O' strain of RPV had a few minute foci of necrosis in the epithelium of the pharynx, appearing as densely eosinophilic necrotic coagula demarcated from normal tissue by a clear area. It had severe lymphocytolysis accompanied by hyperemia in the lymphoid tissue underlying the pharyngeal epithelium resulting in nuclear debris. The oesophagus had small intraepithelial foci of necrosis in the stratum spinosum associated with hydropic degeneration and single cell necrosis. The stomach had extensive necrosis

with complete destruction of the glandular epithelial structure. The small intestines had severe epithelial and cryptal necrosis with complete sloughing off of villi. Severe congestion and multi-focal haemorrhage were observed in the lamina propria in some areas. Severe lymphocytolysis was evident in the Peyer's patches especially in the ileum with numerous nuclear debris and very few remaining intact lymphocytes. In the large intestine extensive epithelial and glandular necrosis was observed in many areas. Extensive eosinophilic masses of necrotic coagula were common in the ileocaecal valve. Severe multifocal to extensive haemorrhage was evident in many areas of the gastro-intestinal tract.

There was severe depopulation of lymphoid follicles in lymph nodes removing the usual demarcation between the cortex and medulla and making stromal tissue more prominent (Fig.4.27g). Remnants of lymphoid follicles, where present, were very small and comprised mainly of nuclear debris (Figs.4.27h). Severe congestion and multi-focal haemorrhage were common in the lymphoid tissues.

The other warthog (TWX1) that was parenterally inoculated with the virulent Kabete 'O' strain of RPV had a few foci of intraepithelial necrosis in the stratum spinosum of the pharynx and oesophagus. Extensive epithelial and glandular necrosis was observed in the stomach, characterized by extensive epithelial sloughing and the presence of debris of nuclei in densely eosinophilic coagula. Severe hyperemia, oedema and multifocal haemorrhage were evident in the mucosa and sub-mucosa of the stomach accompanied by infiltration with many plasma cells and macrophages some of which had engulfed

nuclear debris. Extensive epithelial and cryptal necrosis was evident in the small intestines appearing as diffusely scattered debris of nuclei in a matrix of oedema and densely eosinophilic necrotic coagula. The mucosa of the small intestine was infiltrated with several macrophages and plasma cells and a moderate number of neutrophils. Severe hyperemia, extensive haemorrhage and oedema were evident in the mucosa and submucosa of the small intestine. There was marked sloughing off of sub-mucosal glandular epithelium into the lumen in many areas. The sloughed off cells were rounded and had pyknotic or karyorrhectic nuclei or no nuclei. Extensive severe lymphocytolysis was observed in the Peyer's patches and in the diffuse lymphoid tissues of the lamina propria of the intestines. Multi- nucleated giant cells were evident in some Peyer's patches with complete sloughing of the mucosa overlying the latter in some areas. The changes in the large intestine were similar to those in the small intestine comprising mostly of extensive necrosis accompanied by haemorrhage and oedema.

Severe lymphocytolysis with depopulation of lymphoid follicles was evident in the lymph nodes. The usual demarcation between the cortex and medulla was lost resulting in increased prominence of the stromal matrix of lymph nodes and the red pulp of the spleen. Lymphoid follicles were destroyed resulting in abundant nuclei debris and multinucleated syncytia in some areas. Severe hyperemia was present in lymphoid tissues accompanied with infiltration of plasma cells, macrophages and oedema fluid into the peri-trabecular sinuses.

4.4.2.4 Histopathological lesions in the uninfected control warthog

The uninfected control warthog (TW11) had moderate congestion of the duodenum, and mild congestion of the stomach, jejunum, ileum, ileo-caecal valve, prescapular lymph node and liver. No other lesions were observed in this animal.

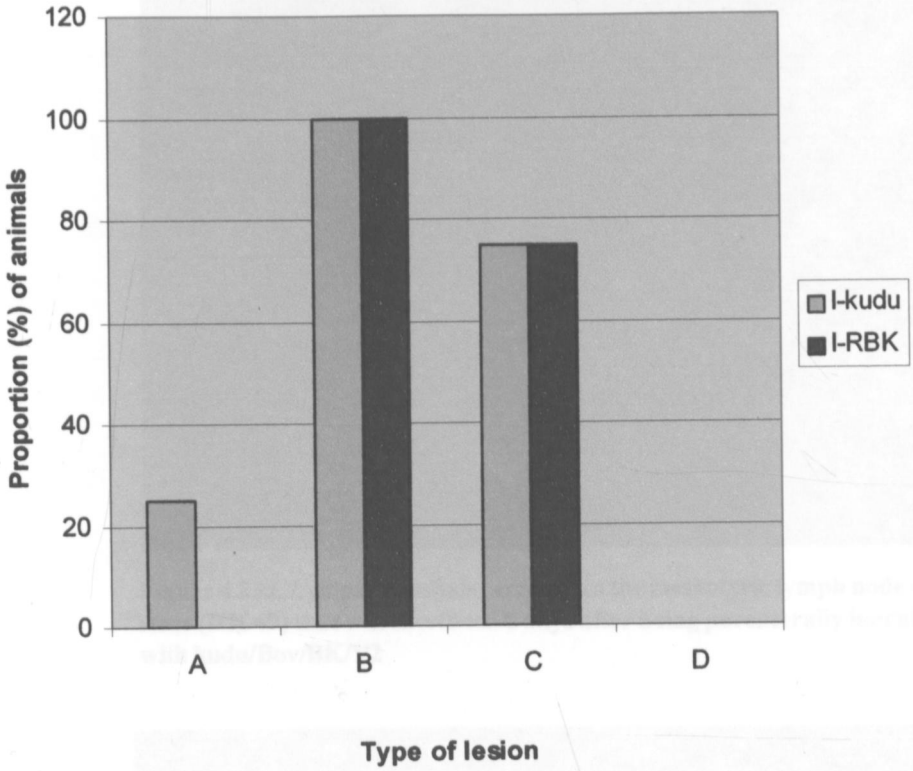


Figure 4.24. Proportion of animals with various types of lesions by strain of virus in cattle that were inoculated with kudu/Bov/BK/V2 or RBK/WP/86/1

Key

A-Focal necrosis in stratified squamous epithelium **B**-Congestion, haemorrhage or necrosis in the stomach and all intestinal tract **C**-Congestion, haemorrhage or necrosis in lymphoid organs **D**-Congestion in major internal organs **I-kudu**-Animals inoculated with kudu/Bov/B2/V2 **I-RBK**-Cattle inoculated with RBK/WP/86/1

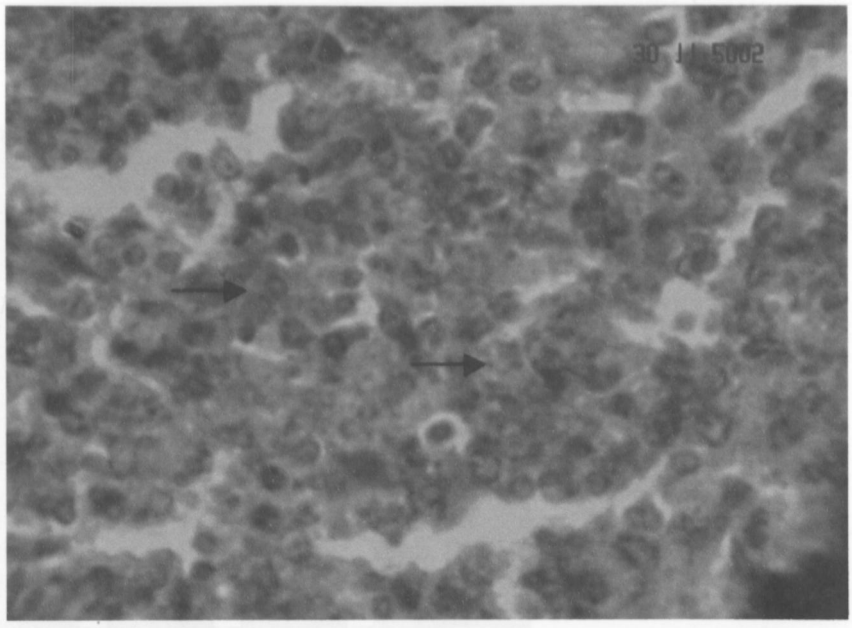


Figure 4.25a. Lymphocytolysis (arrows) in the mesenteric lymph node of a zebu steer (HK 49) that was sacrificed 8 days after being parenterally inoculated with kudu/Bov/BK/V2

