[1]Experimental Infection of Common Warthogs (*Phacochoerus africanus*) and Bushpigs (*Potamochoerus larvatus*) with Classical Swine Fever Virus. II : A Comparative Histopathological Study.

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SUMMARY

Wild African *Suidae*, the common warthog (*Phacochoerus africanus*) and bushpig (*Potamochoerus larvatus*), were experimentally infected with classical swine fever (CSF) virus following the diagnosis of CSF subtype 2.1 in domestic pigs in South Africa in 2005. No data regarding the susceptibility or potential lesions of these African wild suids are available. Seven subadult warthogs and six bushpigs were captured and infected intranasally with the South African isolate. Two in-contact control animals of the same species in each experiment verified intra-species transmission. Surviving animals were euthanized after 44 days. Formalin-fixed tissue samples collected from them as well as animals euthanized during the trial were evaluated for histological lesions. The warthogs, which were clinically normal throughout the study, developed histological lesions that were inconsistently present and sometimes subtle. Three individuals, including one in-contact control, developed distinct lympho-plasmacytic cuffing in their brains. Subtle lesions included scant lympho-plasmacytic infiltration of various organs, occasionally accompanied by perivascular cuffing. In contrast, the bushpigs developed overt clinical signs similar to CSF in domestic pigs. Four animals out of six, including two in-contact controls, died or were euthanized during the trial.

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On post mortem examination, intestinal necrosis and ulceration, purulent rhinitis and pneumonia were present. Affected animals developed lymphoid necrosis and depletion whilst surviving individuals showed perivascular cuffing in multiple organs. From the present work, we conclude that these wild *Suidae* are susceptible to CSF virus and intra-species transmission under experimental conditions can occur.

Keywords: classical swine fever, wild suids, common warthog, bushpig, intra species transmission, pathology.

INTRODUCTION

Classical swine fever (CSF) is a highly contagious disease of pigs and is listed as an important transboundary disease by the World Organisation for Animal Health (OIE). The causative agent is a small, enveloped, single-stranded, positive-sense RNA virus belonging to the family Flaviviridae, genus *Pestivirus*. There are no defined serotypes, although three genotypes can be distinguished (Artois et al., 2002). Under natural conditions, CSF infection occurs in domestic pigs (*Sus scrofa domesticus*) and wild boars (*Sus scrofa*). The latter species is regarded as a reservoir for CSF virus in Europe and serves as a possible source of infection for domestic pigs (Artois et al., 2002; Moennig et al., 2003; Paton and Greiser-Wilke, 2003). The disease causes considerable economic loss, especially in countries with industrialised pig production. Control strategies depend on the status of the country in question. In newly infected countries, effective control depends upon rapid, accurate diagnosis and is usually aimed at eradication if possible, while vaccination is widely used to control and contain CSF in endemically infected countries.

In post-natal infection in domestic pigs, the incubation period ranges from 7 to 10 days with peracute, acute and chronic disease courses described (Liess, 1988). In wild boars, only acute disease has been described to date (Artois et al., 2002; Moennig et al., 2003). In peracute cases, animals die 2 to 5 days post-infection, while animals surviving for more than 30 days are considered chronically infected. Clinical signs in acute cases develop 2 to 6 days post infection. Comprehensive histological and electron microscopic studies of the lesions in domestic pigs infected with CSF have been published (Liess, 1988). The structures most commonly affected are the walls of blood vessels, lymphoid tissue, kidneys, adrenal glands and the central nervous system. Secondary bacterial infections are often reported which

exacerbate and even mask the pathological manifestations of CSF in subacute to chronic infections, resulting in a confusing clinical and pathological picture.

Classical swine fever was diagnosed in a commercial pig unit in the Western Cape Province of South Africa in June 2005, followed by a subsequent diagnosis in the Eastern Cape Province in August after being absent from the country for 87 years. The high morbidity and mortality across all age groups, severity of clinical signs in younger animals, poor response to antibiotic treatment and histopathological changes prompted a presumptive diagnosis of CSF (Sandvik et al. 2005). The identified subtype, 2.1 has previously caused several outbreaks of CSF in Europe, but unlike CSF virus subtypes 2.2 and 2.3, has never become enzootic in European wild boars. CSF virus subtype 2.1 has never been found in the Americas (Sandvik et al. 2005). Clinical signs observed during the South African outbreak, especially in younger animals, included cyanosis and petechial haemorrhages in the skin over the distal extremities, pyrexia, anorexia, lethargy, conjunctivitis, nasal discharge, constipation followed by diarrhoea, muscle tremors, convulsions, difficulty in locomotion progressing to staggering movements and hindquarter paresis. Despite the severe clinical manifestations, macroscopic lesions were subtle and inconsistently present. Occasional cases with petechial haemorrhages in the kidneys and urinary bladder, extensive multifocal necrotic and ulcerative tonsillitis and fibrino-haemorrhagic pneumonia were noted (S. Gers unpublished observations).

