PATHOLOGICAL ANATOMY STUDY OF A HEMORRHAGIC DISEASE IN FOUR (4) PIGS.

Estudio Anatomopatológico de una Enfermedad Hemorrágica en Cuatro (4) Cerdos.

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ABSTRACT

Many diseases occur in pigs causing widespread hemorrhages around the world. Among them are viral or bacterial such as, Classical Swine Fever (C S F) which is produced by a Pestivirus (RNA), Togavirus group of the Flaviviridae family, besides African Swine Fever (ASF) produced by an Iridovirus and which has not been diagnosed in Venezuela, according to the literature. Among bacterial diseases are Salmonellosis and Erysipellosis, being the latter uncommon in Venezuela. In this paper, the morphological aspects of a hemorrhagic disease in four pigs are described. The reported lesions are not too dissimilar to those occurring in cases of C S F, which is characterized by affecting many vital organs, producing death in many pigs. C S F often follows an acute course characterized by generalized hemorrhages, with high morbidity and mortality, but it may be chronic, in that case clinical signs and lesions are often minimal or absent. The disease in naturally infected pigs produces lethal lesions causing death in many unvaccinated animals as well as in vaccinated pigs. The gross and microscopic changes of a hemorrhagic systemic disease in four (4) cases under study are reported from two naturally infected unvaccinated pigs and two postvaccinated animals against C S F. Pathologic changes are correlated with clinical signs and the most relevant morphological gross changes and histopathology are reported from naturally infected unvaccinated and postvaccinated pigs. The hemorrhagic lesions reported in vital organs highly resemble and are very similar to those morphologic changes occurring in C S F, although, other hemorrhagic viral or bacterial diseases induced a more severe and different pattern in lesions though being hemorrhagic. Despite lab tests (Elisa) were not determined in this work, they must be ruled out to show the etiological agent of C S F in spite of the typical and very similar morphological changes reported in this paper.

Key words: Pigs, hemorrhagic disease, pathologic anatomy study.

RESUMEN

Hay muchas enfermedades con hemorragias difusas que ocurren en cerdos alrededor del mundo. Estas pueden ser de origen viral como el Cólera Porcino (C P) conocido como Fiebre Porcina Clásica (F P C) o Peste Porcina Clásica (P P C), producido por un Pestivirus (ARN) del grupo Togavirus, de la familia Flaviviridae; o también la Peste Porcina Africana (P P A) producida por un Iridovirus y la cual de acuerdo a la literatura no ha sido diagnosticada en Venezuela. Entre las de origen bacteriano están la Salmonelosis y Erisipelosis porcina, siendo ésta última poco frecuente en Venezuela. Los cambios hemorrágicos observados en los cerdos bajo estudio son muy similares a aquellos que ocurren en la F P C caracterizada ésta, por afectar varios órganos vitales e induciendo una alta tasa de mortalidad. La F P C cursa en forma aguda con hemorragias generalizadas, alta morbilidad y mortalidad o en forma crónica con signos clínicos y lesiones mínimas o ausentes. El agente etiológico de F P C tiene un efecto letal en animales infectados de forma natural y en animales postvacunados induce lesiones letales, que aunque más suaves producen una alta mortalidad, debido a factores de la vacuna o al estatus inmunológico del cerdo. En el presente trabajo, se reportan los signos clínicos y hallazgos macroscópicos e histopatología de cuatro (4) casos de una enfermedad hemorrágica sistémica muy similar a la F P C en dos animales no vacunados y dos de reciente vacunación contra el F P C. De esta manera se reportan, las lesiones típicas macroscópicas y microscópicas en cerdos infectados no vacunados y post-vacunados, en contraposición a las lesiones que ocurren

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en las otras enfermedades virales o bacterianas mencionadas; de acuerdo con la literatura, estas últimas son de mayor severidad y con patrones morfológicos diferentes, aún siendo hemorrágicas. A pesar de que no se realizaron pruebas de laboratorio en este estudio, las pruebas serológicas como ELISA deben ser llevadas a cabo para la identificación del agente etiológico de F P C, debido a que se podría tratar de una enfermedad de declaración obligatoria.

Palabras clave: Cerdos, enfermedad hemorrágica, estudio anatomopatológico.