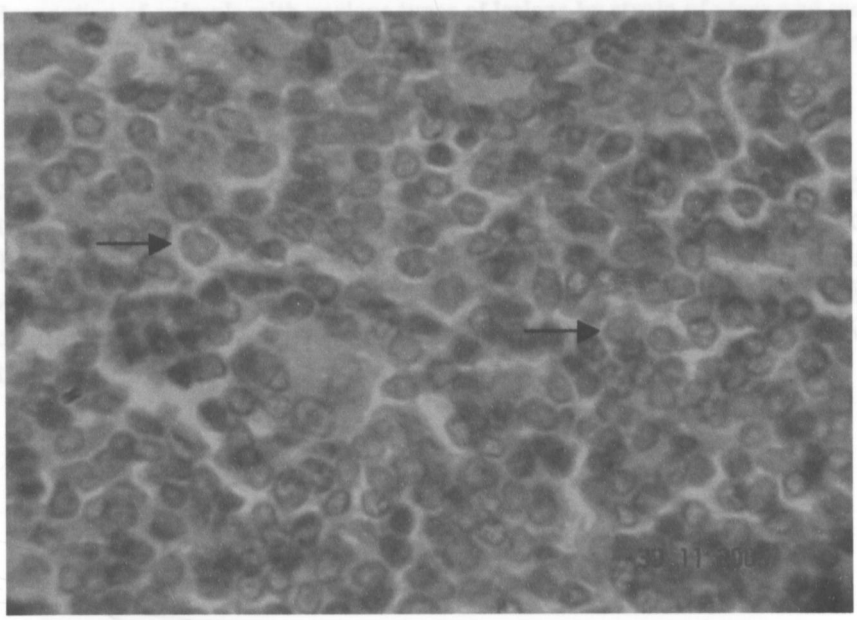


Figure 4.25b. Normal lymphocytes (arrows) in a more intact area of the mesenteric lymph node of zebu steer HK 49 that was sacrificed 8 days after being parenterally inoculated with kudu/Bov/BK/V2

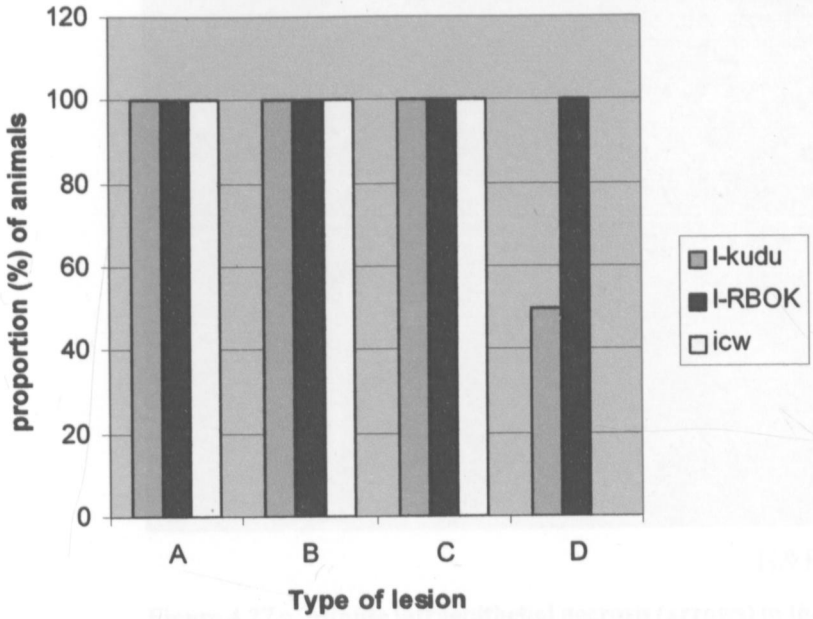
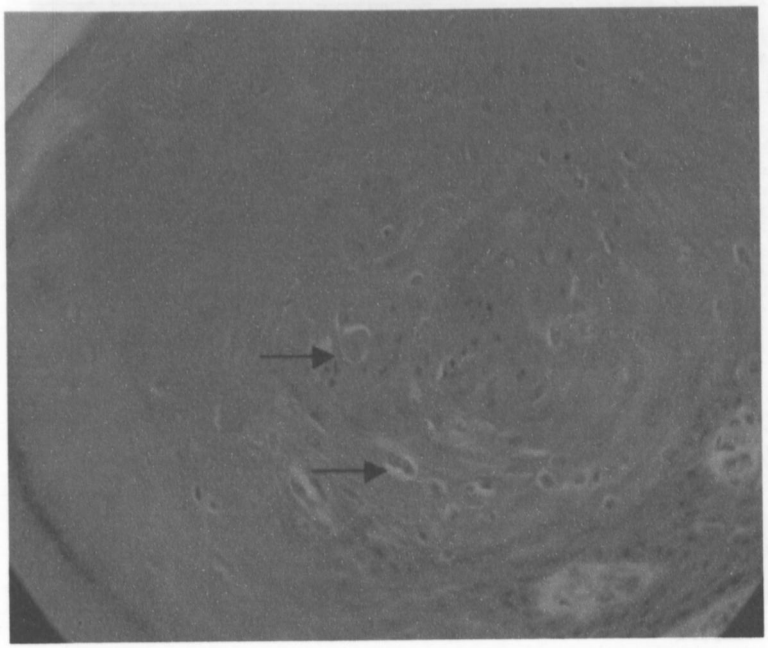


Figure 4.26. Proportion of animals with various types of lesions by strain of virus and mode of infection in warthogs that were challenged with kudu/bov/bk/v2 and the virulent Kabete 'O' strain of RPV

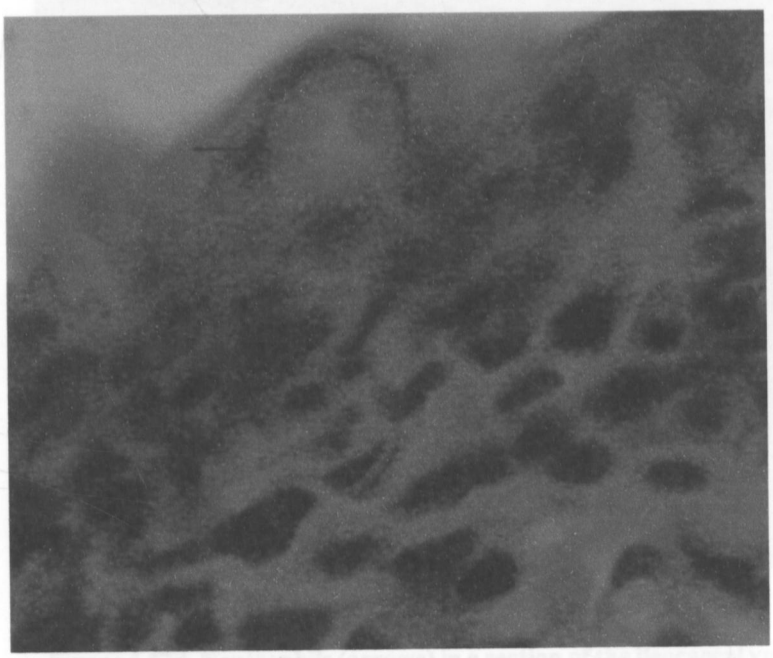
Key

A-Focal necrosis in stratified squamous epithelium **B**-Congestion, haemorrhage or necrosis in the stomach and all intestinal tract **C**-Congestion, haemorrhage or necrosis in lymphoid organs **D**-Congestion in major internal organs **I-kudu**-Animals inoculated with kudu/Bov/B2/V2 **I-RBK**-Cattle inoculated with RBK/WP/86/1 **I-RBOK**-Warthogs inoculated with the virulent Kabete 'O' strain of RPV **icw**-Warthogs kept with other warthogs inoculated with kudu/Bov/B2/V2



