Sveriges lantbruksuniversitet
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Patho-anatomical studies on African Swine Fever in Uganda

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Patologanatomiska studier av afrikansk svinpest i Uganda

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ABSTRACT

African Swine Fever (ASF) is one of the most serious transboundary swine diseases because of its high lethality for pigs, socioeconomic consequences, rapid and international spread and the absence of either treatment or vaccine. This Sida Minor Field Study (MFS) was carried out during fall 2010, as part of a larger collaborative research project called ASFUganda, focusing on the epidemiology of the disease in Uganda. The aim with this study was to get more knowledge about the pathology in cases of ASF in Uganda, by studying macroscopic and microscopic lesions in pigs with acute and chronic ASF and to detect the ASF virus (ASFV) in tissues by immunohistochemistry.

The pigs were selected from the two different geographical locations in Uganda, Mityana and Gulu district, both with on going confirmed outbreaks of ASF. Necropsies were performed in the field, and the laboratory procedures at the JICA (Japan International Cooperation Agency) Veterinary Pathology Laboratory, Entebbe, Uganda, and at the Department of BVF, SLU, Uppsala, Sweden. Three pigs from an outbreak of ASF in Mityana district showed both the history and the clinical symptoms typical for ASF. The macroscopic and histopathological findings were consistent with the lesions described in the literature for acute ASF. ASFV antigen was detected by immunohistochemistry in the lymphoid tissues from these pigs, indicating that the lesions were the direct result of the virus infection. Three pigs from Gulu district did not show any clinical symptoms of ASF disease before slaughter but were positive for ASFV on RT-PCR and ELISA. The macroscopic and histopathological findings resembled the lesions previously described for subacute and chronic ASF.

SAMMANFATTNING

Afrikansk svinpest (ASF) är en av de mest allvarliga sjukdomarna hos svin på grund av dess höga dödlighet, socioekonomiska konsekvenser, snabba och internationella spridning samt frånvaron av varken behandling eller vaccin.

Det här Sida-finanserade MFS-arbetet (MFS=Minor Field Study) utfördes hösten 2010 som en del av ett större samarbetsprojekt kallat ASFUganda, med fokus på sjukdomens epidemiologi i Uganda. Syftet med studien var att få ökad kunskap om patologiska förändringar vid fall av ASF, genom att undersöka makroskopiska och mikroskopiska förändringar hos svin med akut och kronisk ASF samt att påvisa ASF viruset (ASFV) i vävnader med hjälp av immunohistokemi.

Grisarna i studien var utvalda från två olika geografiska platser i Uganda, distriktet Mityana och distriktet Gulu, båda med verifierade pågående utbrott av ASF. Obduktioner utfördes i fält och laboratoriearbetet på JICAs (Japan International Cooperation Agency) Veterinary Pathology Laboratory i Entebbe, Uganda, samt på institutionen för biomedicin och veterinär folkhälsovetenskap (BVF), SLU, Uppsala, Sverige. Tre grisar från ett pågående utbrott av ASF i distriktet Mityana (oktober-december 2010) visade både anamnes och kliniska symptom typiska för akut ASF. De makroskopiska och histopatologiska fynden överensstämde med de förändringar beskrivna i litteraturen för akut ASF. Påvisandet av ASFV via immunohistokemi i den lymfatiska vävnaden från dessa grisar indikerar att lesionerna orsakats av virusinfektionen. Tre grisar från distriktet Gulu visade inte några kliniska symptom på ASF före slakt men var RT-PCR-positiva och ELISA-positiva för ASFV. De makroskopiska och histopatologiska fynden liknade de förändringar beskrivna i litteraturen för subakut och kronisk ASF.

INTRODUCTION

This Sida Minor Field Study (MFS) was carried out during fall 2010, as part of a larger collaborative research project called ASFUganda, focusing on the epidemiology of the disease in Uganda. The ASFUganda consortium includes scientists from the Swedish University of Agricultural Sciences (SLU), the Ugandan Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Makerere University, Uganda Wildlife Authority (UWA) and the International Livestock Research Institute (ILRI). ASF is included among the transboundary animal diseases (TADs) which are notifiable to the World Organisation for Animal Health (OIE) according to the Terrestrial Animal Health Code (http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/). TADs are defined as diseases that are of significant economic, trade and food security importance with potential for rapid international spread, and that require international cooperation for control and management. ASF is one of the most serious transboundary swine diseases because of its high lethality for pigs, its socioeconomic consequences, its rapid and international spread and the absence of either treatment or vaccine [1].

Background – African Swine Fever

World distribution

After being described for the first time 1921 in Kenya, ASF was subsequently reported in most sub-Saharan countries in Africa. In 1957 the disease spread to Portugal, most likely by swill feeding from airports. A second introduction during the 1960´s resulted in spread throughout the Iberian peninsula to several other countries in Europe, including France, Italy, Belgium, Malta, and the Netherlands. It is suspected that the disease was spread through the transportation and distribution of infected pig products via airports and harbours. ASF became well established only in Sardinia (Italy), Spain and Portugal where eradication by stamping out was accomplished some 30 years later, except for Sardinia, where it remains endemic. During the 1970´s ASF was also spread to the islands of the Caribbean and to Brazil, but was successfully eradicated. It has not been established whether these outbreaks originated in Europe or Africa. In June 2007, ASF was reported again in Europe, starting in Georgia and spreading to Armenia, Azerbaijan and to Russia [1]. In 2012 it is endemic in Russia, representing a threat to the European Union (http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10395). ASF has been reported from most of the eastern and southern African countries, and is endemic in Uganda [2]. For a global distribution of ASF in 2011, see Figure 1.

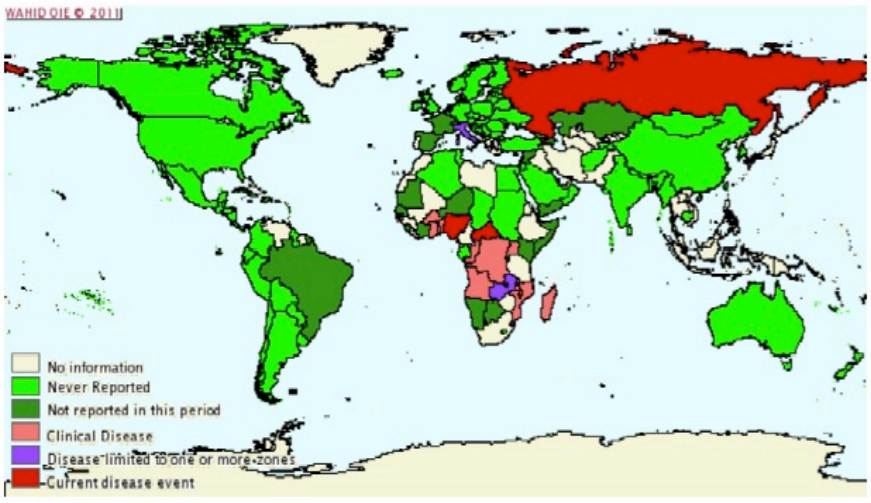

Figure 1. Global Distribution on African swine fever 2011 (OIE, 2011).