In the South African outbreak, the most consistent histological lesion was lympho-plasmacytic perivascular cuffing of blood vessels in the central nervous system of affected pigs. Widespread necrotizing vasculitis was present in numerous organs, sometimes accompanied by thrombosis and infarction. The lymphoid tissue was markedly activated, with follicular lymphoid necrosis found occasionally (S. Gers unpublished observations).

Rural communities, practising communal or informal farming, populate large areas of southern Africa. Domestic pigs form an integral part of the socio-economic structure in some of these communities, particularly in the Eastern Cape Province. Not only are they regarded as a source of fresh meat but their monetary value becomes evident when they are sold live to provide instant income. Transportation of pigs and their products across provincial borders often occurs and sick pigs are slaughtered as soon as

possible to avoid their meat going to waste. Establishment of disease-free zones in the event of outbreaks is often difficult and disease dissemination is aggravated by these practices (G Bührmann, personal communication 2005). Communal farming entails a variety of domestic animal species belonging to different owners grazing together, with no formal boundaries or fences. Under these conditions, direct contact between domestic pigs belonging to different owners and feral pigs and even African wild suids is a distinct risk.

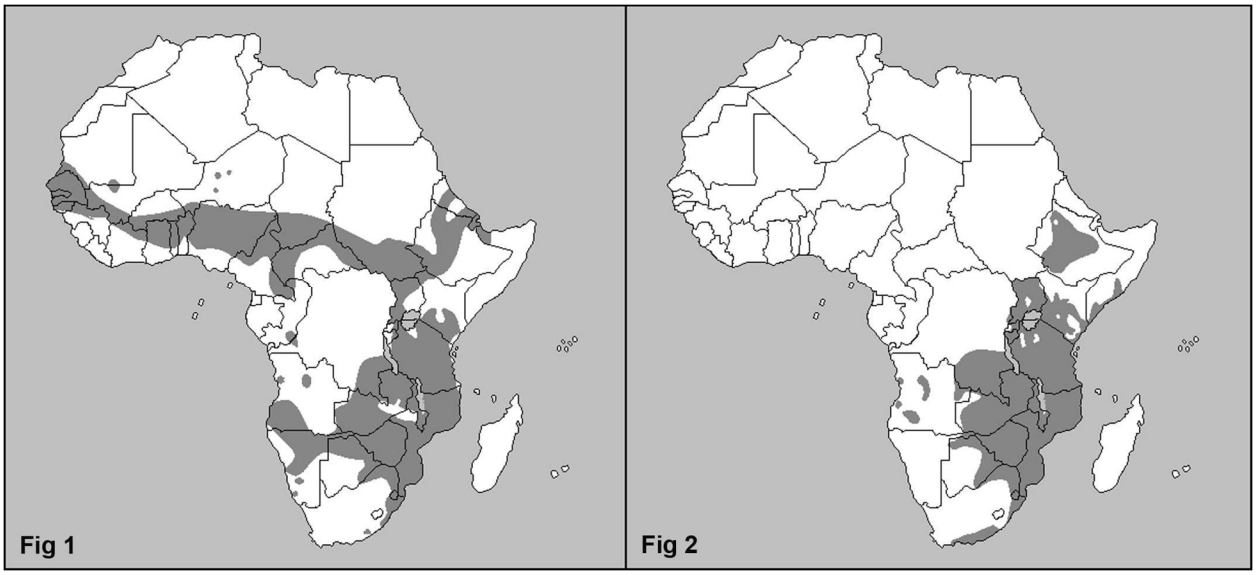

FIG. 1 Distribution of the common warthog (*Phacochoerus africanus*) (after Skinner & Chimimba 2005)

FIG. 2 Distribution of the bushpig (*Potamochoerus larvatus*) (after Skinner & Chimimba 2005)

Two indigenous wild suid species are widely distributed throughout southern Africa, the common warthog (*Phacochoerus africanus* Fig. 1) and the bushpig (*Potamochoerus larvatus*; Fig. 2). The characteristic Albany Thicket vegetation of large parts of the Eastern Cape Province harbours not only large numbers of bushpigs, but feral pigs are also commonly sighted (Skinner and Chimimba 2005). The question arose as to whether these wild African suids might be susceptible to infection by CSF virus, and if so, whether disease and lesions would develop. No data regarding their susceptibility and intra- or interspecies transmission are available. A study was initiated by the National Department of Agriculture, South Africa to assess the susceptibility and development of pathological lesions of common warthogs

and bushpigs to infection by the South African CSF viral isolate. In addition to various tissue samples for viral isolation (Everett et al., E-pub. ahead of print) buffered formalin-fixed tissue samples were collected for histopathological evaluation.

Virus used for infection

The CSF viral isolate (genotype 2.1) used in the experiments originated from the 2005 outbreak in the Western Cape Province (Sandvik et al. 2005). It was grown to a concentration of 10^4 $TCID_{50}$ (tissue culture infective dose) on PK15 cells (porcine kidney cells) and stored at –70 ^{0}C until further use (described in Everett et al., E-pub. ahead of print).