INTRODUCTION

Among viral diseases in swine, there are two important that occur with widespread hemorrhages and being both fatal "Classical Swine Fever (C S F) and African Swine Fever (A S F)" [13] . Classical Swine Fever, also called Swine Cholera is a viral disease produced by a Pestivirus of the Flaviviridae family, Togavirus genus (RNA). It is a contagious acute to chronic infectious disease of pigs. The disease was first described beginning the XIX century in U.S.A., following an outbreak of swine fever occurred in Europe in 1862, which had the same pathological changes during the disease. Actually, the disease is called Swine Cholera (SC) or C S F [13] to distinguish it from African Swine Fever (ASF), which is clinically similar and morphologically different, although the lesions are most striking and the death rate is greater in the latter. Both diseases are caused by different viruses [2-4, 6, 7, 9 - 13, 15] and ASF has not been reported in Venezuela. In underdeveloped countries where the disease has not been eradicated or vaccines are not applied or not available, CSF produces devastating effects on swine susceptible population. There have been many reports of C S F cases after vaccination, when immune depletion ocurrs or vaccines are not well preserved [2-4, 9, 10, 11, 12, 14-16, 22, 25]. On the other hand, many bacterial diseases (Erysipellosis, Salmonellosis and Pasteurellosis) are also hemorrhagic but they induce different patterns of morphological changes which allow for differentiating them from C S F.

The disease (CSF) is produced by a small RNA virus (Pestivirus) which belongs to Togavirus genus of Flaviviridae family [2, 3, 6, 7, 9, 10, 12, 13], and only affect wart hogs and domestic pigs, despite experimental infections can ocurrs in few other species (Peccaries). Togavirus is moderately labile, worldwide spreaded and penetrates by natural ingestion or inhalation, also by skin or semen [2, 3, 9, 10, 13, 15, 18, 19, 21, 22, 25]. The virus invades lymphoid tissues (tonsils, lymph nodes, spleen, and liver); it also damages endothelial cells of blood vessels producing hemorrhages with lymphocytic infiltrates in meninges, brain, cerebellum, kidneys and skin [2, 6, 7, 9, 10, 13-15, 17, 22].

C S F may be acute, subacute or chronic, having each form specific signs. In the acute form, animals present fever (40.5 to 42°C), lethargy, progressive weight loss and mostly incoordination. Purple discoloration on the tips of ears, belly and back are common [9, 10,13,15,16, 22, 25]. Though mortality is high, some animals may recover going to the chronic form, showing general depression and respiratory problems with diarrhea. Acute cases with evident lesions suggestive of C S F have been reported in the literature when massive vaccination is performed, showing similar clinical signs as those of animals with natural infection [2-4, 6, 9, 10, 13, 14, 15, 17, 22, 25].

MATERIALS AND METHODS

Animals

A total of four (4) cross York x Landrace pigs, from 2-7 months of age; 2 females and 2 males, having clinical signs of weakness, incoordination, yellowish diarrhea and dysentery, cyanosis on the ears, belly and back, were referred to the pathology service at veterinary policlinic of the University of Zulia to be necropsied. These animals have come from different geographical zones of Zulia State, La Cañada de Urdaneta. County and Villa del Rosario of Perijá County, and died while were translated on a truck to be relocated in other pig farms. These animals were sent to the pathologic anatomy laboratory to be studied. Two (2) out of four (4) pigs were referred as being vaccinated against C S F a week before the onset of clinical signs, while the other two pigs were not vaccinated. All pigs showed similar clinical signs (Purple discoloration of the tips of ears, belly end back, lethargy, dysentery and incoordination) being more severe in those unvaccinated pigs, because of the sudden death, the body temperature was not taken.

Pathologic Anatomy

A gross evaluation was done during necropsy by routine procedures in all animals [1, 5]. The morphological relevant gross changes were observed in organs, such as: skin, kidneys, large intestine, lymph nodes, spleen and brain [13]. Tissue samples were taken for histopathological evaluation by routine procedures [1, 5, 8]; tissue staining techniques with hematoxilin and eosin (H-E) were done on tissue samples [8]. Gross and microscopic photographs were taken to record all morphological relevant changes for diagnostic. Tissue samples were saved for ulterior laboratory tests, blood seroaglutination, Indirect Inmunofluorescent Assay (IIA) or (Elisa) evaluation. These tests will be ruled out to show the etiological agent.

