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DUBDUBA SYNDROME: AN EMERGING NEUROLOGICAL DISEASE OF CAMELS WITH A POSSIBLE VIRAL ETIOLOGIC AGENT

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ABSTRACT

Following reports on the emergence of a new neurological disease in camels at the north-eastern region of Saudi Arabia, a study was carried out to investigate the magnitude of the problem and to identify its etiologic agent. Based on results of a questionnaire study, the disease appeared for the first time during the rainy season in the year 2004. It affected she-camels of 3 years or more and was limited to those herds that had been grazing the Dubduba area of north-eastern Saudi Arabia. For this reason the disease has been given the name "Dubduba syndrome" by the investigation team. The disease was sporadic, affecting 1 or 2 camels in the herd at a time, but the mortality rate in those affected animals was very high (>90%). The clinical signs were fever, Parkinsons-like tremors of the head, paralysis of the lower lip, motor paralysis, recumbency and animals usually died within 3-5 days of appearance of symptoms. Three camels that died of the disease were autopsied. Congestion and haemorrhages were evident in the brain and meninges. Congestion, haemorrhages and necrosis were seen in the liver, kidneys, heart and stomach. Histopathological examination of the brain and spinal cord showed non-purulent meningoencephalomyelitis, nerve cell necrosis, gliosis and presence of intracytoplasmic inclusion bodies in the nerve cells. The liver was massively fatty degenerated and necrotic. Coagulation necrosis was seen in the renal tubular epithelium which also contained intracytoplasmic inclusion bodies. Since the histopathological findings of Dubduba disease supported a virus infection, virus isolation was attempted on SPF embryonated eggs and laboratory rats but the results were negative. Immunohistochemical studies on paraffin tissue-sections of the brain were negative for the following viruses that are likely to cause the above lesions i.e. Rabies, Crimean-Congo fever, Equine herpse, West Nile Fever (WNF), Panflavivirus, paramyxovirus and Rift Valley Fever. Listeriosis was also negative. A novel viral encephalitis disease was most likely to be the cause of Dubduba syndrome of camels.

Key words: Camel, Dubduba syndrome, hepatitis, meningo-encephalomyelitis

Diseases that cause neurological signs in animals are many and are usually caused by a variety of etiological agents, such as viruses, parasites, bacteria, neuro-toxic chemicals and plant poisons as well as genetic abnormalities and nutritional deficiencies. Acute lymphocytic encephalitis is usually caused by viruses that invade the central nervous system (Jones *et al,* 1997). With the exception of rabies (Al-Dubaib, 2007; Afzal *et al,* 1993; Atta *et al,* 1993; El-Mardi and Ali, 2001; Ali *et al,* 2004), viral diseases that cause clinical neurological signs in the camel are poorly documented in the literature. Of the bacteria, lymphocytic encephalitis is caused by *Listeria monocytogenes,* which is abundant in nature and in animal secretions and excretions (Hurst and Zee, 1999). Listeriosis is not known to occur in camels.

Acute lymphocytic encephalitis or encephalomyelitis is common in many viral infections in domestic animals as well as man. Histopathologically, it is characterised by perivascular lymphocytic cuffing of blood vessels in the brain and/or spinal cord and usually accompanied by gliosis and meningitis. Rabies virus, Borna disease virus, Flaviviruses, Buynaviruses, paramyxoviruses, herpesviruses and many others can cause lymphocytic encephalitis (Jones *et al,* 1997). Al-Hizab and Abdelsalam (2007) reported non-purulent encephalitis in 5 camels at the eastern region of Saudi Arabia and attributed the cause to an unknown viral infection. They excluded rabies virus as an etiological agent of the disease because of lack of typical clinical signs of the disease as well as absence of Negri bodies in histopathological sections of the brain, which are pathognomic for rabies virus infection.

Viruses have always mutated to infect a new host or become more virulent, and the emergence of

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new viral diseases in man and animals is a known worldwide problem. Medani (2005) described a new neurological and fatal viral disease in humans handling animals in Saudi Arabia. A flavivirus closely related to the tick-borne Kyasanur Forest Disease virus was isolated and named Alkhurma Haemorrhagic Fever virus (AHFV). To date, AHFV has been isolated from human samples and genetic and serologic characterisations have grouped the agent with tick-borne flaviviruses (Zaki, 1997; Charrell *et al,* 2007). The virus has been isolated from ticks collected from camel resting points and this supported the theory of the role of camels in AHFV transmission cycle as well as the zoonotic nature of the disease (Charrell *et al,* 2007).

One of the viruses that crossed its geographic limits is Rift Valley Fever virus and is known to infect humans, sheep and cattle in Africa causing acute and sometimes fatal encephalitis and hepatitis (Rippy *et al,* 1992). The virus might also cause nerve cell necrosis, intranuclear inclusion bodies in the brain cells as well as renal tubular necrosis in newborn lambs (Coetzer, 1977). Rift valley Fever virus is endemic in western region of Saudi Arabia (Elfadil, 2006). Antibodies against the virus have not been demonstrated in sera of camels before 2003 (Al-Afaleq *et al,* 2003). However, it is now known to infect camels without producing the clinical disease in this species (Abbas and Omer, 2005; Al-Afaleq *et al,* 2007).