Infection of the wild suids and collection of samples

In two separate experiments, five randomly selected warthogs and four bushpigs were anaesthetised and infected intranasally with 1 *ml* of 10^4TCID_{50} of the CSF viral strain Elsenburg 2005 (Sandvik et al. 2005). Intramuscular injection of a combination of azaperone ("Stresnil" [*Bayer Animal Health*]) at 2 - 2,5 mg/kg (warthogs) and 2 – 4 mg/kg (bushpigs), and tiletamine and zolazepam ("Zoletil 100" [*Virbac*]) at 2,5 mg/kg (warthogs) and up to 5 mg /kg (bushpigs) achieved adequate sedation to allow infection and sampling procedures during the trial. Intraspecies transmission was assessed by two in-contact control animals of the same species in each experiment (WH 6 and 7; BP 1 and 2).

Animals were observed daily for the manifestation of any overt clinical signs and anaesthetised at intervals ranging from 2 to 5 days to collect saliva, nasal secretions, faeces, heparinized blood and serum. To avoid undue stress to these wild suids, animals were not handled daily to measure various clinical parameters. Intravenous euthanasia by an overdose of sodium pentobarbitone ("Eutha-Naze" [*Bayer Animal Health*]) was used to euthanize diseased animals during the trial and surviving animals after 6 weeks. The tissues that were collected unpreserved and in 10% neutral buffered formalin from all animals during post mortem examination for viral isolation and histopathological examination respectively included tonsil, nictitating membrane, various lymph nodes, spleen, stomach, duodenum, jejunum, ileum, ileocaecal valve, caecum, pancreas, adrenal, lung, heart, diaphragm, liver, kidney, urinary bladder, skin and brain. Any macroscopic lesions present were recorded and then sampled for histological evaluation.

After thorough disinfection and decontamination of the sample jars containing the tissues for microscopic examination, they were taken to the Pathology Section of Veterinary Science, University of Pretoria at Onderstepoort for processing and staining. Tissue sections were prepared by conventional methods and routinely stained with haematoxylin and eosin (HE).

RESULTS

Clinical observations and pathology of the warthogs infected with CSF virus

The intranasally infected warthogs were clinically normal for the duration of the trial and there was similarly no clinical evidence of disease in the two in-contact controls. WH 1 never regained consciousness after infection, and WH 2 was euthanized for humane reasons due to poor adaptation to captivity on Day 14 post infection. WH 3, 4 and 5 that were infected intranasally and the in-contact controls, WH 6 and 7, survived for the duration of the experiment (42 days). Post mortem examination of the warthogs revealed no significant macroscopic lesions in any of them. Histological lesions were inconsistently present and, in some cases, subtle. They consisted of varying degrees of interstitial and perivascular lympho-plasmacytic infiltrations in many organs (Table 1a). The most outstanding lesion was noted in the brains of WH 3, 5 and 6 (Fig 3), which showed distinct multifocal perivascular cuffing by lymphocytes and plasma cells. Other lesions included mild to severe lymphoid atrophy and depletion in various lymphoid organs; moderate adrenal cortical hyperplasia (all infected warthogs); scanty, scattered single parenchymal cell necrosis and scanty lympho-plasmacytic cells within the adrenal glandular cortex (WH 3, 6 and 7; Fig 4). Increased numbers of intra- and subepithelial lymphocytes were observed diffusely in the third eyelids of WH 2, 3, 4, 5 and 6 with moderate amounts of lympho-plasmacytic cells surrounding the Harderian glandular ductules of WH 3, 5 and 6 (Fig. 5). Scattered lymphocytes and plasma cells were present within the bladder epithelium and the tunica muscularis of WH 2, 3, 5 and 6. Perivascular cuffing was present in bladder walls of WH 2 (Fig 6) and 5. The diaphragmatic serosal surface of WH 3 was thickened by scattered multifocal perivascular to focally extensive submesothelial lympho-plasmacytic infiltrates. Incidental lesions observed included multiple abscessation and cellulitis of skin lacerations in some of the warthogs (WH 1, 4 and 7) and minor multifocal gastric erosion to ulceration in the pars glandularis of gastric sections from WH 1.

Table 1. Days surviving post infection; presence and severity of perivascular cuffing and lympho-plasmacytic infiltration in organs of warthogs.

	WH1	WH2	WH3	WH4	WH5	WH6■	WH7■
Days surviving post infection	**1**	**14**	**42**	**42**	**42**	**42**	**42**
Organ							
Brain	-	-	++◊	-	++◊	++◊	-
Cardiac muscle	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-
Pancreas	-	-	-	-	-	-	-
Kidney	-	+◊*	+◊*	-	+◊*	+◊*	+◊*
Bladder	-	+◊*	+*	-	+◊*	+*	-
Gastro intestinal tract	-	-	+◊	-	-	++*	-
Adrenal	-	-	+*	-	-	+*	+*
Skeletal muscle	-	-	+◊	-	-	-	0
Skin	0	-	-	-	-	-	0
3rd eyelid	-	+*	+*	+*	+*	+*	-
Harderian gland	-	-	+*	-	+*	+*	0

Key:

-	No lesions observed	*	Lympho-plasmacytic infiltration
+	Mild	◊	Perivascular cuffing
++	Moderate	0	Tissue not available
+++	Severe	■	Uninfected in-contact control animal

Table 2. Nature and relative severity of lymphoid damage in the tissues of warthogs.