RESULTS AND DISCUSSION

Gross and microscopic lesions

The gross and microscopic changes were compatible with a hemorrhagic disease, resembling to CSF which is an infectious pathologic entity of viral etiology, that occurs often in pigs and is characterized in most cases for an acute course

with high mortality in naturally infected animals; few infected pigs may recover of the disease being later carriers of virus. These animals (carriers) play an important role in spreading the disease and may cause epidemic outbreaks in susceptible populations [2, 3, 6, 7, 9, 10, 13, 15, 22, 25], which could have happened in the four cases reported in this paper, due to the onset of the disease during translation of pigs.

The clínical signs, such as incoordination, lethargy and dysentery showed by all pigs are closely related to the gross and microscopic changes reported in this work, as well as are very similar to those lesions referred in the literature as CSF [2, 3, 6, 9, 10, 13, 15, 16, 22]. The four cases reported revealed typical, relevant and consistent clinical signs and lesions which are in agreement with those reported by other authors in CSF [2, 3, 6, 7, 9 - 13, 15, 16, 21, 22, 25]. Even though, Indirect Inmunofluorescent Assays (IIA) test were not run on tissue samples, due to the lack of kits as well as their cost for this kind of test in Venezuela. Nevertheless, the IIA or ELISA tests should be ruled out to demonstrate the viral causal agent as it is stated by International Epizootics Organization (IEO) [13, 15, 22, 24, 25] in order to say it is CSF.

The pathologic anatomy study revealed many morphological changes consistent with a systemic hemorrhagic disease in organs, such as: skin, kidneys, intestine, lymph nodes, spleen and brain, showing a pattern that suggests a viral infection resembling CSF. The skin had purple discoloration consistent with hemorrhagic lesions on belly, tips of ears and back (FIG. 1). The kidney had many petequial and ecchymotic subcapsular and cortical hemorrhages, giving an image as a “turkey egg” appearance (FIGS. 2 and 3). Microscopically, kidney tissue had interstitial and glomerular hemorrhages (FIG. 4) along with interstitial lymphocytic inflammatory cells infiltrate [2, 5, 15]. Submandibular lymph nodes were swollen with cortical petequial and diffuse hemorrhages (FIG. 5), lymph nodes had the cut surface appearance of strawberry like (FIG. 6). Microscopically, there were areas of subcapsular hemorrhages and depletion of lymphocytes in lymphoid germinal centers, along with necrosis and inflammatory cell infiltrated of neutrophils (PMNN) and macrophages (FIG. 7). There was severe splenomegaly as compared with the large curvature of stomach and the spleen showed areas of hemorrhagic infarcts of variable size and shape (FIGS. 8 and 9). Microscopically, there was depletion and necrosis of white pulp, while the red pulp had focal and diffuse areas of necrosis and hemorrhages with leukocytes infiltrated (FIG. 10); there was also necrosis and hyalinization of follicular and trabecular blood vessels walls. The brain had meninges with blood vessels dilatation and hemorrhages, these changes were less severe in vaccinated pigs (FIG. 11). Microscopically, there were areas of hemorrhages and focal perivascular cuffing of lymphocytes on white matter (FIG. 12) as well as moderate gliosis. Intestinal loops were distended and redness with multiple petequial serous hemorrhages and necrosis (FIG. 13). The ileocecal mucosal area had bottom like ulcers (FIG. 14) this has been considered a very

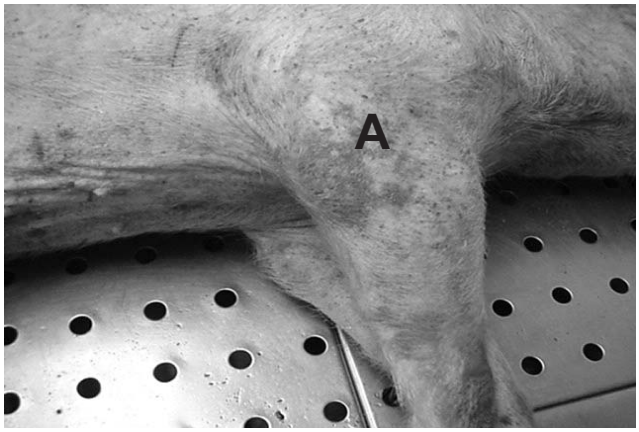

FIGURE 1. MACROPHOTOGRAPH. SKIN. NOTE BLUISH COLORATION ON CRANEAL LIMBS, BELLY AND NECK (A) / MACROFOTOGRAFÍA. PIEL. NÓTESE COLORACIÓN VIOLÁCEA EN MIEMBROS CRANEALES, ABDOMEN Y CUELLO (A).