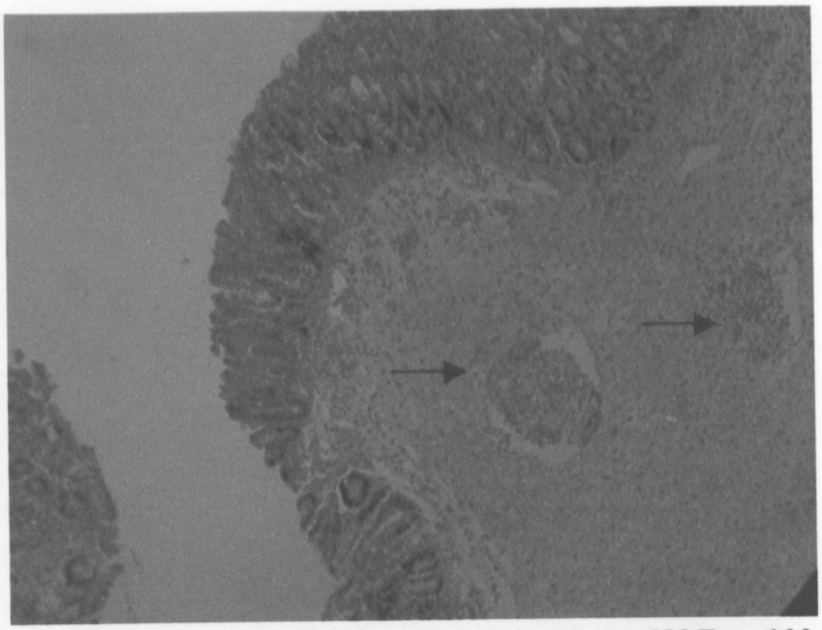
H&E x 200

Figure 4.27 a. Minute intraepithelial necrosis (arrows) in the lip of a warthog (TW33) that was sacrificed eight days after being inoculated with kudu/Bov/BK/V2



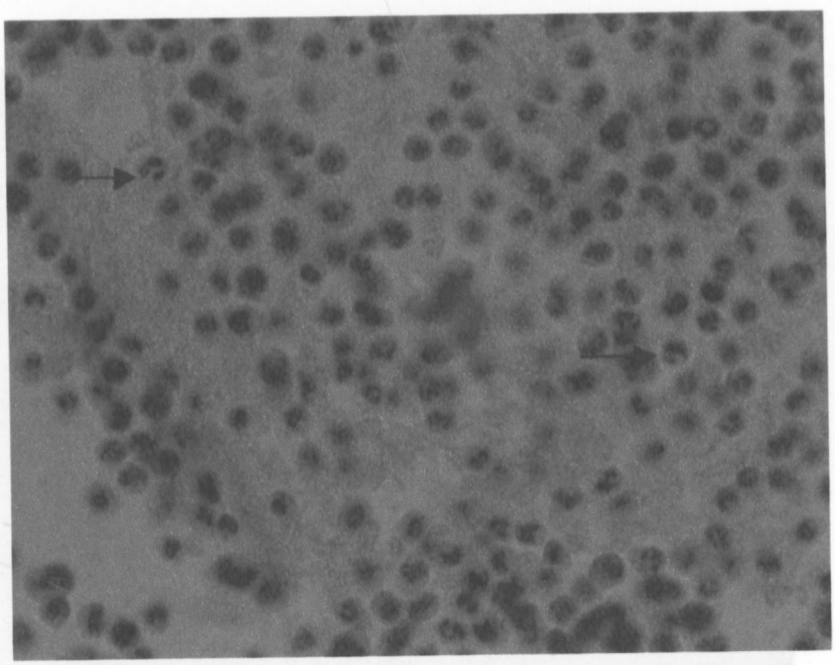
H&E x 400

Figure 4.27 b. Ballooning degeneration (arrow) in the epithelium of the lip of warthog TWY that was sacrificed 9 days after being inoculated with kudu/Bov/BK/V2



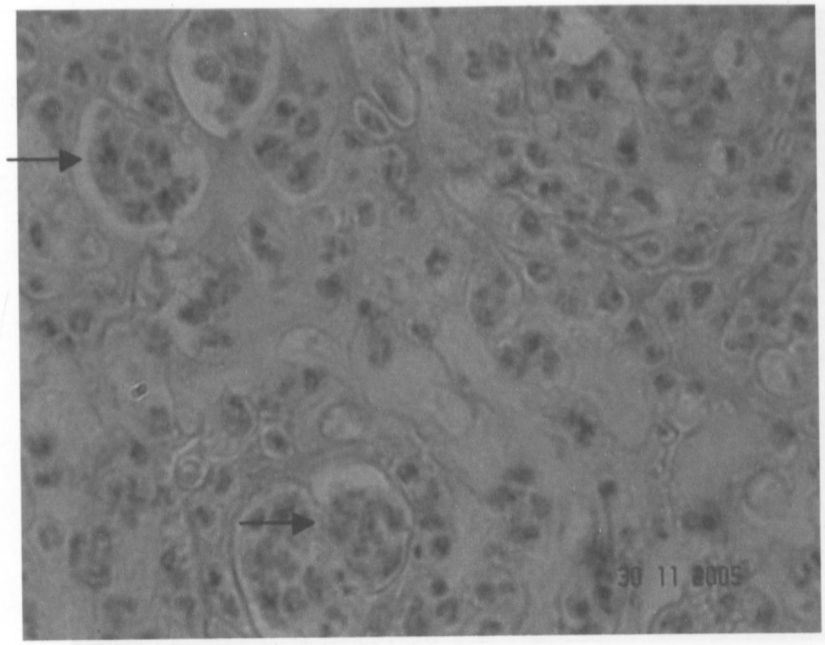
H&E x 100

Figure 4.27 c. Focal infiltration of neutrophils (arrows) in a section of the ileocaecal valve of a warthog (TW33) that was sacrificed 8 days after being inoculated with kudu/Bov/BK/V2



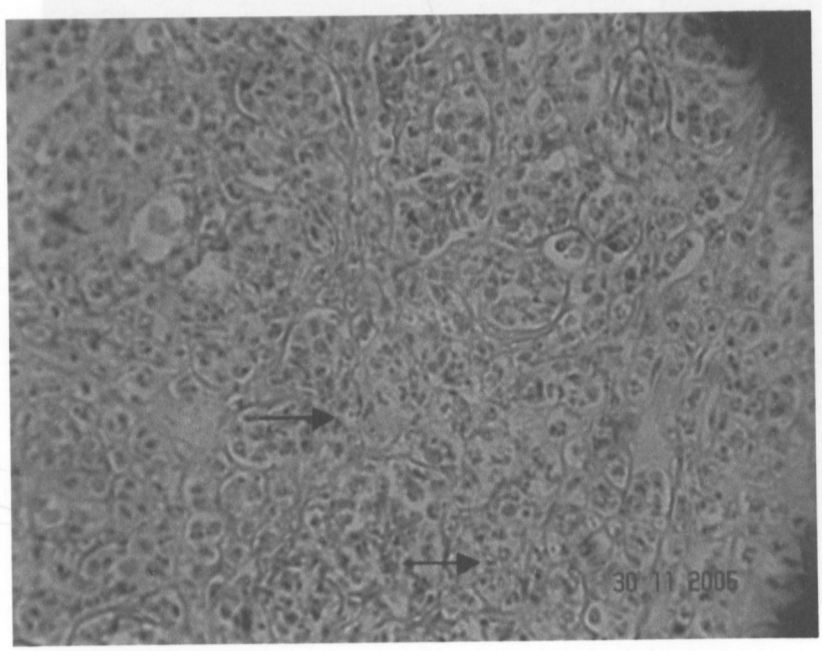
H&Ex 400

Figure 4.27d. Neutrophils (arrows) in a section of the ileocaecal valve of warthog TW33 (Fig.4.27 c.) under higher magnification