		WH1	WH2	WH3	WH4	WH5	WH6■	WH7■
Lymphoid necrosis	Lymph node	-	-	-	-	-	-	-
	Tonsil	-	-	-	-	-	-	-
	Spleen	-	-	-	-	-	-	-
	GALT	-	-	-	-	-	-	-
Lymphoid atrophy	Lymph node	++	+++	+	+++	++	++	+++
	Tonsil	-	0	-	++	++	0	+
	Spleen	-	-	-	-	+	-	+++
	GALT	+	+++	+	+	++	+	-

Key:

-	No lesions observed	0	Tissue not available
+	Mild lymphoid depletion	■	Uninfected in-contact control animal
++	Moderate lymphoid depletion		
+++	Severe lymphoid depletion	GALT	Gut Associated Lymphoid Tissue.

Table 3. Cortical hyperplasia and single cell necrosis observed in the adrenal gland sections of warthogs.

	WH1	WH2	WH3	WH4	WH5	WH6■	WH7■
Hyperplasia	++	++	++	++	++	++	++
Single cell necrosis	-	-	+	-	+	+	-

Key:

+	Scanty single cell necrosis	-	No lesions observed
++	Moderate adrenal cortical hyperplasia	■	Uninfected in-contact control animal
+++	Severe adrenal cortical hyperplasia		

Lesion severity, extent, organ distribution and days surviving post infection are summarized in Tables 1, 2 and 3.

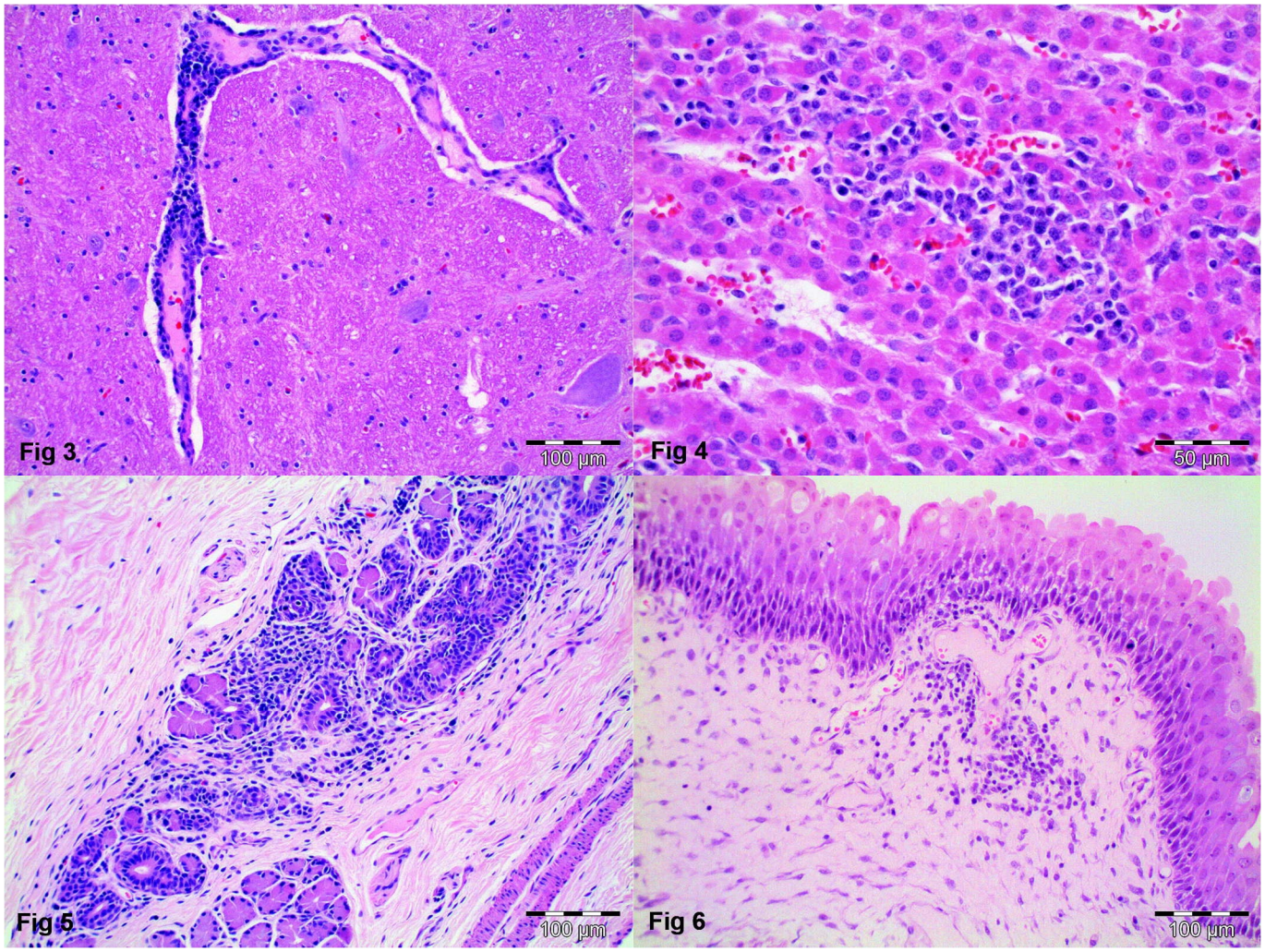

FIG. 3 WH 6. Brain. Infiltration and perivascular cuffing of the bloodvessel wall by lymphocytes and plasma cells. (HE 200X)