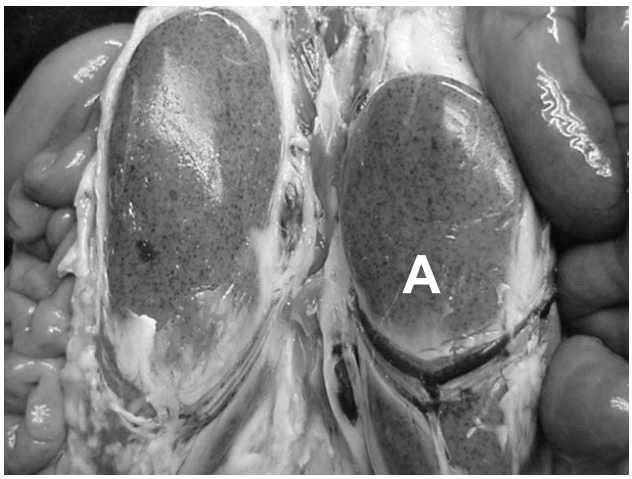

FIGURE 2. MACROPHOTOGRAPH. KIDNEYS. NOTE MANY SUBCAPSULAR PETEQUIAS(A) / MACROFOTOGRAFÍA. RIÑONES. NÓTESE PETEQUIAS SUBCAPSULARES (A).

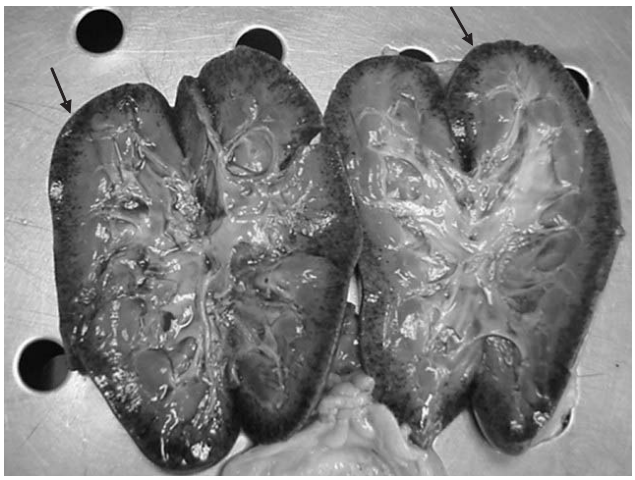

FIGURE 3. MACROPHOTOGRAPH. KIDNEYS. NOTE PETECHIAL HEMORRHAGES ON CORTEX (ARROWS) / MACROFOTOGRAFÍA. CORTE SAGITAL DE RIÑONES. NÓTESE HEMORRAGIAS PETEQUIALES EN CORTEZA (FLECHAS).

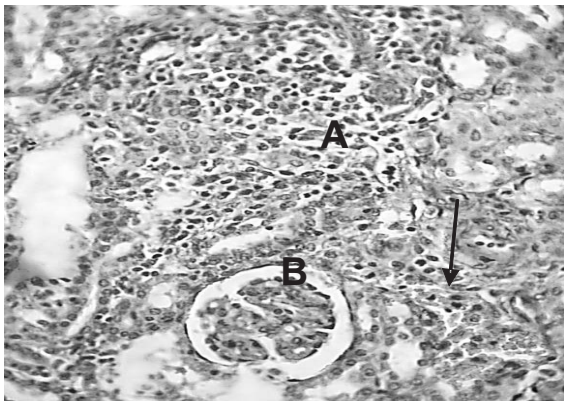

FIGURE 4. MICROPHOTOGRAPH. KIDNEY. NOTE INTERSTITIAL HEMORRHAGE (Arrow), ALONG WITH INTERSTITIAL LYMPHOCITIC INFILTRATED (A) AND GLOMERULAR HIPERPLASIA (B). H-E. X40 / MICROFOTOGRAFÍA. RIÑÓN. NÓTESE HEMORRAGIA INTERSTICIAL (FLECHA), CON INFILTRADO CELULAR LINFOCÍTICO (A) E HIPERPLASIA DEL GLOMÉRULO (B). H-E. X40.