Uganda

Set at the equator, Uganda is a sub-Saharan country in East Africa with a rapidly growing population of around 30 million people. Since the independence from Great Britain in 1962 the history of Uganda has been afflicted by internal conflicts and war. Things are improving steadily but still 31 % of the population is living in poverty, and the country is rated as number 161 out of 187 countries by the Global Human Development Report 2011 (http://hdrstats.undp.org/en/countries/profiles/UGA.html). The economy depends mostly on agriculture in form of small farms, with around 85 % of the population living in rural areas. The lack of proper infrastructure prevents the people living in the rural areas from the benefits of the country's economic growth and modernization (http://www.ruralpovertyportal.org/web/guest/country/home/tags/uganda)

African Swine Fever in Uganda

Uganda has the largest and most rapidly growing pig production in East Africa, with over 3.1 million pigs in 2009 (http://www.ubos.org. 2009). Most of the pigs are kept by smallholder farmers in the rural areas, either free ranging or in small housing systems. In the urban or peri-urban areas there are also bigger, commercial pig farms [2]. ASF is endemic in Uganda and only during the period between July 2010 and December 2011 more than 40 outbreaks were investigated and confirmed within ASFUganda. An outbreak of ASF often has disastrous consequences both for the larger farms with intense pig production and for the smallholders who often cannot afford proper biosecurity. Many farmers in the rural areas lack knowledge about routes of transmission of the disease, and mix pigs from different markets and on transports. It is common that as soon as their pigs are starting to show symptoms of a severe disease they hurry to sell or slaughter the animals, thereby contributing to the spread of the infection [3]. A useful method to achieve producer-based systems of surveillance and control of ASF are the education programs by pig farmers associations, community leaders and rural extension officers, implementing knowledge about prevention and control of the disease in rural and peri-urban areas [2].

The ASF virus

ASFV is an icosahedral double stranded DNA Asfivirus that is presently the sole member of the family of Asfarviridae [4]. ASFV replicates mainly in the cytoplasm of the infected cells, and is the only known DNA virus to be an arbovirus (arthropod-borne virus), able to multiply in both vertebrate and invertebrate hosts [2]. Although there is only one serotype, more than 20 genotypes and numerous subtypes of ASFV of varying virulence have been identified. The genome of the virus has been sequenced, and up to 113 viral proteins have been described, one of which, VP73, is often used as an antigen in detection tests. In a suitable environment the ASFV is stable over a wide range of temperatures and pH values. It may remain infectious in serum for 18 months when kept at room temperature, in refrigerated blood for 6 years and in blood at 37° C for one month. It is necessary to heat serum at 60° C for 30 minutes to inactivate the virus. Viability is greatly reduced in the absence of a protein medium. ASFV is stable over a pH range of 4 to 10, but has been shown to remain active at lower and higher values for up to 3 days in serum. Sunlight and desiccation rapidly inactivates the ASFV. The virus may remain infective in raw, smoked or frozen pork for at least 15 weeks up to several months, which means that undercooked pork, blood or carcass meal derived from pigs must be regarded as dangerous if fed to pigs [1]. In pig faeces ASFV can persist over 100 days [2].

Transmission of ASFV infection

ASFV infects only species within the pig family (*Suidae*), where the virus is spread through two different cycles: the domestic and the sylvatic [1].

In the domestic cycle the virus is spread between domestic pigs through direct or indirect contact. The most important route of transmission is the direct contact between domestic pigs with a predominantly oronasal infection route. Transmission also occurs through indirect contact with aerosols, faeces, and urine or other body fluids. After infection with ASFV, domestic pigs may shed infective amounts of virus for 24 to 48 hours before clinical signs appear. Pigs that survive the acute disease may shed high amounts of virus for about 30 days, even though the infection may persist longer. The exact length of time over which infective levels of virus are maintained in lymphoid tissues is unknown, but does not appear to exceed three to four months. Current studies indicate that vertical transmission of ASFV is not possible [5].

Pigs that recover from acute or subacute ASF may become subclinical persistent carriers of the virus. The virus levels in recovered pigs are considered to be too low for the pig to transmit the virus to other pigs by direct contact, unless the virus becomes reactivated by stress. Other routes of transmission, especially important for spreading to new areas, are spread through contaminated fomites and swill feeding with uncooked pork [1].

The sylvatic cycle involves wild species of swine spreading the virus by soft ticks of the Ornithodoros moubata complex. Infected wild pigs are believed to have an infective virus level in other tissues than the lymph nodes for about two months after infection, but stay infected for life [6].

The spread of ASFV between wild and domestic pigs occur when wild and domestic pigs share the same feeding area and tick from the wild pigs bites the domestic pigs. Carcasses from wild pigs, brought by humans, are also considered to play a role of introducing ticks to the domestic pigs. Direct contact between infected warthogs and domestic pigs has not been shown to cause transmission, and experiments with domestic pigs fed with carcasses from infected warthogs did not lead to infection [6].

Epidemiological features

Irrespective of age or gender, domestic pigs are highly susceptible to ASF, and the mortality can be as high as 100%. However, in central parts of Africa and in certain local breed populations of pigs, higher than expected survival rates have been observed, even when ASF outbreaks were caused by virulent strain. The endemic persistence of the virus may drive the selection of some inherent resistance in the exposed pig population. Any of the wild African suids are susceptible to infection with ASFV, but most of them do not develop clinical disease. Warthogs (*Phacochoerus africanus* and *Phacochoerus aetiopicus*) are considered as the major host for ASFV. Bushpigs (*Potamochoerus porcus* and *Potamochoerus larvatus*) and giant forest hog (*Hylochoerus meinertzhageni*) have been found to be infected with ASFV, but their role in the epidemiology of the disease is unknown [1].

Immunity

Antibodies are formed after about 7–12 days after an acute infection in both warthogs and domestic pigs. They do not protect fully against a new infection but some immunity against the same strain may develop. Virus replication can continue in the presence of antibodies in subacutely or chronically infected pigs, and the deposition of immune complexes in tissues may account for many of the lesions in these forms of the disease. The antibodies persist for a long time, and serologically positive sows transmit antibodies to the piglets through the colostrum. As no vaccine is available for ASF and no serological cross-reactions with other viruses is known, the detection of antibodies in pigs confidently correlates to exposure to natural infection [1].