FIG. 4 WH 3. Adrenal gland. Interstitial lymphocyte and plasma cellular infiltration. (HE 400X)

FIG. 5 WH 6. Harderian gland of the third eyelid. Interstitial and intra tubular lymphocyte and plasma cellular infiltrates. (HE 200X)

FIG. 6 WH 2. Bladder wall. Perivascular cuffing by lymphocytes and plasma cells. (HE 200X)

Clinical observations and pathology of the bushpigs infected with CSF virus

The bushpigs, in contrast to the warthogs, developed overt clinical signs resembling those of CSF in domestic pigs. Bushpigs 1, 2 (both in-contact controls) and 3 were euthanized during the trial on days 20,

32, and 19 post infection respectively and BP 6 died on day 4 post infection without manifesting any apparent clinical signs. Bushpigs 1, 2 and 3 developed diarrhoea, became depressed, pyrexic and anorexic, and deteriorated rapidly before euthanasia. Post mortem examinations revealed lesions that included multifocal areas of colonic necrosis and ulceration (BP 1 and 2), rhinitis (BP 3), and severe bronchopneumonia (BP 2). Histologically, the lymphoid tissues of these three bushpigs showed widespread severe (lymph node BP 3; Fig. 7) to scattered single cell necrosis of lymphocytes against a background of extensive lymphoid follicular depletion and atrophy. Focally extensive areas of lytic necrosis were present in the gut-associated lymphoid tissues of BP 1 (Fig. 8), 2 and 6 while multifocal, extensive lytic necrosis and ulceration of the colonic mucosa was present in BP 1 (Fig. 9) and 2. Acute, purulent, lobular bronchopneumonia, with the presence of intralesional bacteria was present in BP 2. Rhinitis in BP 3 manifested histologically as multifocal, necrotic and ulcerative.

The two surviving bushpigs (BP 4 and BP 5) developed pyrexia post infection, with severe adrenal cortical hyperplasia being noted at post mortem examination. Histological examination of their tissues revealed varying degrees of interstitial and perivascular lympho-plasmacytic infiltrations in several organs and tissues, including cardiac muscle (BP 5 Fig 10), kidney, urinary bladder, adrenal cortex, various parts of the intestinal tract, the third eyelid (around the Harderian glandular ductules as well as intra- and subepithelial), brain (BP 4 Fig. 11and BP 5) and lung (BP 1, BP 4 Fig. 12 and BP 6). All the bushpigs developed moderate to severe adrenal cortical hyperplasia with scanty, scattered single parenchymal cell necrosis. Incidental lesions occurring in individual animals included minor multifocal gastric erosion to ulceration in the pars glandularis of gastric sections from BP 3 and BP 6 and a mesenteric lymph node manifesting caseous lymphadenitis, which was found in BP 1. Mild orthokeratotic hyperkeratosis and peri-folliculitis with superficially embedded yeast-like organisms resembling *Candida* sp. was demonstrated in the skin section of BP5. Multifocal necrotizing posthitis was present in BP 2.

Lesion severity, extent, organ distribution and days surviving post infection are summarized in Tables 4, 5 and 6.

Table 4. Days surviving post infection; presence and severity of perivascular cuffing and lympho-plasmacytic infiltration in organs of bushpigs.

	BP1■	BP2■	BP3	BP4	BP5	BP6
Days surviving post infection	**20**	**32**	**19**	**44**	**44**	**4**
Organ						
Brain	-	-	-	+◊	+◊	-
Cardiac muscle	-	-	-	++◊*	++◊*	-
Lung	+*	-	-	++◊*	-	+*
Liver	-	-	-	-	-	-
Pancreas	-	-	-	-	-	-
Kidney	-	++◊*	+◊*	+◊*	+◊*	-
Bladder	-	-	-	+*	+*	-
Gastro intestinal tract	+◊	-	-	++◊	+++◊*	-
Adrenal	-	-	-	+*	+*	-
Skeletal muscle	-	-	-	-	-	-
Skin	-	-	-	-	-	-
3rd eyelid	+*	-	-	+*	+*	-
Harderian gland	-	-	-	+*	+*	+*

Key:

-	No lesions observed	*	Lympho-plasmacytic infiltration
+	Mild	◊	Perivascular cuffing
++	Moderate	■	Uninfected in-contact control animal
+++	Severe		

Table 5. Nature and relative severity of lymphoid damage in the tissues of bushpigs.