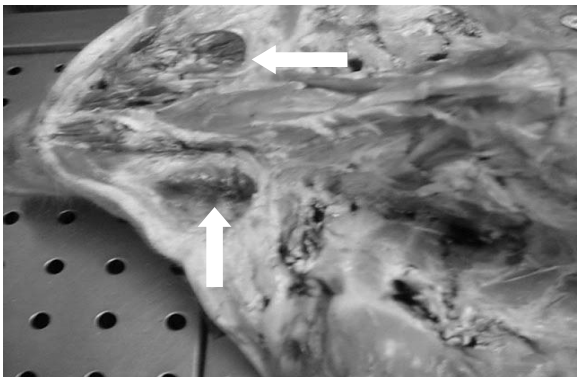

FIGURE 5. MACROPHOTOGRAPH. NOTE SUBMANDIBULAR LYMPHOID NODES (White Arrows) SWOLLEN AND HEMORRHAGIC / MACROFOTOGRAFÍA. NÓTESE NÓDULO LINFOIDE SUBMANDIBULAR TUMEFACTO Y HEMORRÁGICO (FLECHAS BLANCAS).

FIGURE 6. MACROPHOTOGRAPH. LYMPHOID NODES. NOTE BRILLIANT RED CORTICAL HEMORRHAGES ON CUTSURFACE STRAWBERRYLIKE (White arrows) / MACROFOTOGRAFÍA. GRUPO DE NÓDULOS LINFOIDES SUBMANDIBULARES, FARÍNGEOS, PREESCAPULARES Y POPLÍTEOS. OBSERVE APARIENCIA DE FRESA CON HEMORRAGIAS EN CORTEZA DE COLOR ROJO BRILLANTE (FLECHAS BLANCAS).

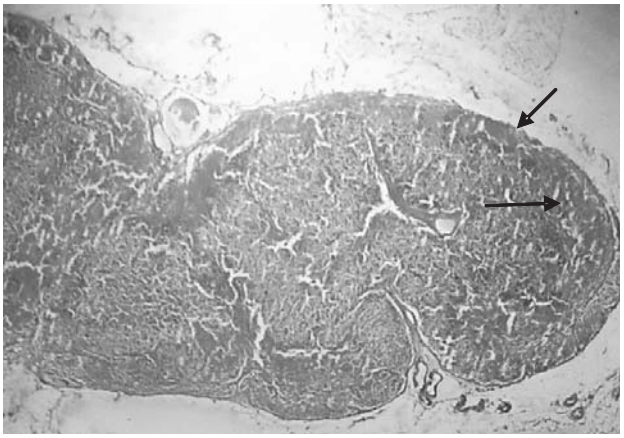

FIGURE 7. MICROFOTOGRAPH. LYMPHOID NODE. NOTE SUBCAPSULAR HEMORRHAGE (Arrows). H-E. X 10/ MICROFOTOGRAFÍA. LYMPH NODE. NÓTESE HEMORRAGIAS SUBCAPSULARES (FLECHAS) H-E. X10.

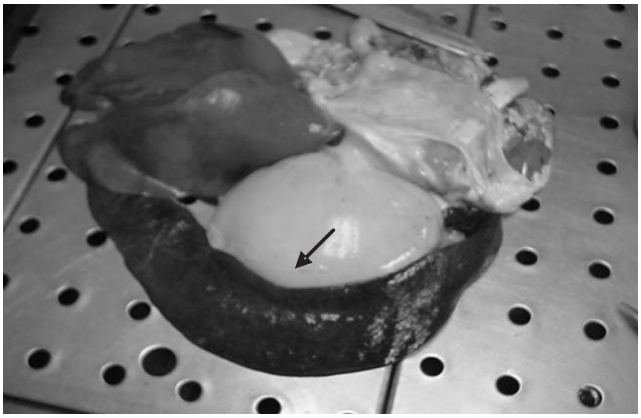

FIGURE 8. MACROPHOTOGRAPH. SPLEEN. NOTE SWELLING AND INCREASE SIZE (A) AS COMPARED TO THE LARGE CURVATURE OF STOMACH (Arrow). / MACROFOTOGRAFÍA. BAZO. NÓTESE ESPLENOMEGALIA (A) AL COMPARAR SU LONGITUD CON LA CURVATURA MAYOR DEL ESTÓMAGO (Flecha).