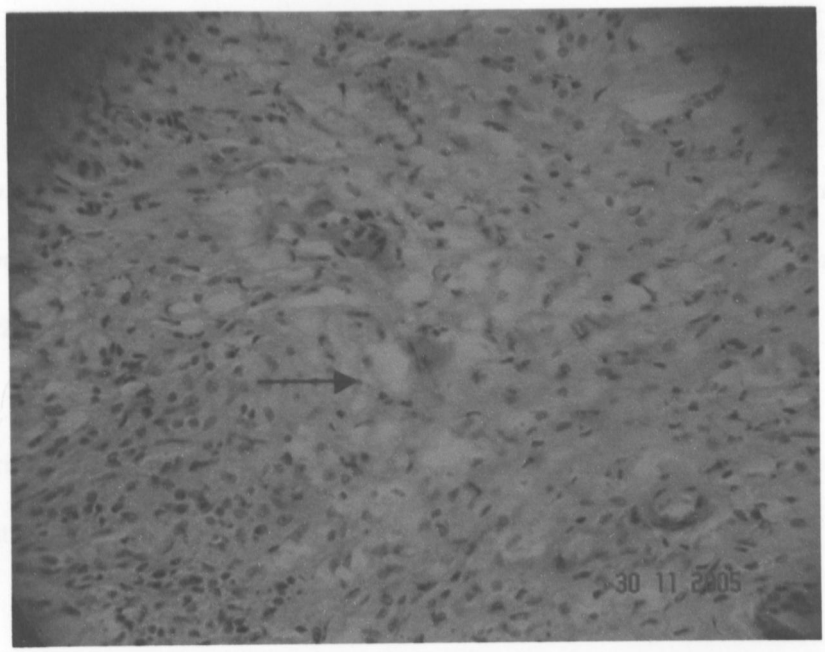
H&E x 400

Figure 4.27e. Several multinucleated syncytia (arrows) in the epithelium of a warthog (TWY), 22 days after being kept in contact with other warthogs that had been inoculated with kudu/BK/V2



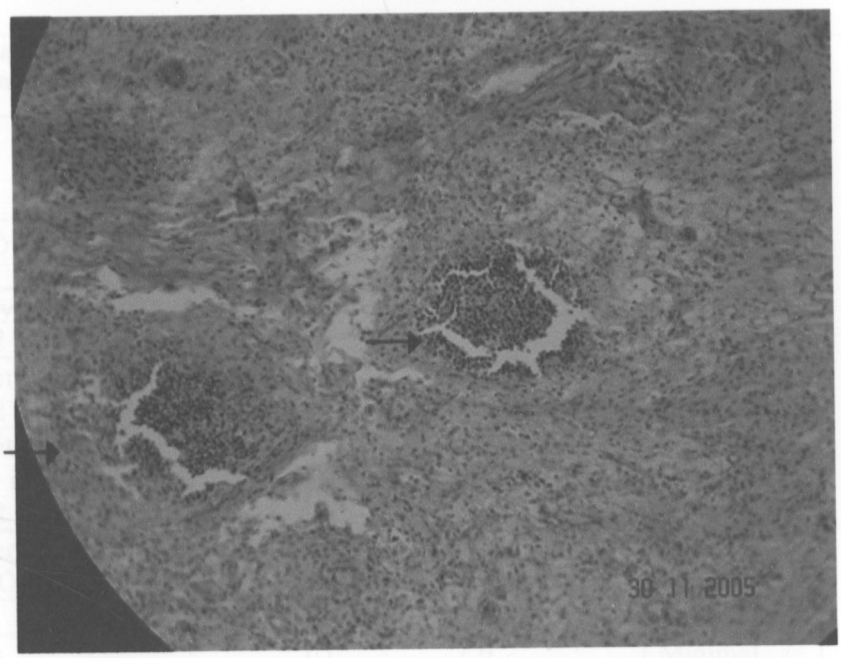
H&E x 400

Figure 4.27f. Extensive intraepithelial necrosis (arrows) in the epithelium of warthog TWY, 22 days after being kept in contact with other warthogs that had been inoculated with kudu/Bov/BK/V2



H&E x 400

Figure 4.27g. Severe lymphoid depopulation with prominence of stromal tissue (arrow) in the prescapular lymph node of a warthog (TWA), 4 days after being inoculated with the virulent Kabete 'O' strain of RPV



H&E x 200

Figure 4.27h. Severe reduction in size of lymphoid follicles (arrows) in the prescapular lymph node of warthog TWA, 4 days after inoculation with the virulent Kabete 'O' strain of RPV

4.4.3. Comparisons between the severity of histological lesions in zebu cattle experimentally induced by kudu/Bov/BK/V2 with those in warthogs experimentally induced by kudu/Bov/BK/V2 and the virulent Kabete 'O' isolates of RPV

The relative severity of histopathological lesions in cattle and warthogs that were infected with various isolates of RPV is illustrated in Table 4.10 and Figures 4.28 and 4.29.

4.4.4. Summary of histopathological reaction

The overall histopathological reaction by strain of virus and mode of challenge in the various groups of animals is summarized in table 4.11.

Table 4.11 A summary of histopathological lesions in cattle and warthogs by isolate of rinderpest virus and mode of infection

Group	Number of animals	Proportion of reactors	Overall severity of lesions
Cattle parenterally inoculated with RBK/WP/86/1	4	4/4	Mild
Cattle parenterally inoculated with kudu/Bov/BK/V2	4	3/4	Mild
Warthogs parenterally inoculated with kudu/Bov/BK/V2	4	4/4	Mild
Warthogs kept with other warthogs that were parenterally inoculated with kudu/Bov/BK/V2	1	1/1	Moderate
Warthogs parenterally inoculated with virulent Kabete 'O' strain of RPV	2	2/2	Severe
Control cattle	1	0	Minimal
Control warthogs	1	0	Minimal

Table 4.10 a. Mean scores of severity of histopathological lesions by tissue type in cattle and warthogs that were infected with three isolates of RPV.

Tissue Type	Cattle			Warthogs			
	I-kudu	C	I-WP	I-kudu	icw	C	I-RBOK
A	0.75	5	0.00	6.25	28	0	23.50
B	11.00	0	10.75	15.25	2	6	102.00
C	1.75	0	2.25	12.50	16	1	42.50
D	0.00	0	0.00	2.25	0	1	5.00
Overall mean score	**13.50**	**5**	**13.00**	**36.25**	**46**	**8**	**173.00**

Scoring criterion

Each organ tissue examined was scored for the severity of congestion, haemorrhage or oedema (general vascular responses) on a scale of 1-4 and for severity of necrosis on a scale of 2-8. The same organ tissues were examined in all the animals for comparison. The scores of all the organ tissues of each type were summed to obtain the cumulative score of severity of that tissue type in the animal. The mean cumulative score of each tissue type in each group was computed by summing up the cumulative scores of all animals in the group and dividing the sum by the number of animals in the group. Mean score of severity of histopathological lesions for each group of animals was obtained by summing the mean scores of all the tissue types (Appendix 3).

Scoring scale

Vascular responses:

1 - Mild congestion; **2** - Moderate congestion; **3** - Marked congestion; **4**-Severe congestion

Necrosis with or without cellular inflammatory response:

2 -A few minute foci of necrosis; **4** - A few small- moderate size foci of necrosis; **6** - Large foci of necrosis with or without cellular inflammatory response; **8** - Extensive necrosis with or without cellular inflammatory response

Key

Tissue types

A-Stratified squamous epithelium **B**-Stomach and intestinal tract **C**-Lymphoid organs **D**-Major internal organs

Animal groups

I-kudu-animals inoculated parenterally with kudu/Bov/BK/V2 isolated of RPV; **I-WP**-Animals inoculated with RBK/WP/86/1 isolate of RPV; **I-RBOK**-Animals inoculated parenterally with the virulent Kabete 'O' strain of RPV; **C**-Uninfected controls; **icw**-One warthog in contact with cattle that had been inoculated parenterally with kudu/Bov/BK/V2