		BP1■	BP2■	BP3	BP4	BP5	BP6
Lymphoid necrosis	**Lymph node**	SCN	-	SCN	-	SCN	SCN
	Tonsil	SCN	-	SCN	-	-	0
	Spleen	SCN	-	SCN	-	SCN	-
	GALT	LN	LN	-	-	-	LN
Lymphoid atrophy	**Lymph node**	+++	+	+	+	+	+++
	Tonsil	++	-	+	+	+	0
	Spleen	+++	+	+	-	+	-
	GALT	LN	LN	+	+	++	LN

Key:

-	No lesions observed	0	Tissue not available
+	Mild lymphoid depletion	■	Uninfected in-contact control animal
++	Moderate lymphoid depletion	SCN	Single cell necrosis
+++	Severe lymphoid depletion	LN	Lytic necrosis
		GALT	Gut Associated Lymphoid Tissue

Table 6. Cortical hyperplasia and single cell necrosis observed in the adrenal gland sections of bushpigs.

	BP1■	PB2■	PB3	BP4	BP5	BP6
Hyperplasia	++	++	+++	++	++	+
Single cell necrosis	-	-	-	+	+	-

Key:

+	Scanty single cell necrosis	-	No lesions observed
++	Moderate adrenal cortical hyperplasia	■	Uninfected in-contact control animal
+++	Severe adrenal cortical hyperplasia		

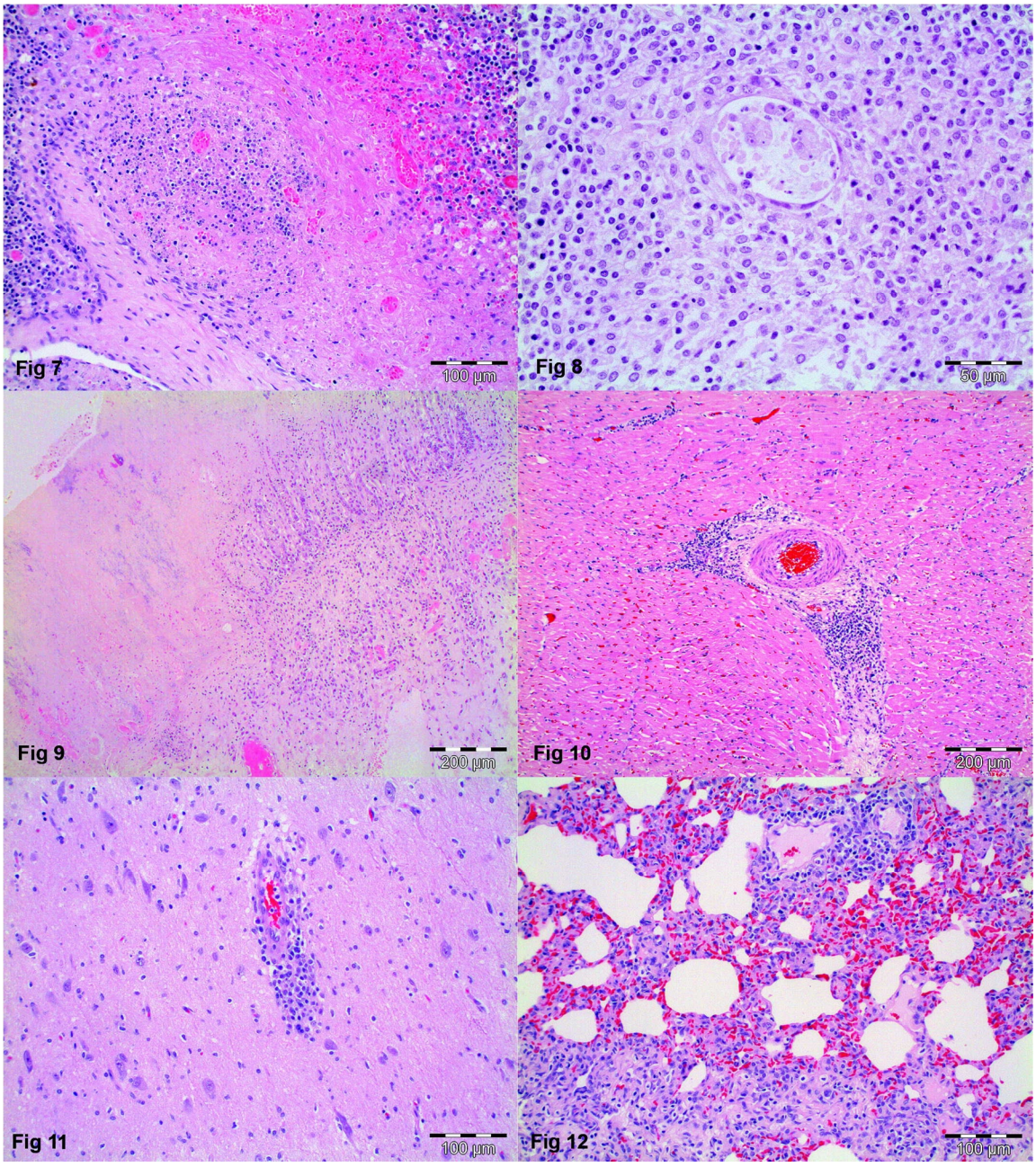

FIG. 7 BP 3. Lymph node. Extensive lymphoid follicular necrosis. (HE 200X)