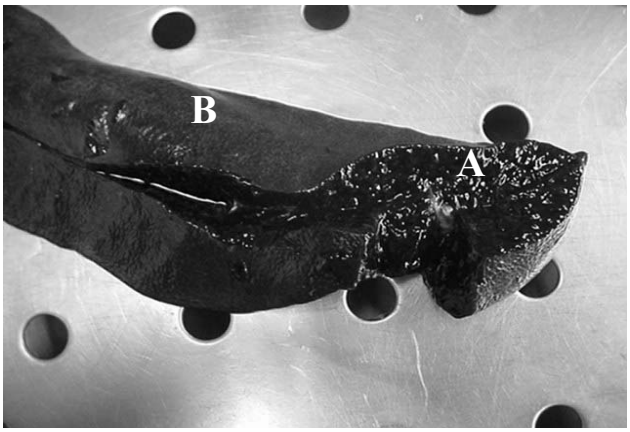

FIGURE 9. MACROPHOTOGRAPH. SPLEEN. NOTE BULGING CUTSURFACE (A) AND AREA OF INFARCT (B)/ MACROFOTOGRAFÍA. BAZO. NÓTESE SUPERFICIE DE CORTE PROTRUYENTE (A) Y ÁREA DE INFARTO (B).

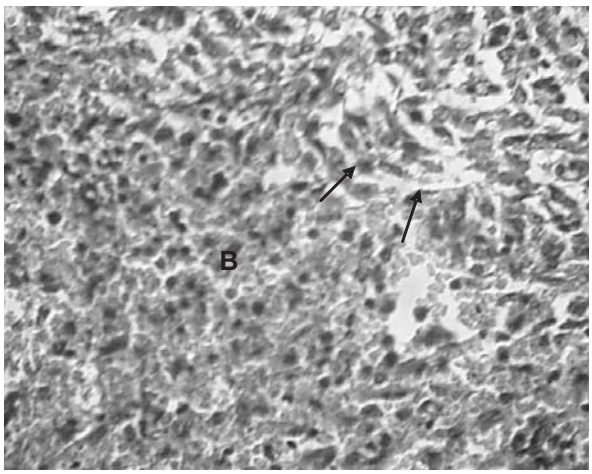

FIGURE 10. MICROPHOTOGRAPH. SPLEEN. NOTE WHITE PULP DEPLETION WITH LACK OF LYMPHOCITES (Arrows) AND RED PULP WITH FOCAL AND DIFUSE AREAS OF HEMORRHAGIC NECROSIS ALONG WITH LEUKOCYTES INFILTRATED (B). H-E. X60/ MICROFOTOGRAFÍA. BAZO. OBSÉRVESE DEPLECIÓN DE PULPA BLANCA CON ESCASO TEJIDO LINFOIDE (FLECHAS) Y ÁREAS DE HEMORRAGIA DIFUSA Y NECROSIS CON INFILTRADO CELULAR DE LEUCOCITOS (B) H-E. X60.

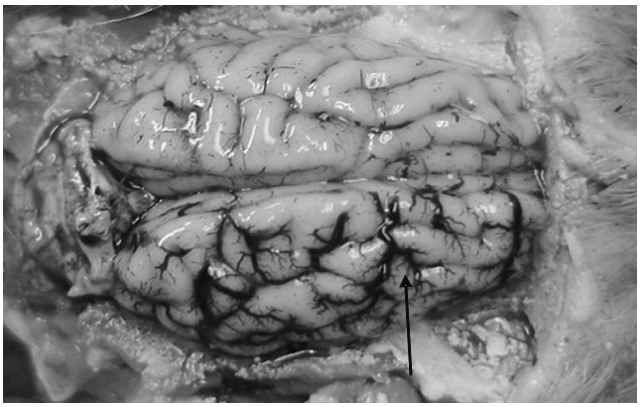

FIGURE 11. MACROPHOTOGRAPH. BRAIN. NOTE CONGESTION AND HEMORRHAGE OF LEPTOMENINGEAL BLOOD VESSELS / MACROFOTOGRAFÍA. CEREBRO. NÓTESE CONGESTIÓN Y PLÉTORA SANGUÍNEA CON HEMORRAGIAS DE VASOS EN LEPTOMENINGES.