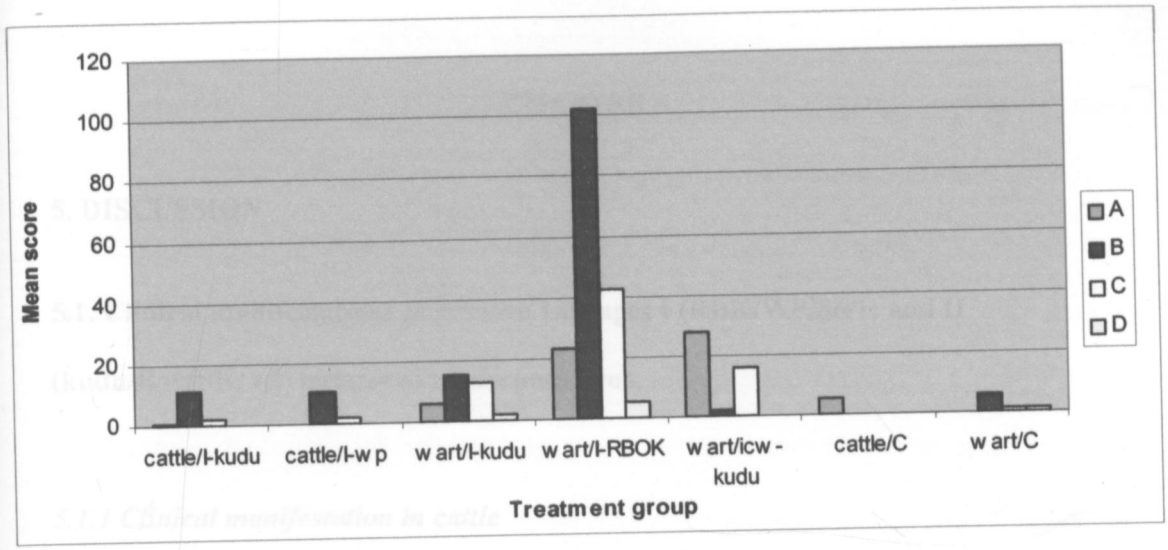


Figure 4.28. Mean score of severity of histopathological lesions by tissue type,strain of virus and mode of infection in warthogs and cattle that were infected with various isolates of RPV and the uninfected controls.

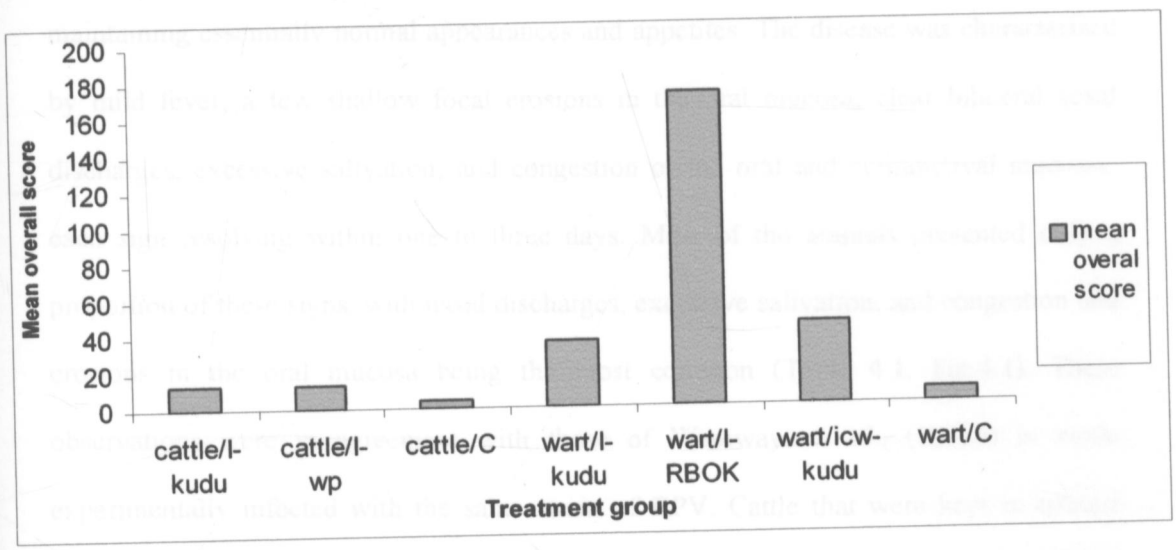


Figure 4.29. Mean overall score of severity of histopathological lesions by strain of virus and mode of infection in warthogs and cattle that were challenged with various strains of RPV and the uninfected controls.

Key:
A-Stratified squamous epithelium **B**-Stomach and all intestinal tract **C**-Lymphoid organs **D**-Major internal organs **Cattle/I-kudu**-Cattle inoculated with kudu/Bov/B2/V2 **Cattle/I-wp**-Cattle inoculated with RBK/WP/86/1 **wart/I-kudu**-warthogs inoculated with kudu/Bov/B2/V2 **Wart/I-RBOK**-Warthog inoculated with the virulent Kabete 'O' strain of RPV **wart/icw-kudu**-warthog in contact with other warthogs inoculated with kudu/Bov/B2/V2 **Cattle/ C**-uninfected control cattle **wart/C**-uninfected control warthogs

CHAPTER 5

5. DISCUSSION

5.1. Clinical manifestations of African Lineages I (RBK/WP/86/1) and II (kudu/Bov/BK/V2) isolates of rinderpest virus.

5.1.1 Clinical manifestation in cattle

The wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of RPV produced very mild disease in both the parenterally inoculated and the in-contact cattle with the animals maintaining essentially normal appearances and appetites. The disease was characterized by mild fever, a few shallow focal erosions in the oral mucosa, clear bilateral nasal discharges, excessive salivation, and congestion of the oral and conjunctival mucosae, each sign resolving within one to three days. Most of the animals presented only a proportion of these signs, with nasal discharges, excessive salivation, and congestion and erosions in the oral mucosa being the most common (Table 4.1, Fig.4.1). These observations were in agreement with those of Wamwayi *et al.*, (1995b) in cattle experimentally infected with the same strain of RPV. Cattle that were kept in contact with warthogs inoculated with the wildlife-derived African lineage II isolate of RPV developed mild clinical disease by day 9 characterized by clear bilateral oculonasal discharges, congestion of the oral mucosa, and a few small shallow erosions in the latter, indicating that infected warthogs are capable of transmitting the virus to cattle.

The cattle-derived African lineage I (RBK/WP/86/1) isolate of RPV induced mild signs of disease in both the parenterally inoculated and in-contact cattle characterized by mild fever, excessive salivation, congestion of ocular and oral mucosae and a few shallow focal erosions in the latter. Some of the clinical signs were present in only a proportion of the cattle, most occurring in the parenterally inoculated group (Table 4.1, Fig.4.1). These observations were consistent with those made by Wamwayi (1993) in experimentally infected Boran cattle using the same strain of virus. Wamwayi (1993) reported one death in Boran cattle experimentally infected with the RBK/WP/86/1 isolate of RPV, which was associated with respiratory signs but this was not observed in the current study.

5.1.2 Clinical manifestation in warthogs

The wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of RPV induced mild clinical disease in two out of the four parenterally inoculated warthogs within nine days of examination indicating that warthogs are susceptible to the virus. The disease was mostly characterized by congestion of the ocular and oral mucosae, cyanosis of the lightly pigmented parts of the skin, and dullness. These signs were present in only a proportion of the animals (Table 4.2, Fig.4.5) Warthog-to-warthog transmission of the virus resulted in moderately severe disease characterized clinically by fever, diarrhoea, marked dullness, marked erosive stomatitis, joint inflammation, and cutaneous eruptions. On the other hand, cattle-to-warthog transmission of the virus resulted in very mild clinical disease characterized by transient ocular congestion and lacrimation. These observations suggest that the wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of RPV does not induce severe disease when transmitted from cattle to warthogs

but it induces a moderately severe disease when transmitted from warthog to warthog. This may partly explain why warthogs were not affected during an epidemic of rinderpest in wildlife in Tsavo National Park (Kock *et al.*, 1999) where the isolate was recovered from the tissues of an infected kudu (Wamwayi, 1995a). Similarly, Robson *et al.*, (1959) reported an eland-derived isolate of RPV that did not infect pigs under experimental conditions. These observations support the suggestion by Plowright (1963) that changes in pathogenic phenotype of the RPV can occur on passage through different animal species, but the molecular factors responsible for such changes are yet to be determined (Barret *et al.*, 1998). The erosive stomatitis and vesicular cutaneous eruptions observed in the warthogs are consistent with the observations reported in domestic pigs during natural outbreaks of rinderpest (Anderson *et al.*, 1996). The joint inflammation observed in one warthog was similar to the observations made in lesser kudu during an epidemic of rinderpest in wildlife in Tsavo National Park (Kock *et al.*, 1999).