FIG. 8 BP 1. Gut associated lymphoid tissue. Lymphoid necrosis with a cystic area lined by enterocytes and filled with necrotic cellular debris. (HE 400X)

FIG. 9 BP 1. Colon. Focally extensive lytic mucosal necrosis. (HE 100X)

FIG. 10 BP 5. Heart. Perivascular and interstitial lymphocyte and plasma cellular infiltration. (HE 100X)

FIG. 11 BP 4. Brain. Lymphocytic perivascular infiltrate. (HE 200X)

FIG. 12 BP 4. Lung. Mononuclear interstitial pneumonia, signified by a widened interstitium and perivascular spaces by lymphocytes, plasma cells and oedematous fluid. (HE 200X)

The two domestic pigs used to demonstrate viability and virulence of the CSF virus used in each of the trials developed clinical signs typical of CSF and were euthanized on Days 16 and 13 for the warthog and bushpig trials respectively. Clinical and pathological findings were consistent with those of CSF (data not shown).

DISCUSSION

This is the first description of experimental CSF virus infection in two of the wild African suid species (bushpigs and common warthogs) that occur in sub-Saharan Africa. Since these two animal species are abundant in certain parts of the continent, it became imperative to elucidate their potential role in the epidemiology of CSF, should it become established. Their involvement would impact severely on disease control strategies and would make eradication (and control) of the disease considerably more complex. Although all indications are that CSF has been eradicated from domestic pigs in South Africa, it was necessary to experimentally ascertain whether warthogs and bushpigs can become infected in order to assess their potential as reservoirs of the virus if they were susceptible.

No overt clinical manifestation or macroscopic lesions of CSF were observed in the warthogs despite becoming infected and also transmitting disease to the in-contact animals as was demonstrated with virus isolation and detection of genomic material from excretions as well as sero-conversion (Everett et al., E-pub. ahead of print). In contrast, the intranasally infected and in-contact bushpigs showed overt clinical signs similar to those of CSF in domestic pigs. These included pyrexia and diarrhoea, with three out of six animals requiring euthanasia for welfare reasons because of the disease. Histological lesions in the warthogs were inconsistently present and sometimes subtle, while the bushpigs developed distinct lesions.

In the acutely to sub-acutely affected bushpigs that died or were euthanized (BP 1, 2, 3 and 6), necrosis of various epithelial linings (intestine and nasal epithelium) necrosis and depletion of lymphoid tissues and secondary bacterial pneumonia were the major lesions observed. Surviving bushpigs and warthogs (BP 4 and 5; WH 2, 3, 4, 5, 6 and 7) developed lympho-plasmacytic infiltrations in various organs, frequently occurring as multifocal perivascular cuffs. This lesion was particularly pronounced in the brains of WH 5 and 6, and BP 4. It is of particular significance to note that WH 6 was an in-contact control.

Lymphocytes and plasma cells also occurred as diffuse infiltrates in interstitial tissues of organs such as the lungs of BP 1, 4 and 6 (the so-called mononuclear interstitial pneumonia), and in some around the secretory ducts of certain glands, such as, the Harderian glands in the third eyelid of WH 3, 5 and 6, and BP 4, 5 and 6. Diffusely scattered lymphocytes were found in the urinary bladder wall of WH 2, 3, 5 and 6, and BP 4 and 5 with perivascular cuffing also being present in WH 2 and 5. Increased numbers of intra- and subepithelial lymphocytes were observed throughout the tissues of the third eyelids of WH 2, 3, 4, 5 and 6, and BP 4 and 5.

Infiltration of tissues and perivascular cuffing of blood vessels by lymphocytes and plasma cells are an immunological reaction to antigen, in this case CSF virus. In the bushpigs, this lesion was observed in many organs. In the warthogs, however, lympho-plasmacytic infiltrations were observed mainly around blood vessels in the brain of infected animals, and occurred in the absence of noticeable clinical signs. Interestingly, perivascular cuffing in the brain was the single most consistent lesion observed in domestic pigs during the 2005 field outbreak (S. Gers, personal observation).

Small numbers of lymphocytes and plasma cells may occur in most tissues of normal, healthy animals. In the tissue samples examined during this trial, increased numbers of these cells were observed. The presence of lymphocytes and plasma cell infiltrations is often considered a non-specific change, especially within organs such as the kidney or mucosa of the gastro-intestinal tract. In these locations it is therefore often regarded as an incidental finding. Within the brain, perivascular accumulations of lymphocytes and plasma cells are considered highly significant because they are frequently associated with viral encephalitis. Similarly, mononuclear (lympho-plasmacytic) interstitial pneumonia is regarded as strong supportive evidence of a viral aetiology (Maxie 2007).