Pathogenesis

The main infection route is via the upper respiratory tract, and initial virus replication occurs within 24 hours of infection in the pharyngeal tonsil and in the lymphoid tissue draining the nasal mucosa [2]. Viremia follows, and the virus affects the vascular endothelium and causes lymphopenia, followed by thrombocytopenia. After a few days the endothelial lesions involve basement membranes and death usually follows due to pulmonary oedema. ASFV replicates primarily in monocytes and macrophages but replication has also been described in a range of other cells such as megakaryocytes, endothelial cells, glomerular mesangial and tubular epithelial cells, hepatocytes, thymus reticulum-epithelial cells, fibroblasts, smooth muscle cells of venules and arterioles, neutrophils and lymphocytes [2, 7-16]. The most striking histopathological feature of ASF is massive karyorrhexis in lymphoid tissues, often accompanied by haemorrhage and disseminated intravascular coagulation (DIC) [1]. Pigs infected with ASF generally suffer severe lymphopenia, which occurs during the initial-middle phase of the disease [17]. The lymphopenia is attributed to apoptosis of lymphocytes [18, 19]. The haemorrhage is caused by cytokines and other mediators released by infected macrophages, resulting in the activation of the clotting cascade and DIC [20]. Endothelial necrosis from viral replication in endothelial cells is another possibility [2, 12, 21]. The thrombocytopenia has been attributed to consumption of platelets due to coagulopathy or as a direct effect of the virus on megakaryocytes and is generally observed in the final phase of acute disease [21-23]. The massive destruction of macrophages also plays a major role in the impaired haemostasis [24]. The pathogenesis of ASFV in chronic infections is not well characterized. Some authors suggest that these forms of disease have an autoimmune component and that lesions might result from the deposition of immune complexes with complement in tissues such as kidneys, lungs and skin [25].

Clinical signs

The incubation period is from 3 to 15 days, and clinical signs vary depending on the virulence of the strain [2]. Acute ASF is usually caused by highly virulent virus isolates [1]. The infected pigs develop a persistent fever of up to 42 C, become listless and anorexic, and erythema of the ears, tail, distal extremities and ventral areas of chest and abdomen is common. Vomiting, bloody diarrhoea and ataxia due to hind-limb weakness usually develops, followed by difficult breathing indicative of the lung oedema that is often the primary cause of death. Pigs that survive longer than 6 up to 20 days may develop neural symptoms. Abortion may occur in pregnant sows. Low virulent isolates produce mainly subclinical and nonhaemorrhagic infection with seroconversion. Chronically infected pigs usually get more diffuse symptoms like emaciation, joint ill, respiratory distress and skin lesions. Viremia can reoccur with periods of fever, and secondary bacterial infections are common. The mortality is approximately 30 %, and pigs usually die within a couple of months [2].

Pathology

Macroscopic lesions

Acute ASF

Pigs that die peracutely often do so before any lesions develop, but skin flushing of the ventrum and extremities may be seen in white-skinned pigs. Also a general congestion of organs with fluid in body cavities and fibrin strands on organ surfaces may be present. The lesions of acute ASF may depend on the virus isolate, but most common are cyanosis of the extremities and the ventral surface in white-skinned pigs, areas of cyanosis in hairless parts, cutaneous ecchymosis on the legs and abdomen and mucosal congestion and haemorrhage [2]. Several internal organs are congested and oedematous. There is often accumulation of straw-coloured fluid in pleural, pericardial and peritoneal cavities and widespread

haemorrhages in the renal cortex, spleen and the lungs, and on parietal surfaces. Petechiae in the larynx, bladder mucosa and on visceral surfaces of organs are common. Congestive splenomegaly occurs frequently, but infarction only rarely. Oedema may be present in the mesentery of the colon and in the gall bladder. In particular, hepatogastric and mesenteric lymph nodes may be swollen and haemorrhagic. The mucosa of the stomach is often congested to haemorrhagic, sometimes necrotic [1]. Hydropericardium and epi- to endocardial haemorrhages are described in both acute and chronic cases [26].

Subacute and chronic ASF

In the subacute form, serofibrinous pleuritis and pericarditis are frequently observed. The lymph nodes are often enlarged and haemorrhagic to fibrous, and joint and tendon sheath effusions with oedema of periarticular tissues may be present. In the chronic form, there may be local or generalized skin eruptions. Oedema, lobar consolidation and necrosis of the lungs may develop. Fibrinous pericarditis and purulent to serofibrinous arthritis may occur as in the subacute form. Regional lymphadenopathy, with enlarged and firm lymph nodes often accompanies visceral lesions, but haemorrhages are rare. The spleen may show varying lesions including splenomegaly, haemorrhage and necrosis.

Microscopic lesions

Acute ASF

Acute ASF affects mainly lymphoid tissues, lungs, kidneys and the liver and brain. Pathologic characteristics of the lymphoid tissues, particulary the lymph nodes, tonsil and spleen, is massive necrosis with prominent karyorrhexis. The tonsils in both acute and chronic cases may have infiltrates of necrotic mononuclear cells and reticular cells in the sinus stroma and in the germinal centres [26]. General hyperaemia with oedema and haemorrhages is common, and necrosis of the squamous epithelium that overlies the tonsil and lines the crypts has been observed. The lymph nodes are often hyperaemic with peripheral or diffuse oedema. The blood vessels and sinus stroma are usually infiltrated by mononuclear cells, which often are necrotic, as well as the reticulate cells of the sinus stroma and germinal centre cells. These lesions are often observed in all of the lymphoid tissues. Acute spleen lesions are also diffuse degeneration of lymphoid tissue and vascular walls combined with severe congestion or haemorrhage in the red pulp [27]. Lung oedema is common, and is often accompanied by necrotic mononuclear cells in the alveolar septa and lumina [26]. Necrosis may also involve the alveolar septa. Vascular endothelial damage in the lung may cause thrombosis of vessels and thickening of the alveolar walls [28]. Acute proliferative glomerulonephritis may occur, and prominent oedema and macrophage infiltration may be seen in the renal interstitium [29]. Leptomeningitis and encephalitis with oedema and perivascular mononuclear cell infiltrates occur [26]. Thrombosis and necrosis of epithelium may be seen in the choroid plexuses. Lesions described in the liver include degeneration and focal necrosis of hepatocytes, Kupffer´s cell degeneration, [18] and mononuclear cell infiltrates in the sinusoids [26]. In the myocardium, few lesions are reported, mainly intramural haemorrhage and hyperaemia. In both acute and chronic cases, serosal petechiae may appear both in the stomach and in the intestines [26].

Subacute and chronic ASF

Spleen lesions in the subacute and chronic ASF are characterized by diffuse occurrence of myeloid cells in the red pulp. Lymphoid degeneration and atrophy may be present, but less intense compared with the acute form [27]. Chronic hyperplastic changes usually involve visceral and parietal lymph nodes. There may be mild lung oedema, and interstitial pneumonia is common [26]. Various types of glomerulopathy occur, associated with immune-mediated phenomena. The interstitium of the kidney may show lymphohistiocytic and

plasmacytic infiltrates [28].