typical lesion, compatible with CSF. Microscopically, the intestinal tissue had focal mucosal ulcers with hemorrhage and necrosis; a cellular lymphocytic infiltrated was present in lamina propia and submucosa (FIG. 15). All morphological gross and microscopic changes reported are in agreement with other author's publications [9, 10, 13, 15, 16, 21] on CSF. The predominant hemorrhagic lesions found in the four (4) cases are typical and highly suggestive to CSF due to the effect of a viral infection, being the most striking changes, necrosis and hemorrhages of lymphoid tissue, as well as blood vessels walls degeneration and necrosis which have been reported in the literature [2-4, 6, 7, 9, 10, 13, 15, 25]. Endothelial changes are mainly degenerative which produce hemorrhages by rexis of the wall. The severity of lesions depends on susceptibility of

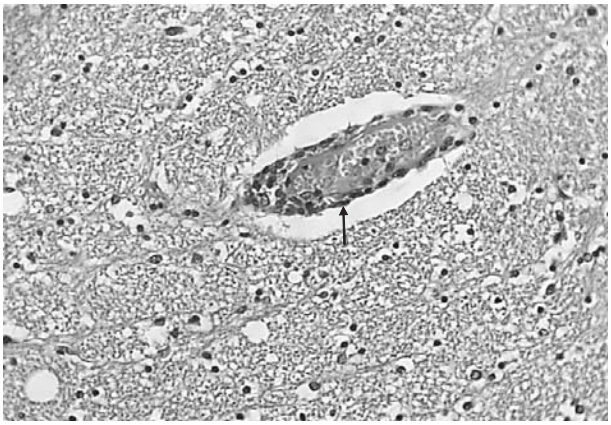

FIGURE 12. MICROPHOTOGRAPH. BRAIN. NOTE LYMPHOCITIC PERIVASCULAR CUFFING (Arrow) AND FOCAL HEMORRHAGE (upper left corner). H-E. X 40/ MICROFOTOGRAFÍA. CEREBRO. OBSÉRVESE INFILTRADO CELULAR LINFOCÍTICO PERIVASCULAR (FLECHA) Y HEMORRAGIA FOCAL (ESQUINA SUPERIOR IZQUIERDA) H-E. X40.

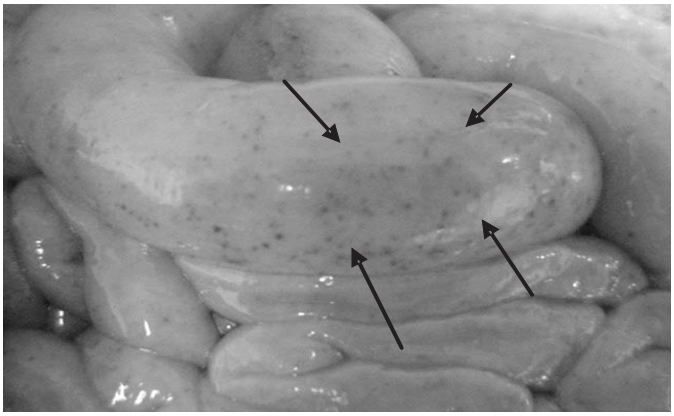

FIGURE 13. MACROPHOTOGRAPH. INTESTINE. NOTE PETECHIAS ON SEROUS LAYER (Arrows) / MACROFOTOGRAFÍA. INTESTINO. NÓTESE HEMORRAGIAS PETEQUIALES EN CAPA SEROSA (FLECHAS).

pigs and their immune status as well as the pathogenicity of virus are reported in two of the four cases in this study, this is in agreement with other authors [11-15, 23, 25]. Therefore, in the present study, the relevant morphological lesions in two unvaccinated cases from natural infection and the reaction of two postvaccinated cases are reported [2, 3, 6, 7, 11- 15, 17, 20, 22] to be a hemorrhagic systemic disease with lesions compatible to CSF.