The two warthogs that were parenterally inoculated with the virulent Kabete 'O' strain of RPV developed a severe fatal disease characterised by oculonasal discharges, extreme weakness, and diarrhoea, beginning on day 2 and terminating fatally by day 5. This is consistent with historical records that state that warthogs are among the most highly susceptible wildlife species to rinderpest (Andeson *et al.*, 1996). These findings also suggest that the virulence of the Kabete 'O' strain that has been consistently replicated in experimental cattle (Plowright and Ferris 1959b, Wafula and Kariuki, 1987) is readily replicated in warthogs. In outbreaks of rinderpest in wild animals in Nigeria in 1983, Shanthikumar and Atiola (1990) reported that 20 warthog carcasses were recovered. This

was the species with the second largest number of recovered carcasses, underscoring its high susceptibility to rinderpest. These observations suggest that the severity of rinderpest in warthogs is partly dependent on the strain of virus. One of the warthogs inoculated with the virulent Kabete 'O' strain of RPV developed hypothermia on day 5 (Fig 4.6). This could have been as a result of hypovolemic shock caused by severe dehydration, which was evidenced by sunken eyeballs (Fig 4.7d) and haemoconcentration (Table 4.5).

5.1.3 Clinical manifestations in buffaloes

The four buffaloes that were kept in contact with two cattle inoculated with African lineage I (RBK/WP/86/1) isolate of RPV showed very mild signs of disease characterized by congested conjunctival mucosae, and a few shallow focal erosions in the lips or gums. Rectal congestion was observed in two of the buffaloes before the beginning of the experiment possibly reflecting stress-related lower gut infections due to causes that were not specified in this study. This showed that rectal congestion due to non-specific causes should always be considered when examining captive buffaloes for experimentally induced rinderpest signs. Wamwayi *et al.*, (2002) reported severe fatal disease in buffalo kept with cattle that had been inoculated with the wildlife-derived African lineage II isolate of RPV. The results from the current study suggest that the clinical manifestation of rinderpest in buffaloes is dependent on the strain of virus and that strains that cause severe disease in cattle may not behave similarly in buffaloes and vice versa.

5.2 Haematological Values

5.2.1 Haematological values in cattle

The mean red blood cell count (rbc) in the group of zebu cattle inoculated with the wildlife-derived African lineage II (kudu/Bov/Bk/V2) isolate of RPV decreased steadily between days 0 and 7 post-inoculation but remained within the normal range over the experimental period (Fig.4.8) The packed cell volume (pcv) and haemoglobin concentration (Hb) for the group similarly remained normal. The relatively higher rbc counts on the first day following infection could have been due to the initial handling fright in the cattle causing adrenalin related release of rbcs from the spleen into circulation. The subsequent decrease might be explained by adaptation of the cattle to handling hence less adrenalin related circulatory changes (Schalm, 1986). High mean white blood cell counts (wbc) and high mean differential lymphocyte counts were recorded in the group on days 6 and 7 respectively possibly reflecting initial lymphocytic leucocytosis in response to the virus in agreement with observations reported by others (Robey and Hale, 1946). Daily variations in haematological parameters in individual inoculated cattle reflected transient lymphocytic leucocytosis in the early stages followed by lymphopaenic leucopaenia, consistent with earlier observations (Robey and Hale 1946). Relative lymphocytosis with normal leucocyte counts was recorded in two out of the four animals possibly reflecting an intermediate stage between lymphocytic leucocytosis and lymphopaenic leucopaenia.

One of the two zebu cattle that were kept with the four cattle parenterally inoculated with the wildlife-derived African lineage II isolate of RPV had a transient lymphocytic leucocytosis on day 10. The other had a leucopaenia on day 11 preceded by relative lymphocytosis on day 10. These observations might be explained as mild responses to the wildlife-derived African lineage II isolate of RPV virus transmitted from the in-contact cattle.

The four cattle in contact with warthogs that were inoculated with the wildlife-derived African lineage II isolate of RPV had sporadic leucocytosis from day 0 to day 15 but there was no leucopaenia. The sporadic leucocytosis was difficult to interpret but could have been stress-related due to disturbance of the cattle by the in-contact warthogs. More marked leucocytosis of above 22.0 x 10^3 cells/µl was recorded in three of the four cattle on day 14 preceded by relative lymphocytosis of above 75% in two of them. This was possibly induced by Rinderpest virus contracted from the infected warthogs. Sporadic leucocytosis and relative lymphocytosis were also observed in the two negative control zebu cattle that were kept in contact with the two negative control warthogs.

5.2.2 Haematological values in warthogs

The mean rbc count in the group of warthogs inoculated with the wildlife-derived African lineage II isolate of RPV remained normal over the entire experimental period whereas the mean wbc rose to a high value of 14.5 x 10^6 cells/µl on day 6, dropping to leucopaenic levels of 7 x 10^6 cells/µl and 7.8 x 10^6 cells/µl on days 7 and 8 respectively. Mean differential lymphocyte count for the group dropped to a low of 23% on day 6 post-

inoculation but was within the normal range for the rest of the experimental period. Variations in blood parameters in individual animals reflected leucopaenia and/or lymphopaenia in three out of the four animals from day 5 to day 9. These observations can be explained as responses to the rinderpest virus, consistent with earlier observations by others (Robey and Hale, 1946).

The mean blood values in the group of four warthogs that were kept in-contact with zebu cattle inoculated with the wildlife-derived African lineage II isolate of RPV remained normal although variations in individual animals showed leucopaenia with relative lymphocytosis in two out of four animals on days 9 and 11, respectively, and relative lymphocytosis with normal leucocyte counts in all warthogs between days 9 and 15 post-contact. These variations could be explained by responses to the wildlife-derived African lineage II isolate of RPV transmitted from the in-contact cattle.

The mean blood values in the two warthogs kept together with four others that were parenterally inoculated with the wildlife-derived African lineage II isolate of RPV reflected leucocytosis with neutrophilia on day 22 post-contact, and relative lymphopaenia on day 15. Individual variations showed neutrophilic leucocytosis in both warthogs on day 22. These observations could be explained as responses in the late stages to rinderpest virus transmitted from the in-contact warthogs. Robey and Hale (1946) also reported neutrophilic leucocytosis at late stages of the disease in animals experimentally infected with RPV.

The mean rbc and wbc in the two warthogs inoculated with the virulent Kabete 'O' strain of RPV remained normal but individual variations showed leucopaenia in both accompanied by normal differential count in one warthog and relative lymphocytosis in the other (Fig.4.16). In one of the warthogs the leucopaenia was preceded by leucocytosis and was accompanied by high rbc pcv and Hb indicative of haemoconcentration (Fig.4.14, Table 4.5). These observations could be explained as response of the warthogs to the virulent Kabete 'O' strain of RPV. The relative lymphocytosis accompanying leucopaenia was atypical since lymphopaenic leucopaenia is normally expected in rinderpest virus infections (Robey & Hale, 1946; Plowright, 1968). This may have been due to an acute high neutrophil demand in tissues undergoing necrosis leading to a sharp reduction of neutrophils from circulation thus resulting in the relative lymphocytosis.

The two negative control warthogs maintained normal blood values except for relative lymphocytosis recorded in one of them on day 5, possibly due to capture stress.