Diagnosis

According to the OIE Manual of Standards for Diagnostic Tests and Vaccines, preferred samples for virus isolation/ antigen detection for ASF are tissue samples from lymphatic tissues, or whole blood from febrile pigs up to five days after the onset of fever. Polymerase chain reaction (PCR) is recommended for the identification of ASFV DNA because of its high sensitivity and specificity. Histopathological examination and detection of virus by immunohistochemical methods are also carried out. Serological tests such as ELISA are recommended in endemic areas or where a primary outbreak is caused by a strain of low virulence [2].

Differential Diagnosis

The most important differential diagnosis for ASF is classical swine fever (CSF) [1]. Other diseases to consider are Porcine Reproductive and Respiratory Syndrome (PRRS), pasteurellosis, salmonellosis and erysipelas [1] (http://epiwebb.se/02.afrikansk_svinpest/index.shtml).

OBJECTIVE

My part of the project in Uganda was to perform field necropsies, make histological sections from tissue samples, compare macroscopic and histopathological lesions and try to detect the ASF virus by immunohistochemistry in a range of tissues from selected pigs from the study of ASFUganda. The aim with this study was consequently to investigate and describe the pathological lesions in verified cases of ASF, to contribute to the understanding of the epidemiology in ASF.

MATERIALS AND METHODS

Animals

The pigs originated from two farms, one in Mityana district in central Uganda and one in Gulu district in northern Uganda (Figure 2).

Figure 2. Map of Mityana and Gulu district, Uganda (Google Earth, 2011).

The pigs from Mityana (No. 1-3) were about 12 weeks old when they died in acute ASF in the

PCR-confirmed ASF outbreak, which started in October 2010[1]. The farmer had 320 pigs in an intensively managed housing system with dry and lactating sows. The 7 of October 2010 one of the sows showed symptoms of fever and anorexia, and a few days later her piglets started to die. The three pigs used in this study were found dead a few hours before being necropsied the 14 of October 2010, and were therefore not blood sampled for RT-PCR. One of the pigs from the litter that was still alive was blood sampled and confirmed as ASFV positive. The sow died the next morning and was not sampled. The chronic cases (No. 1, 11 and 13) were from Binya village in Gulu district where there had been an outbreak starting in July 2010 with deaths until the middle of august. The three surviving pigs had been blood sampled for ASFV once every month for three months (August-October) and were PCR-positive two of the three sampling occasions (Table 1). They were seropositive but did not show any clinical signs of disease until slaughter [30]. The three pigs were not from the same litter, and between six months and one year old at the slaughter date. The pigs were kept on free range, scavenging in the surroundings of the village together, but owned by different families.

Table 1. PCR results from the three chronic cases from Gulu district that were blood sampled at three different occasions with one months interval [30]. After the last blood sampling the pigs were slaughtered for necropsy

Pig	PCR 2010-08-12	PCR 2010-09-28	PCR 2010-10-21
No. 1	+	+	-
No. 11	-	+	+[1]
No. 13	+	-	+

[1] *positive also in spleen and lung tissues*

Necropsy and tissue sampling

The three acute cases from Mityana were necropsied in the field the 14 of October 2010. The three chronic cases from Gulu were slaughtered at the local district veterinary office in Gulu the 21 of October 2010 and necropsied at the same occasion. Tissue samples from spleen, lymph nodes from various sites, tonsil, liver, lung, kidney, brain, heart, intestine and stomach were collected and fixed in 10% buffered formalin for 12-24 hours. After fixation, the samples were trimmed, manually dehydrated through a graded series of alcohol to xylene, and embedded in paraffin wax for subsequent light microscopic studies.

[1] *For more information, please see the thesis "African Swine fever in Uganda-description of a recent outbreak and studies of possible differential diagnoses" written by Malin Andersson (http://stud.epsilon.slu.se/2407/1/andersson_m_110401.pdf)*

Histology and immunohistochemistry

Sections were cut 4 μm thick, deparaffinised and stained with haematoxylin and eosin (HE). Spleens were also stained with Perl´s stain for detection of hemosiderin. Kidneys were stained with periodic acid Schiff (PAS), Martius Scarlet Blue (MSB) for detection of fibrin, and van Gieson for collagen. All the organs were compared with control material constituted of tissues from clinically healthy Swedish pigs of the same age as the pigs in this study.

In order to study the presence of ASFV nucleic acid in monocytes-macrophages, the avidin-biotin-peroxidase (ABC) immunohistochemistry technique was used on the acute cases from the Mityana outbreak. A monoclonal mouse anti-ASF antibody (18BG3; isotype IgG2A) raised against vp 73 (Ingenasa, Madrid, Spain) was used as primary antibody. Vectastain Avidin Biotin Complex (ABC) kit with biotinylated horse anti-mouse IgG and diaminobenzidine (DAB) (Vector Laboratories Inc.) was used for detection of immunoreactivity. The following organs were selected for study: spleen, tonsil, mesenteric lymph node and colon. After deparaffinization and hydration of the sections, endogenous peroxidase was quenched with 3 % hydrogen peroxide in methanol at room temperature (RT) for 30 minutes. Antigen retrieval was performed with pronase/citric acid. To avoid nonspecific binding of the secondary antibody, sections were incubated with 20 % normal horse serum (NHS) for 20 minutes at RT. Sections were incubated with the primary antibody (1:10) overnight at 4^{o}C, then with biotinylated secondary antibody, and Vectastain ABC reagent, followed by DAB according to manufacturer´s instructions. The slides were counterstained with Mayer´s haematoxylin. As nonspecific negative reagent controls, mouse IgG2 was used instead of specific antibody. Preparation of tissues, paraffin embedding, staining with HE and part of immunohistochemistry staining procedures was carried out at JICA´s (Japan International Cooperation Agency) Veterinary Pathology Laboratory in Entebbe, Uganda. Special stains and part of immunohistochemistry procedures were done at Section of Pathology, Department of BVF, SLU.

RESULTS

Macroscopic lesions

Acute cases

All three acute cases (from the Mityana district) had cyanotic ears and extremities (Figure 3 A). The intestines were congested with subserosal haemorrhages, and the lungs had ecchymotic intraparenchymal haemorrhages. Superficial as well as thoracic and abdominal lymph nodes were enlarged and haemorrhagic; particularly the mesenteric, gastric, portal, renal, mesenteric and thoracic, mandibular, retropharyngeal and inguinal nodes were affected (Figure 4). The spleen in two pigs was friable and dark but not remarkably enlarged.

Figure 3. Macroscopic findings in pigs with acute ASF. A. One of the pigs that died in acute ASF in the Mityana District. Note the reddened ears. B. Necropsy in the field. C-F. Pig No. 1. C. Subcapsular petechial haemorrhages in the renal cortex. D. Straw-coloured gelatinous oedema in the renal pelvis (arrow). E. Haemorrhagic gastritis. F. Hydrothorax (arrow).