CONCLUSIONS

- An hemorrhagic severe infectious disease of high mortality is reported in several pigs of Zulia State, Venezuela.
- The gross and microscopic lesions reported in the studied cases are highly suggestive of a viral disease, due to its pathogenicity and affinity for lymphoreticular and endothelial cells of blood vessels in different vital organs,

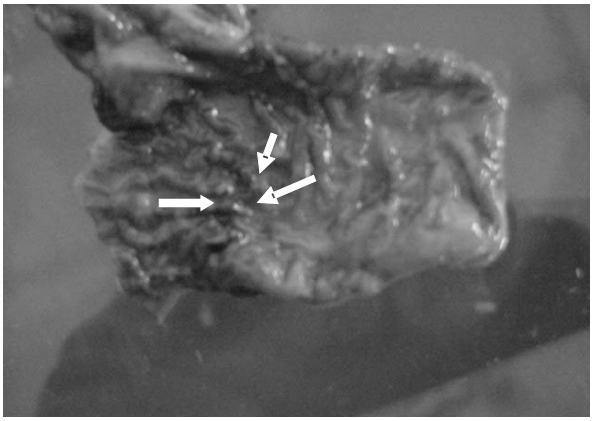

FIGURE 14. MACROPHOTOGRAPH. LARGE INTESTINE (CECUM). NOTE PRESENCE OF BOTTOM ULCERS (White Arrows).TIFLITIS / MACROFOTOGRAFÍA. INTESTINO GRUESO (CIEGO). NÓTESE PRESENCIA DE ULERAS EN BOTÓN Y HEMORRAGIA (FLECHAS BLANCAS). TIFLITIS.

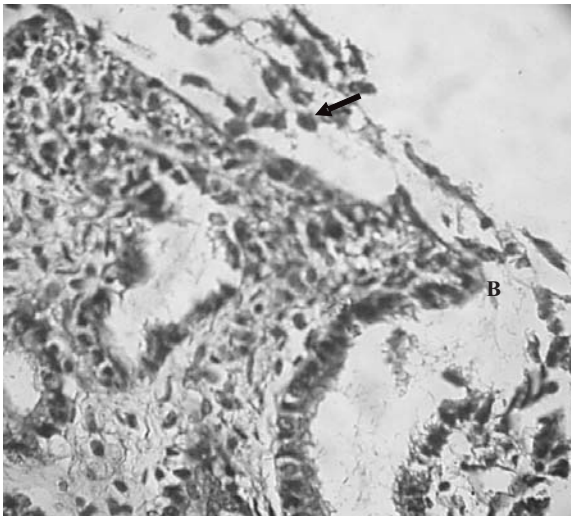

FIGURE 15. MICROPHOTOGRAPH. NOTE PRESENCE OF ULCERS (A) WITH NECROSIS(B) AND CELLULAR LYMPHOCYTIC INFILTRATED IN LAMINA PROPIA (Arrow). H-E. X40 / MICROFOTOGRAFÍA. OBESÉRVESE LA PRESENCIA DE ULCERACIÓN (A) CON NECROSIS DEL EPITELIO (B) E INFILTRADO CELULAR LINFOCÍTICO (FLECHA) EN LAMINA PROPIA. H-E. X40.

while bacterial hemorrhagic diseases do not induce lesions on vessels endothelium.

– The relevant and diagnostic lesions reported in organs like spleen, lymphoid tissue, large intestine, kidneys and central nervous system are very typical of a disease called Classical Swine Fever. African swine fever was not considered because it has not been diagnosed in Venezuela according to the literature, neither bacterial due to the pattern of lesion and lack of bacterial colonies in histological evaluation. Though, it is necessary to run serological and Elisa tests to confirm the diagnoses of C S F.

– Inadequate vaccination in animals may induce signs and lesions similar to those occurring in naturally infected pigs as reported in this paper. This occurred in two out of the four pigs studied in this work. Lesions were found to be more severe in naturally infected unvaccinated pigs than in vaccinated pigs.

RECOMMENDATIONS

– To determine the viral status of Swine Classic Cholera in all pig population at Zulia State, Venezuela.

– To run IIA and Elisa tests on samples from pig farms in Zulia State.

– To establish a vaccination plan under surveillance of veterinarians in all pig farms of Zulia State.

– To keep a good handling and preservation of vaccines as a biological product of high risk.

– To apply vaccines adequately in healthy pigs to avoid any undesirable postvaccination effect, due to immune depletion or deaths in diseased pigs.

– To certificate all vaccinated pigs before moving to slaugther house or

– to other pig farms and request their vaccination certificated for mobilization of pigs in the region.

– To report and notify official government about outbreaks of cases to coordinate control measures periodically.

ACKNOWLEDGMENTS

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