5.3 Gross Post Mortem Lesions

5.3.1 Gross post mortem lesions in cattle

All the four zebu cattle parenterally inoculated with the wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of RPV and all the four Boran cattle inoculated with the cattle-derived African lineage I (RBK/WP/86/1) isolate of RPV had mild enteric lesions comprising of varying degrees of congestion in the mucosa of the small and large intestines, and enlargement of various lymph nodes and Peyer's patches. These observations are consistent with those made by others in cattle experimentally infected

with rinderpest virus isolates that induce mild clinical disease (Wamwayi 1993, Wamwayi *et al.*, 2002). The gross post mortem lesions in both groups of cattle were more marked in the ileum, ileocaecal valve and caeco-colic junction, consistent with observations reported by others (Anderson *et al.*, 1996).

5.3.2 Gross post mortem lesions in warthogs

Warthogs inoculated with the wildlife-derived African lineage II isolate of RPV had mild gross post mortem lesions characterised by varying degrees of congestion of the gastrointestinal mucosa, enlargement and congestion of prescapular and mesenteric lymph nodes and cyanosis of the lightly pigmented skin of the abdomen and inner aspects of the legs. The lesions observed in this group were quite mild compared to those described by Anderson *et al.*, (1996) in domestic pigs infected with other strains of RPV. One of the four warthogs that was found dead on day 4 had marked autolysis of a portion of the proximal ileum, with bloody ingesta in the lumen and extreme engorgement of associated mesenteric blood vessels, consistent with intestinal strangulation. Mild to moderate congestion of the gastrointestinal mucosa, particularly in the duodenum, was observed in the uninfected control warthog and the in-contact warthogs euthanised after convalescence. This observation suggested that gastroenteric congestion induced by the wildlife-derived African lineage II isolate of RPV in warthogs can be masked by or confused with non-specific, possibly stress related congestion.

Characteristic lesions comprising of erosive stomatitis, severe ulcerative gastroenteritis, lymphadenitis, congested urinogenital tract and kidneys, tracheal haemorrhage and

pulmonary congestion were seen in warthogs inoculated with virulent Kabete 'O' strain of RPV on days 4 and 5 post-inoculation. These observations are consistent with those reported by Anderson and others (1996) in domestic pigs infected with rinderpest virus.

5.4 Histopathological Lesions

5.4.1 Histopathological lesions in cattle

All zebu cattle parenterally inoculated with the wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of RPV had varying degrees of enteric congestion and oedema corresponding in severity to the gross lesions observed. Focal lymphocytolysis was seen occasionally in lymph nodes and gut associated lymphoid tissues accompanied by infiltration with neutrophils and rare intracytoplasmic inclusions. Occasional intraepithelial necrosis in the stratified squamous epithelium accompanied with infiltration of neutrophils into the underlying lamina propria was also observed in the inoculated cattle. These observations were comparable to those described by Wohlsein and others (1995) in tissues from cattle infected with mild rinderpest virus isolates. Tissues from the four Boran cattle inoculated with cattle-derived African lineage I (RBK/WP/86/1) isolate of RPV showed non-characteristic mild lesions comprising marked degrees of enteric congestion occasionally accompanied by infiltration with neutrophils or abundance of plasma cells, and varying degrees of congestion and oedema in the lymphoid tissues. These lesions were milder but consistent with those described by Wohlsein and others (1995) in tissues from cattle inoculated with the same virus.

5.4.2 Histopathological lesions in warthogs

Warthogs inoculated with wildlife-derived African lineage II isolate of RPV had mild histologic lesions. These comprised of multifocal intraepithelial necrosis, single cell necrosis, and hydropic to ballooning degeneration in the epithelium of the upper digestive tract. In addition, there was a low degree of lymphocytolysis in lymph nodes and gut associated lymphoid tissues with formation of syncytia and occasional eosinophilic intracytoplasmic inclusions. These lesions were similar to those reported by Wohsein *et al.*, (1995) in tissues from cattle infected with the mild isolates of rinderpest virus. One of the two warthogs that were kept together with those inoculated with the wildlife-derived African lineage II isolate of RPV had similar lesions but with a more marked erosive stomatitis.

Warthogs inoculated with the virulent Kabete 'O' strain of RPV had severe histologic lesions comprising of extensive necrosis of the gastroenteric mucosa with complete destruction of glandular structures and associated lymphoid tissues, severe lymphocytolysis in all lymphatic tissue, intraepithelial necrosis in the upper digestive tract, and formation of syncytia and eosinophilic intracytoplasmic inclusions. These observations were consistent with those made by others in tissues from cattle inoculated with the virulent strains of rinderpest virus (Gathumbi, 1988, Wohlsein *et al.*, 1995).

CHAPTER 6

6. CONCLUSIONS

The clinical, haematological, gross and histopathological findings from this study suggest the following:

1) Warthogs are susceptible to infection with the wildlife-derived African lineage II (kudu/Bov/BK/V2) isolate of rinderpest virus and may develop mild clinical disease.

2) Warthogs are susceptible to infection with the virulent Kabete 'O' strain of rinderpest virus and develop severe acute clinical disease that terminates fatally.

3) Warthogs infected with the kudu-derived African lineage II (kudu/Bov/BK/V2) isolate of rinderpest virus can transmit the virus to other in-contact warthogs resulting in moderately marked clinical disease that can be demonstrated by gross pathology, histopathology and in some cases by haematology

4) Warthogs infected with the kudu-derived African lineage II (kudu/Bov/BK/V2) isolate of rinderpest virus can transmit the virus to cattle resulting in a mild self-limiting clinical disease.

5) Cattle inoculated with the kudu-derived African lineage II (kudu/Bov/BK/V2) isolate of rinderpest virus develop mild clinical disease that can be demonstrated by gross pathology, histopathology and haematology.

6) Cattle infected with the kudu-derived African lineage II (kudu/Bov/BK/V2) isolate of rinderpest virus can transmit the virus to other cattle resulting in mild clinical disease that can be demonstrated by haematology.

7) Cattle infected with the kudu-derived African lineage II (kudu/Bov/BK/V2) isolate of rinderpest virus can transmit the virus to in-contact warthogs resulting in mild self-limiting clinical disease that can be demonstrated by haematology.

8) Buffaloes in contact with cattle parenterally inoculated with the cattle-derived African lineage (RBK/WP/86/1) I strain of rinderpest virus do not develop remarkable clinical signs of rinderpest.

The results from this study suggest that wildlife plays an important role in the epidemiology of rinderpest but the dynamics involved in the maintenance and persistence of the disease are yet to be clarified.

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8.APPENDICES

Appendix 1: Table 4.8. Distribution of histopathological lesions in 4 cattle (HK10, HK45, HK49, HK69) that were inoculated with kudu/Bov/BK/V2, four (550K, 566K, 569K, 558K) that were inoculated with RBK/WP/86/1, and one control (HK62)

Appendix 2: Table 4.9. Distribution of histopathological lesions in five warthogs (TW10, TW13, TW33, TWY, TW1) that were infected with kudu/Bov/BK/V2, two (TWA, TWX1) that were challenged with the virulent Kabete 'O' strain of RPV and one negative control (TW11)

Appendix 3: Table 4.10 Mean score of severity of histopathological lesions by tissue type in cattle and warthogs that were challenged with various isolates of RPV.