The same pig had subcapsular petechial haemorrhages in the renal cortex and a straw-coloured gelatinous oedema in the renal pelvis (Figure 3 C and D). All three pigs had a congested and haemorrhagic stomach mucosa, consistent with acute haemorrhagic gastritis (Figure 3 E). Two of the pigs had pulmonary oedema. One pig had blood- stained serous fluid in the thorax (Figure 3 F). One pig had a slightly enlarged liver. Two pigs had haemorrhagic meninges and necrotic tonsils.

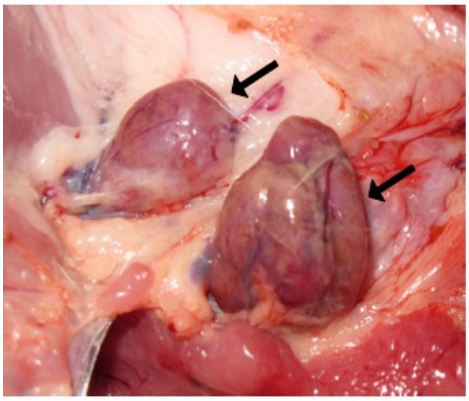

Figure 4. Acute ASF pig No 1. Haemorrhagic and enlarged mesenteric lymph nodes.

Chronic cases

The condition of the three pigs from Gulu at the time of slaughter was good, and they all had a lot of ingesta in their stomachs at necropsy (Figure 5 A). One pig had fibrinous exudate in the pericardial sac (Figure 5 B). All three pigs had slightly enlarged, firm and haemorrhagic lymph nodes, particularly the mesenteric, external and internal inguinal, mediastinal and submandibular nodes (Figure 5 C). All pigs had superficial ulcerations in the stomach mucosa (Figure 5 D), and one of the pigs had a deep gastric ulcer.

Figure 5. A. The three pigs from Gulu after transport from the farm to the local veterinary office just before slaughter, 21 October 2010. Note the good condition of the pigs. B-D. Macroscopic findings in pig No. 1 with chronic ASF. B. Pericarditis. C. Haemorrhagic external inguinal lymph nodes (arrows). D. Superficial ulcers in the stomach mucosa (arrow).

Microscopic lesions

Acute cases

Histologically, the spleens in all of the three acute cases showed massive congestion, even though only one of the spleens was macroscopically enlarged. There was haemorrhagic necrosis of the red and white pulp with severe depletion of the lymphoid tissue, and marked lymphocyte necrosis (Figure 6 A and B). The tonsils showed interfollicular lymphocyte necrosis with karrhyorhexis (Figure 6 C and D), and many tingible body macrophages in the follicles. Lymphocyte depletion was also seen, and necrosis of the crypt epithelium was evident.

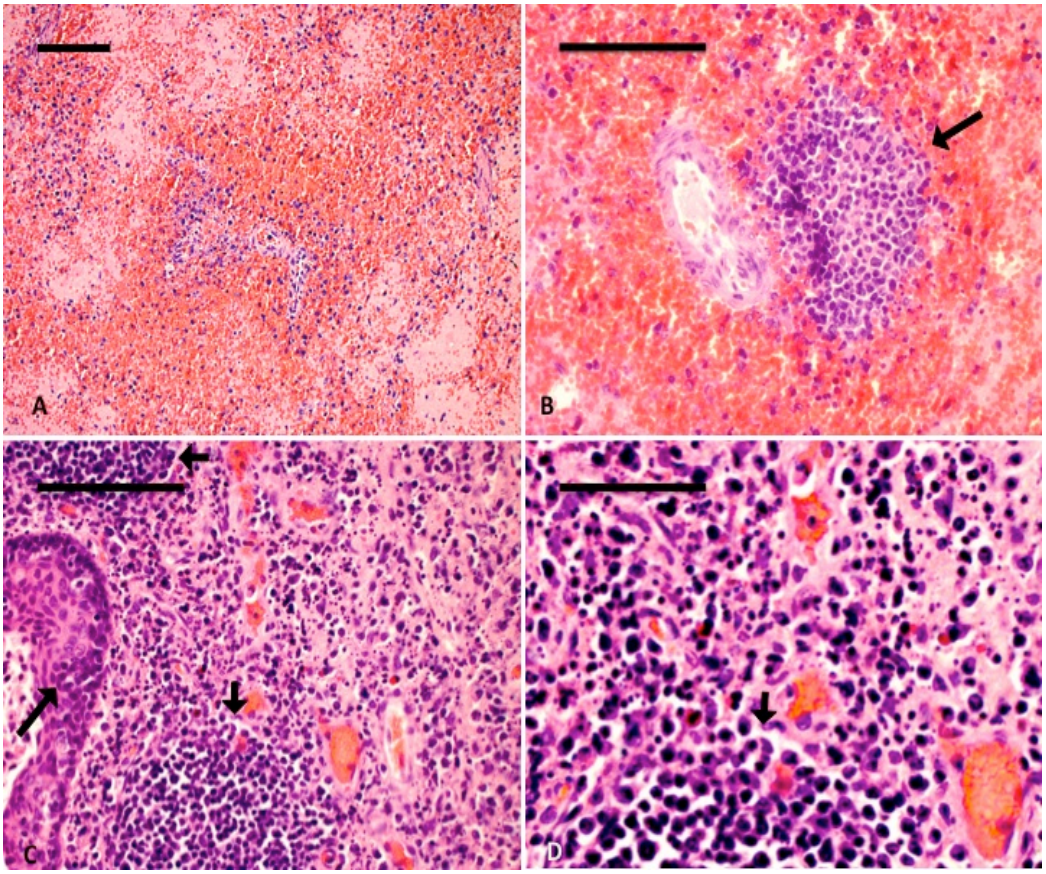

Figure 6. Spleen (A and B) and tonsil (C and D) from pig No.1 with acute ASF. A. Spleen with massive haemorrhagic necrosis of red and white pulp and depletion of the lymphoid tissue. B. Remnant of lymphoid tissue next to a vessel (arrow). C-D. Tonsil with interfollicular lymphocyte necrosis and karyorrhexis. Long arrows point to a part of crypt, short arrows point to part of follicles, in between is an area of lymphocyte necrosis and nuclear fragments. A-D HE stain, A-C Bar=100 µm, D Bar=50 µm.

The lymph nodes were congested. The medullary sinuses were moderately dilated and there was marked lymphocyte necrosis with karyorrhexis and invasion of macrophages in the perifollicular zones and subcapsular area. In the cortical area there was hemosiderosis in many of the lymph nodes (Figure 7 A-C).