CSF virus is known to damage lymphoid tissues of affected pigs, primarily as a result of infection of macrophages and other cells of the myeloid lineage, such as dendritic cells. Infected macrophages in lymphoid tissue locally induce apoptosis and necrosis in uninfected lymphocytes by secretion of mediators such as interleukin-1 α (IL-1 α), interleukin-6 (IL-6) and tumour necrosis factor α (TNF α) during the acute to subacute stages of the disease, progressing to lymphoid depletion or atrophy during the later stages of the infection. Suppression of the immune response is likely a direct consequence of viral injury

to, and suppression of, dendritic cells and lymphocytes [Pauly et al., 1998; Artois et al., 2002; Sánchez-Cordón et al., 2003). Earlier work by Bruschke et al. (1997) has also revealed the viral glycoprotein E^{rns} of CSF virus to be able to induce apoptosis in porcine lymphocytes *in vitro,* but the relevance of this to viral pathogenesis and the disease course in the pig is unknown. In the present experiments, lymphoid tissue samples from bushpigs that died or were euthanized before termination of the experiment (BP 1, 2, 3, and 6) showed lymphoid necrosis (representing an acute manifestation of disease). Their gut-associated lymphoid tissue, in the vicinity of the ileocecal valve in particular, was severely damaged, resulting in focally extensive areas of lytic necrosis of the Peyer's patches. Other lymphoid organs, such as the lymph nodes and spleen, were less severely injured. The lesion in these organs was characterized by scattered single cell necrosis against a background of various degrees of lymphoid depletion. By the time the experiment was terminated, 6 weeks post-infection, only lymphoid atrophy and depletion were noted in the remaining animals (BP 4 and 5).

Lymphoid atrophy and depletion is also associated with other conditions, for example severe prolonged stress, which induces high levels of circulating cortisol (Maxie 2007). Capturing, captivity and handling of wild animals such as these warthogs and bushpigs are potent stressors that probably contributed to the morphological changes observed in the lymphoid organs of the experimental animals. The role of stress in these animals is further substantiated by the pronounced hyperplasia of the *zona fasciculata*, the cortisol secreting zone of the adrenal cortex (Tables 1c and 2c). WH 1, which never regained consciousness after the CSF infection procedure, displayed adrenal cortical hyperplasia and lymphoid depletion and atrophy, similar to that observed in the other warthogs and bushpigs that survived long enough to develop CSF virus-associated signs or lesions. Small, scattered foci of single adrenal cell necrosis (observed in WH 3, 5 and 6 and BP 4 and 5), unlike adrenal cortical hyperplasia, may not only be a stress related change because scant lympho-plasmacytic cellular infiltration was sometimes associated with these foci. Severe acute stress has been known to cause large areas of haemorrhage and necrosis in the adrenal glands of animals (Maxie 2007). According to Liess (1988) the adrenal glands of domestic pigs are targeted by the CSF virus and the lesions there are central to the pathogenesis of the disease.

Most investigators have reported the presence of diarrhoea due to enteritis and multifocal ulceration in the caecum and colon, and secondary bacterial pneumonia in domestic pigs as sequelae of CSF infection. The acutely affected bushpigs (BP 1, 2 and 3) developed diarrhoea, with BP 2 also developing bacterial bronchopneumonia. Intestinal lesions are ascribed to chemical mediators released by infected mononuclear cells, most probably macrophages (Sánchez-Cordón et al., 2005).

Some of the inconsistent additional lesions found, such as caseating necrosis of a mesenteric lymph node in BP 1, posthitis in BP2, dermatitis in BP5 and multifocal gastric erosion and ulceration of the pars glandularis in gastric sections of BP 3 and 6 and WH 1, cannot be ascribed specifically to infection with CSF virus. Gastric ulcers are often associated with animals that are stressed. In this instance, stress has to be considered as one of the main inciting factors for these ulcers. Multiple skin lacerations, with abscessation and cellulitis in some of the warthogs, are regarded as being a consequence of captivity and handling. Posthitis (found in BP2) is described in 25 – 40% of entire and castrated male domestic pigs. The precise cause is unclear, but accumulation and decomposition of urine are considered of primary importance (Maxie 2007).

CONCLUSIONS

From the present work, we conclude that common warthogs and bushpigs are susceptible to experimental infection with CSF virus. Intra-species transmission under experimental conditions occurred. In concurrence with findings in domestic pigs (Elbers et al., 2003), the clinical signs and histological lesions observed in the bushpigs and warthogs were inconsistent and relatively non-specific. It is apparent that the diagnosis of CSF in these wild African suids cannot be made with confidence solely on macroscopic and histological findings. Additional diagnostic procedures, demonstrating antibody and/or antigen, or viral RNA are essential for CSF confirmation.

In domestic pigs, various factors influence the outcome of post-natal CSF infection, including strain virulence, age, health status and breed, as well as infecting dose (Artois et al., 2002). Age might have influenced the course of infection and the development of lesions in the bushpigs. The younger animals that died or were euthanized developed pneumonia, lymphoid and intestinal necrosis, while the older

individuals surviving for the duration of the trial developed only perivascular lympho-plasmacytic cuffing in various organs. The latter might be indicative of chronic CSF, but needs to be substantiated by additional investigative data in a larger number of animals. The ability of the virus to cross the placenta in warthogs and bushpigs, the possible role of these animals as carriers of the virus, and the possibility of interspecies transmission, are unknown, but would be critical determinants of the potential for these species to maintain CSF virus circulation and transmit it to domestic pigs.

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