Table 4.12. Distribution of histopathological lesions in 4 cattle (HK10, HK45, HK49, HK69) that were inoculated with kudu/Bov/BK/V2, four (550K, 566K, 569K, 558K) that were inoculated with RBK/WP/86/1, and one uninfected control (HK62)

Tissue	HK45	HK10	HK49	HK69	HK62	550K	566K	569K	558K
Muzzle	-	-	-	-	-	-	-	-	-
Lips	-	-	-	-	-	-	-	n	-
Cheeks	-	-	-	-	-	-	-	-	-
Gingiva	-	-	-	-	-	-	-	-	-
Dental pad	-	-	-	-	-	-	-	-	-
Tongue	-	-	+	-	-	-	-	n	-
Hard palate	-	-	-	-	-	-	-	-	-
Soft palate	-	-	-	-	-	-	-	n	-
Pharynx	-	-	-	-	-	-	-	-	-
Oesophagus	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	*	-	-
Duodenum	*	*	** ++	-	-	*	***	*	**
Jejunum	*	*	**	*	-	**	*	-	**
Ileum	***	***	*** +++	*	-	***	**	**	**
Ileo-cecal valve	-	*	-	-	-	***	-	***	n
Caecocolic junction	n	*	*	-	-	***	-	**	**
Caecum	**	**	*	-	n	*	*	**	n
Colon	*	*+	*+	-	-	-	-	-	*
Rectum	-	-	-	n	n	-	-	***	-
Spleen	-	-	-	-	-	-	-	-	-
Prescapular lymph node	-	-	-	-	-	-	-	**	*
Messenteric lymph node	*	-	*+	-	-	***	-	n	-
Mandibular lymph node	-	-	-	-	-	-	-	***	-
Gall bladder	-	n	-	-	***	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-
Salivary gland	-	-	-	-	-	-	n	n	-
Adrenal glands	-	-	-	-	-	-	-	-	-
Eyelid	n	-	-	-	-	-	n	-	-
Third eyelid	-	-	-	-	-	-	-	n	-
Nasal mucosa	-	n	*	-	-	-	-	-	-
Trachea	-	-	-	-	-	-	-	n	-
Lung	n	-	-	-	-	-	-	n	-
Heart	-	-	-	-	-	-	-	-	-
Skin	-	-	-	-	-	-	n	-	-
Pancreas	-	-	-	-	-	n	-	-	-
Brain	-	-	-	-	-	-	-	-	-
Thyroid gland	-	-	-	-	-	-	-	-	-
Conjunctiva	-	-	-	-	-	-	n	-	-
Epiglottis	-	-	-	-	-	-	-	-	-
Skeletal muscle	-	-	-	-	-	-	-	n	n
Urinary bladder	-	-	-	-	**	n	-	n	-
Urethra	-	-	-	-	-	-	n	-	-
Uterus	-	-	N/A	N/A	-	-	N/A	n	-
Genital mucosa	-	-	N/A	N/A	-	-	N/A	-	-
Forestomachs	-	-	-	-	-	-	-	-	-

Key:

* - mild congestion

** - moderate congestion

*** - marked congestion

**** - severe congestion

+ - a few minute foci of necrosis

++ - a few small-moderate size foci of necrosis

+++ - Large foci of necrosis with/ without cellular inflammatory response

++++ - Extensive necrosis with/ without cellular inflammatory response

e 4.9. Distribution of histopathological lesions in five warthogs (TW10, TW13, TW33, TWY, TW1) that were challenged with kudu/Bov/Bk/V2, two (TWA, X1) challenged with the virulent Kabete 'O' strain of RPV, and one uninfected control (TW11).

sue	TW10	TW13	TW33	TWY	TW11	TW1	TWA	TWX1
ut	-	-	-	+	-	-	-	-
s	-	-	+	+	-	++	-	-
eks	-	-	-	+	-	-	-	+
giva	-	+	+	+	-	-	-	-
tal pad	-	n	n	n	-	++	-	-
ngue	-	-	-	+	-	+++	-	-
d palate	-	-	+	+	-	-	-	-
t palate	-	-	-	-	-	+++	-	-
rynx	n	-	+	-	-	+++	++++ ****	+
ophagus	-	-	+.	-	-	+	++	+
mach	***	-	*	**	*	*	++++	++++ ****
odenum	*	***	***	*	**	n	++++ ***	++++ ****
num	*	**	**	**	*	-	++++ ****	++++ ****
m	***	***	**	***	*	n	++++ ****	+++ ****
-cecal valve	**	*	**	-	*	-	++++ ****	+++ ***
cocolic junction	***	-	**	**	-	-	++++ ****	++++ ****
cum	**	-	*	*	-	-	++++ ****	++++ ****
on	**	***	**	*	-	-	++++ ****	+++ ***
ctum	**	*	*	*	-	*	++++ ****	++++ ***
een	-	***	-	-	-	*++	++++ ****	++++ ***
scapular lymph node	****	**+	***+	***+	*	*	++++ ****	++++ ***
ssenteric lymph node	****	****+	***+	n	-	***++	++++ ****	++++ ***
ndibular lymph node	****	***+	***+	***	-	*+	++++ ****	++++
l bladder	-	-	-	-	n	-	n	-
dney	**	**	-	-	-	-	**	-
ver	-	-	-	-	*	n	-	+++ **
ivary gland	-	-	-	-	-	-	-	-
renal glands	-	-	-	-	-	-	-	-
elid	-	-	-	-	-	-	-	-
ird eyelid	-	-	-	-	-	n	***	+
sal mucosa	-	-	-	-	-	-	-	-
achea	-	-	-	-	-	-	-	-
ng	***	**	-	-	-	-	-	-
art	-	-	-	-	-	-	-	-
in	-	-	-	-	-	-	-	-
ncreas	-	-	-	-	-	-	-	-
ain	-	-	-	-	-	-	-	-
yroid gland	-	-	-	-	-	-	-	-
njunctiva	-	-	-	-	-	-	*	++++
iglottis	*	-	-	-	-	-	-	-
eletal muscle	-	-	-	-	-	-	-	n
ethra	-	-	-	-	-	-	-	+
inary bladder	-	-	-	-	-	-	-	+
erus	-	-	-	N/A	n	N/A	-	-
nital mucosa	-	-	-	N/A	n	N/A	-	+

* - mild congestion, ** - moderate congestion, *** - marked congestion, ****- severe congestion, + -a few minute foci of necrosis, ++ - a few small- moderate foci of necrosis, +++ - Large foci of necrosis with/ without cellular inflammatory response, ++++ - Extensive necrosis with/ without cellular inflammatory response

8.3 APPENDIX 3

Table 4.10. Mean scores of severity of histopathological lesions by tissue type in cattle and warthogs that were infected with three isolates of RPV.

	Cattle											Warthogs									
	I-kudu					C	I-WP					I-kudu					icw	C	I-RBOK		
Tissue Type	HK 45	HK 10	HK 49	HK 69	Mean score	HK 62	550 K	566 K	569 K	558 K	Mean score	TW 10	TW 13	TW 33	TW Y	Mean score	TW 1	TW 11	TW A	TW X	Mean score
A	0	0	3	0	0.75	5	0	0	0	0	0.00	1	2	10	12	6.25	28	0	25	22	23.50
B	8	12	22	2	11.00	0	13	8	13	9	10.75	19	13	16	13	15.25	2	6	103	101	102.00
C	1	3	3	0	1.75	0	3	0	5	1	2.25	12	18	15	5	12.50	16	1	48	37	42.50
D	0	0	0	0	0.00	0	0	0	0	0	0.00	5	4	0	0	2.25	0	1	2	8	5.00
Overall scores	9	15	28	2	13.50	5	16	8	18	10	13.00	37	37	41	30	36.25	46	8	178	168	173.00

Scoring criterion

Each organ tissue examined was scored for the severity of congestion, haemorrhage or oedema (general vascular responses) on a scale of 1-4 and for severity of necrosis on a scale of 2-8. The same organ tissues were examined in all the animals for comparison. The scores of all the organ tissues of each type were summed to obtain the cumulative score of severity of that tissue type in the animal. The mean cumulative score of each tissue type in each group was computed by summing up the cumulative scores of all animals in the group and dividing the sum by the number of animals in the group. Mean score of severity of histopathological lesions for each group of animals was obtained by summing the mean scores of all the tissue types.

Scoring scale

Vascular responses:
1 - Mild congestion **2** - Moderate congestion **3** - Marked congestion **4**- Severe congestion,

Necrosis with or without cellular inflammatory response:
2 -A few minute foci of necrosis **4** - A few small- moderate size foci of necrosis **6** -Large foci of necrosis with or without cellular inflammatory response **8** - Extensive necrosis with or without cellular inflammatory response

Key

Tissue types
A-Stratified squamous epithelium **B**-Stomach and intestinal tract **C**-Lymphoid organs **D**-Major internal organs

Animal groups
I-kudu-animals inoculated parenterally with kudu/Bov/BK/V2 isolated of RPV **I-WP**-Animals inoculated with RBK/WP/86/1 isolate of RPV **I-RBOK**-Animals inoculated parenterally with the virulent Kabete 'O' strain of RPV **C**-Uninfected controls **icw**-One warthog in contact with cattle that had been inoculated parenterally with kudu/Bov/BK/V2