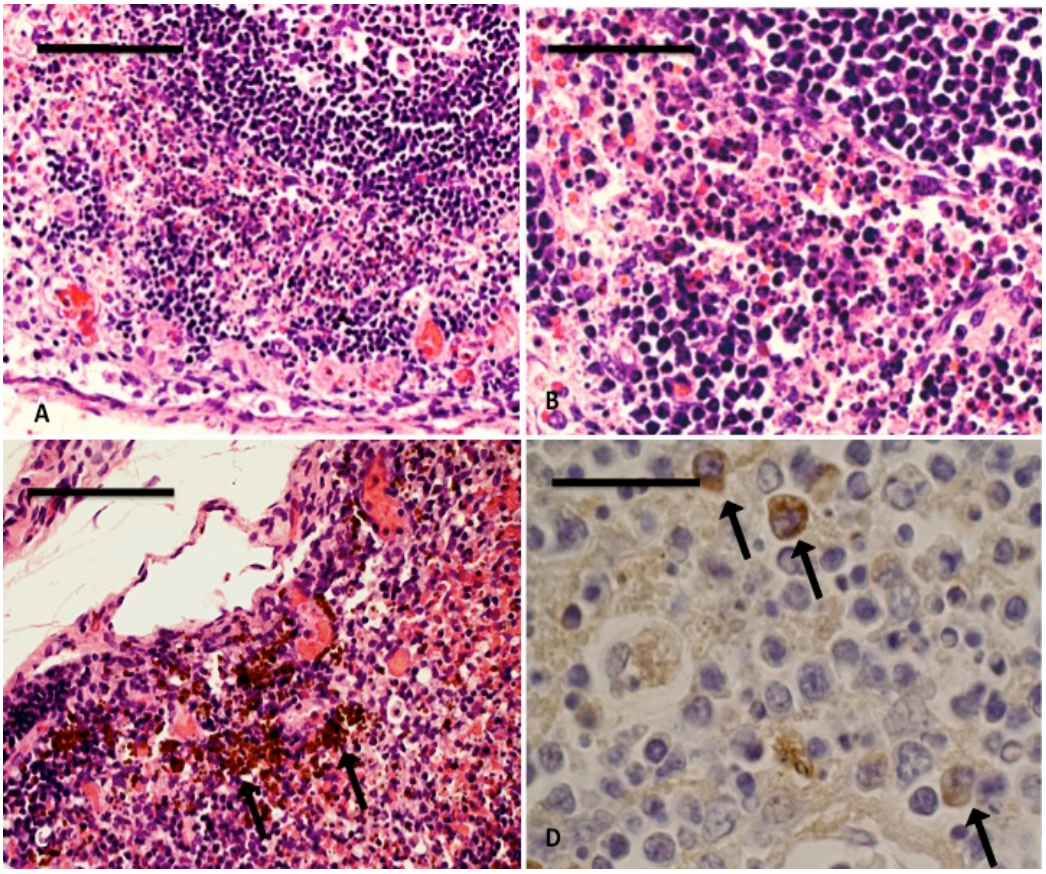

Figure 7. Mesenteric lymph node (A-D) from pig No.1 with acute ASF. A and B. Lymphocyte necrosis and karyorrhexis (A Bar=100 µm, B Bar=50 µm). C. Deposits of hemosiderin in sub capsular area (arrows) (Bar=100 µm). A-C HE stain. D. Immunohistochemistry with Anti-ASFV (1:10) with positive stained macrophage like cells (arrows) Bar=50 µm.

The lung parenchyma was congested and there was interstitial and intraalveolar oedema. There were infiltrates of mononuclear cells in the alveolar septa and lumina and peribronchial infiltrates of lymphocytes with lymphocyte necrosis. Also necrosis of the alveolar capillary endothelium and dilated blood vessels occluded by thrombocyte thrombi was seen (Figure 8 A). The brain parenchyma and meninges were congested. In the meninges and choroid plexus there were also abundant, partly perivascular, lymphohistiocytic infiltrates, with necrosis of mononuclear cells (Figure 8 B). In the white matter there also were perivascular mononuclear cell infiltrates with lymphocyte necrosis (Figure 8 C and D). In one of the cases focal vacuolar degeneration of the brain parenchyma and vascular thrombosis with mainly thrombocytes and erythrocytes were observed.

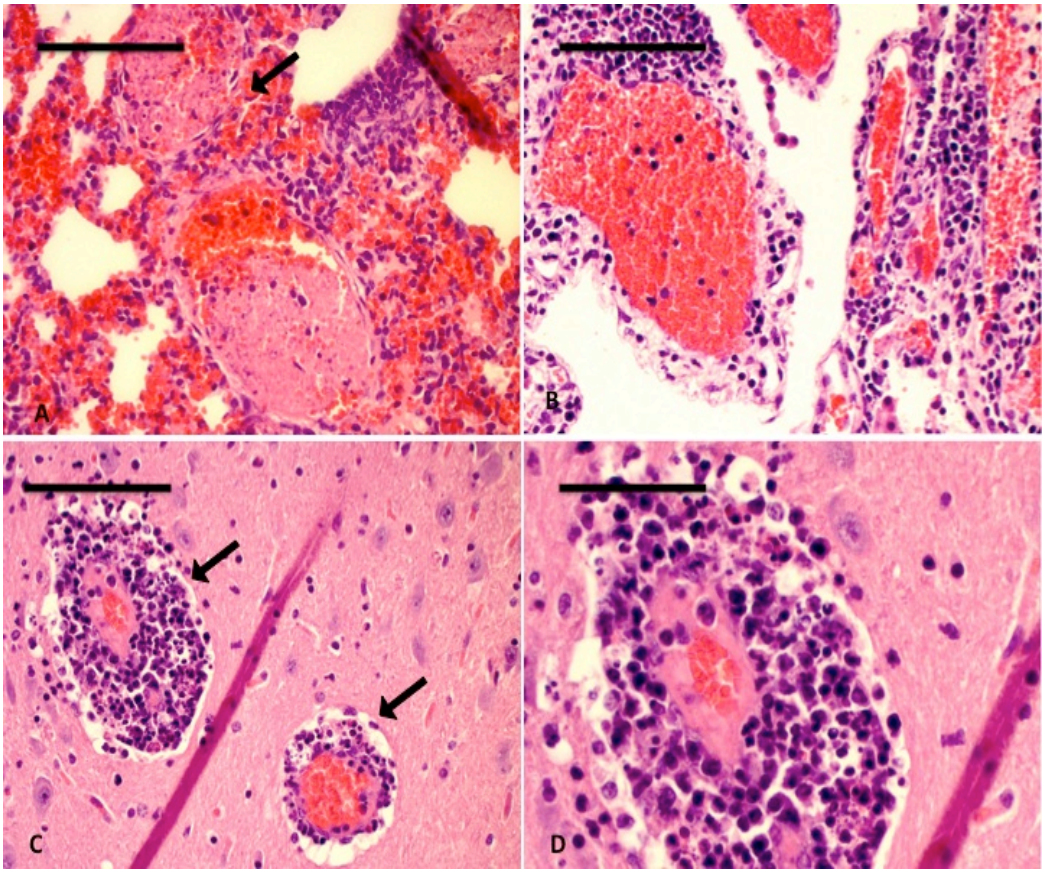

Figure 8. A. Lung with severe congestion and dilated blood vessels occluded by thrombi (arrows). B. Choroid plexus of the brain with severe congestion and abundant, partly perivascular, mononuclear cell infiltrates. C and D. Brain parenchyma with perivascular necrotic mononuclear cell infiltrates showing karyorrhexis (arrows). A-D HE stain, A-C Bar=100 µm. D Bar=50 µm.

The kidneys from all acute cases were severely congested. There was thrombocyte thrombosis in the interstitial vessels, focal necrosis of renal tubules and necrosis of the endothelium in the glomeruli consistent with acute haemorrhagic glomerulonephritis. One of the pigs had abundant PAS-positive protein-rich droplets in the tubules (Figure 9 A). In two acute cases there was fresh fibrin in the glomeruli, shown with MSB-staining (Figure 9 B).

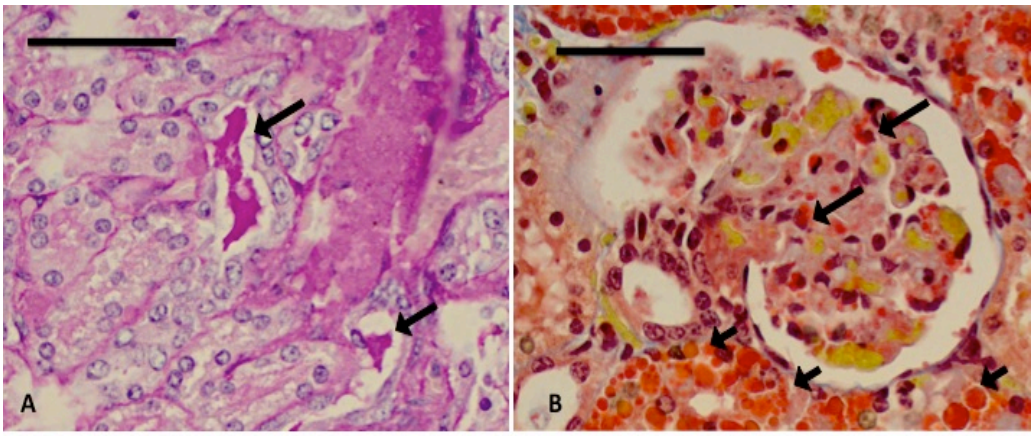

Figure 9. Kidney from pig No. 3 with acute ASF (A, B). A. Tubule with PAS positive protein material (arrows). PAS stain. B. Glomerulus with fibrin (arrows). Droplets of protein material in tubular epithelium (short arrows). MSB stain, fibrin stains red, collagen stains blue, nuclei stain blue/black, erythrocytes stain yellow. A, B Bar=50 µm.

The liver was congested and with focal hepatocyte necrosis. Nuclei in affected hepatocytes

showed pyknosis and karrhyorhexis. Some affected hepatocytes were swollen, but the hepatic cell cord architecture was intact. In the periportal zones and in the interlobular septa there were prominent lymphocyte infiltrates and lymphocyte necrosis. In the heart the only finding was haemorrhages between the muscle fibres. No significant histological lesions were seen in the intestines or in the stomach.

Chronic cases

In the chronic cases the deep inguinal lymph node from one pig had prominent haemorrhages in the perifollicular areas of the cortex, and another lymph node showed prominent oedema with cyst like spaces containing lymphocytes, presumably dilated lymphatic vessels (Figure 10 A, B). One of the pigs had fibrinous deposits mixed with chronic inflammatory infiltrates consisting of lymphocytes, plasma cells and macrophages in the pericardium (Figure 10 C, D). All three pigs had the parasite *Sarcocystis* sp. in the heart muscle. No significant findings were detected in other organs studied as compared to normal controls.

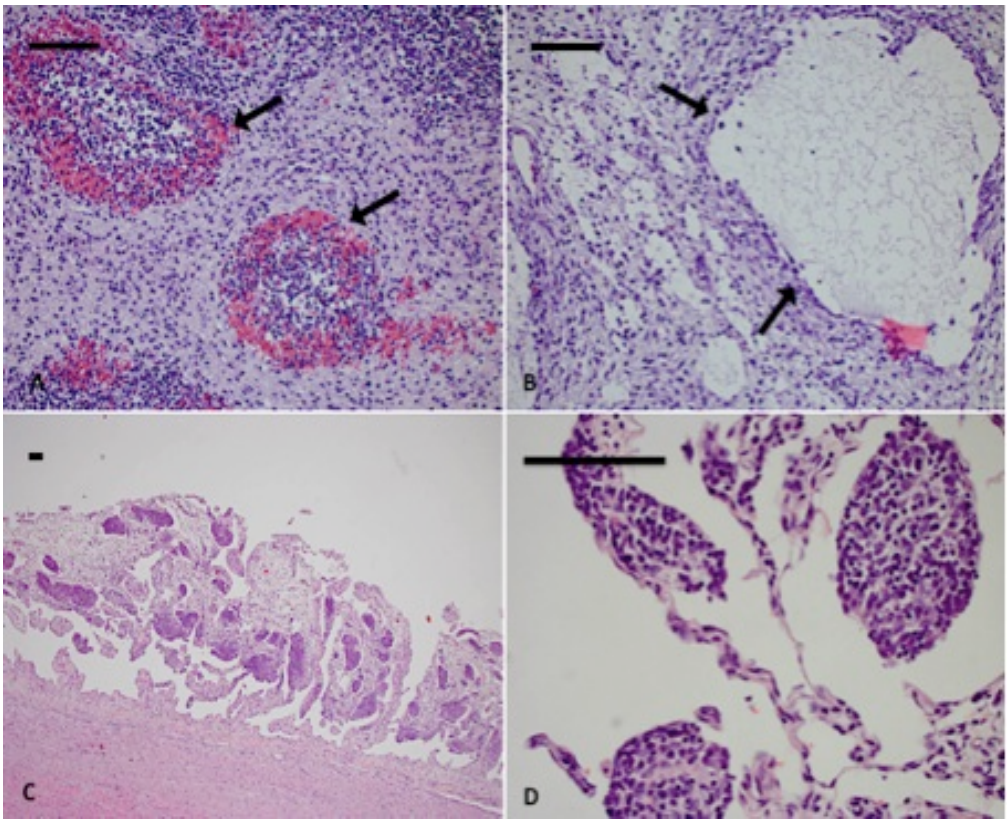

Figure 10. A-D. Deep inguinal lymph nodes and heart from pig No.1 with chronic ASF. A lymph node with prominent hemorrhages in the perifollicular areas of the cortex (arrows). B. Edematous changes with cyst-like spaces in lymph node, presumably a dilated lymphatic vessel (arrows). C-D. Heart with prominent fibrinous pericarditis and chronic inflammatory infiltrates, shown in higher magnification in D. A-D HE stain, A-D Bar=100 µm.

Immunohistochemistry findings in acute ASF

The most prominent finding was moderate to abundant amount of diffusely spread positive stained round cells of macrophage type in the lymphatic tissue (Figure 7 D). These cells were localised in lymph follicles and in the pericortical areas.

DISCUSSION

African Swine fever is one of the highly contagious and serious transboundary animal diseases (TADs) in Uganda, with a significant economic, trade and food security importance for the country.

ASFV affects the same organs and tissues as the Classical swine fever virus (CSFV). Because the clinical signs and macroscopic lesions may be identical in cases of ASF and CSF, virologic diagnosis is essential in any case of suspected swine fever [1]. Until today, Uganda has never reported a case of CSF. The last case of CSF in Africa was reported in South Africa 2007 [30].

According to the literature, the macroscopic lesions in cases of CSF and ASF may be different. In acute CSF, the lesions do not develop as rapidly as in acute ASF. The splenomegaly and hematoma-like visceral lymph nodes characteristic for ASF are usually not seen in CSF [28]. Also pulmonary oedema, common in acute ASF, is rarely seen in CSF. However, microscopic findings such as degeneration of vascular endothelium, fibrinoid arterial changes and extensive necrosis of cells of the MPS (Macrophage Phagocyte System) can be identical in both ASF and CSF. However, the necrosis with karyorrhexis in lymphoid tissues seen in ASF is quite rare in CSF. Renal tubular degeneration with amorphous casts in the medulla is frequent in ASF and rare in CSF. The same applies to presence of necrotic periportal hepatocytes and hepatic lymphocyte infiltration. Another difference between ASF and CSF is that there is much more necrotic cells in the perivascular inflammatory cell infiltrates in the brain in ASF than in CSF cases [28].

The pigs in this study were selected from two different geographical locations in Uganda, the Mityana and the Gulu district, both with RT-PCR confirmed outbreaks of ASF. The cases from Mityana showed both the history and the clinical symptoms typical for acute ASF. The macroscopic lesions were identical with the lesions described in the literature for acute ASF, such as cyanotic skin, generally congested organs with haemorrhages on parietal and serosal surfaces, petechial haemorrhages in the renal cortex, enlarged and haemorrhagic lymph nodes, lung oedema, congested gastric mucosa, haemorrhagic gall bladder mucosa, hydrothorax, hydropericardium and splenomegaly [26]. Also the microscopic findings in the acute cases are consistent with those described in the literature for acute ASF. All spleens showed prominent congestion, even though only one of the spleens was enlarged. It has been described that hyperaemia or haemorrhage is present only in the red pulp [27], while in our cases there was haemorrhagic necrosis of both the red and the white pulp with severe depletion of lymphoid tissue and marked necrosis of mononuclear (macrophage and lymphoid) cells. The dilated medullary sinuses seen in the lymph nodes may be related to the diffuse oedema described in lymph nodes in acute ASF [26]. In the lungs, necrosis of alveolar capillary endothelium, dilated blood vessels occluded by thrombocyte thrombi and lung oedema was present. Those lesions are correlated with the prominent vascular endothelial damage in acute ASFV [26], [28].

The chronic cases from Gulu did not show any clinical symptoms of disease, however, they were all positive for ASFV with RT-PCR two of three times with repetitive blood sampling during the last three months before slaughter. Two pigs were also RT-PCR positive at the time for slaughter and all pigs were seropositive [30].The macroscopic findings in the Gulu pigs, such as enlarged and hyperaemic lymph nodes, ulceration in the stomach mucosa and pericarditis are consistent with the lesions described for subacute and chronic ASF [26]. Also the microscopic findings in the lymph nodes such as haemorrhages in the perifollicular areas of the cortex and dilated lymphatic vessels, as well as the pericarditis are described in this

form of the disease. No immunohistochemistry was made on the chronic cases, but the macroscopic and microscopic findings together with RT-PCR results and the history of a recent ASF outbreak are consistent with chronic ASF.

Symptoms and lesions found in pigs from ASF outbreaks may vary with virulence of the virus [2]. The varying survival rates in outbreaks of ASF are considered to originate from an interaction between the virus, the environment and the host, theoretically due to genetically inherited resistance evolving from a natural selection in the exposed pig populations or a decrease in virulence of the virus in enzootic areas [30].

Chronic ASF is generally caused by less virulent strains of ASFV than acute ASF and chronically infected pigs usually get more diffuse and unspecific symptoms and lesions than pigs with acute ASF [2]. It is believed that subclinical or chronic disease may be due to a genetically inherited resistance to the infection [1].

Different genotypes of ASFV are detected in Uganda. However, the outbreaks in Mityana and Gulu were caused by ASF viruses of the same genotype (gt IX) (Karl Ståhl, personal communication). Different outcome of infection with ASFV with identical genotype in Mityana and Gulu is an interesting finding, which may be explained by differences in virulence of the virus in different geographical locations, differences in susceptibility to the virus in different pig populations, and environmental factors. It may also be a possibility that there was a combination of ASF with other infections. In Gulu, the virulence of the virus may have decreased or/and the pigs may have evolved a genetically inherited resistance to the infection, making them chronically infected or survivors of the acute stage of the disease. Occurrence of ASFV- infected pigs with decreased mortality rates and unspecific clinical symptoms makes it harder to control such cases of ASF compared to acute forms of the disease, because of the higher number of animals surviving the infection spreading the disease combined with the higher risk that the farmers will not report the outbreak to the veterinary authorities.

The detection of ASFV antigen in the tissues of pigs from the acute outbreak in Mityana by immunohistochemistry shows that this technique is useful as a diagnostic method for ASF. However, for reliable immunohistochemistry results it is important that the materials used are correctly stored and that the procedure is performed under optimal conditions. This may be difficult in the current economic situation in the veterinary laboratories in Uganda, possibly limiting the applicability of this diagnostic technique under the present circumstances.

CONCLUSIONS

The macroscopic and microscopic lesions seen in three cases of acute ASF and three cases of chronic ASF resemble the lesions described in the literature for ASF.

The same genotype of ASFV may cause acute or chronic ASF in different geographical locations of Uganda, probably depending on the virulence of virus and/or the resistance of the host.

The detection of ASFV antigen in tissue from the acute cases by immunohistochemistry shows that this was a useful aid in confirming the etiology of the disease in necropsied pigs.

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