West Nile Fever virus (WNFV) is one of the haemorrhagic fever viruses that was first reported in Uganda, in the 30th of the last century, but it is now of worldwide distribution. What made this virus very successful in spreading worldwide is that, it is harboured in birds, that can take it across borders, and it is transmitted by mosquitoes, which can spread the infection actively to many humans or animals in the area. Antibodies against WNFV have been detected in llamas manifesting a clinical neurological disease (Whitehead *et al,* 2006). Antibodies against WNFV have been demonstrated in sera of healthy dairy camels at United Arab Emirates by Wernery *et al* (2007). However, the virus has not been isolated from camels suffering from a clinical neurological disease in the area.

Crimean-Congo haemorrhagic fever (CCHF) virus is a tick-borne member of the Bunyaviridae family (Nairovirus genus) that causes severe haemorrhagic disease in humans. Many vertebrates are able to replicate the virus. The virus was first observed in the Crimean of Russia in 1944 and isolated in Congo in Africa in 1956. It is of worldwide distribution and is reported in about 30 countries in Africa, Asia and Europe (Drosten *et al,* 2003). In mammals, the infection is usually subclinical and asymptomatic. Antibodies against the virus has been demonstrated in serum of healthy camels from Sudan and Kenya imported to Egypt (Morrill *et al,* 1990).

Due to the harsh and inaccessible habitat in which the camel lives, many of its diseases skipped reporting. People had the erroneous belief that camels are resistant to infectious diseases. Dirie and Abdulrahman (2003) reported on some unknown disease of camels in the Horn of Africa. They described a fatal neurological disease, which they called ''Laaba''. The disease appeared after the *El-Nino* phenomenon of 1997-1998. The pathology and etiology of this disease remained unknown.

Following reports of emergence of a highly fatal neurological disease in adult camels grazing the Dubduba area of the north-eastern region of Saudi Arabia, an investigation team was formed to identify the etiological agent as well as control its spread. The disease was believed to be the result of the Gulf war by the camel herdsmen, and since its etiology has not been identified, it was given the name "Dubduba syndrome of camels". The results of the pathological studies carried on 3 camels dying of the disease are described below and they point to a viral infection that is likely to belong to the haemorrhagic fever group. There is a strong belief by the veterinarians in the area that the disease is tick-transmitted.

Materials and Methods

Three she-camels that died with neurological signs were autopsied and pieces of tissues from organs showing lesions were fixed in formol saline. These were later processed in wax, sectioned and stained with hematoxylin and eosin for routine histopathology.

Liver, kidneys, spleen and brain were sliced open and impression smears were made from them. They were stained with Gram stain for demonstration of bacteria.

Immunohistochemical methods were carried out on paraffin sections to investigate possible viral infections by standard methods (Emmons and Riggs, 1977; Tsai, 1999; Ellis *et al,* 2005). Considering the geographical location of Saudi Arabia, immuno-histochemistry investigation was carried out for the following viruses that are known to invade the nervous system in farm animals: Rabies, Rift Valley Fever, West Nile Fever, Crimean-Congo fever, Pan flavivirus, Herpsevirus and Paramyxovirus. *Listeria monocytogenes,* a bacterium that is known to infect the brain of farm animals was also investigated by immunohistochemistry.

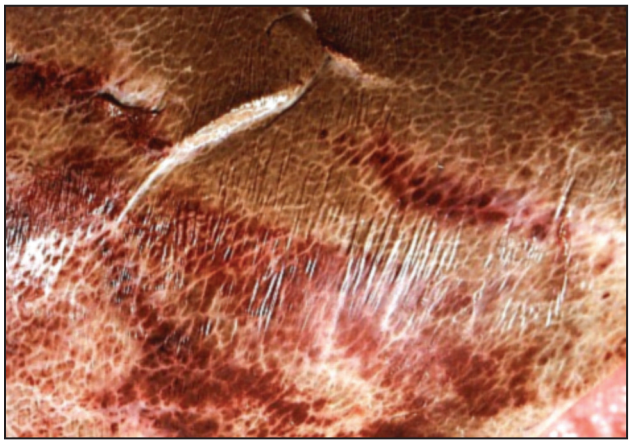

Fig 1. Haemorrhages on the liver capsule.

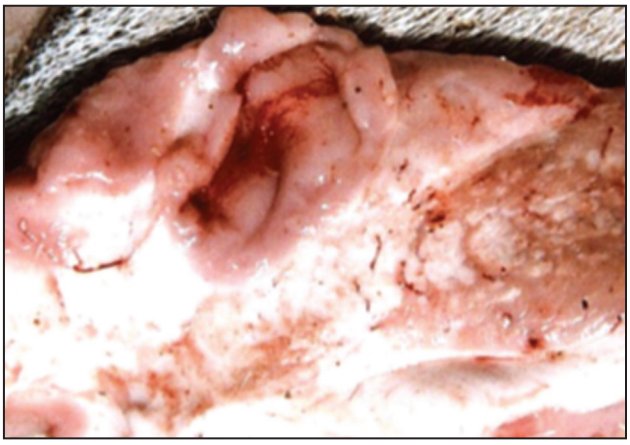

Fig 2. Congestion and haemorrhages in the brain.

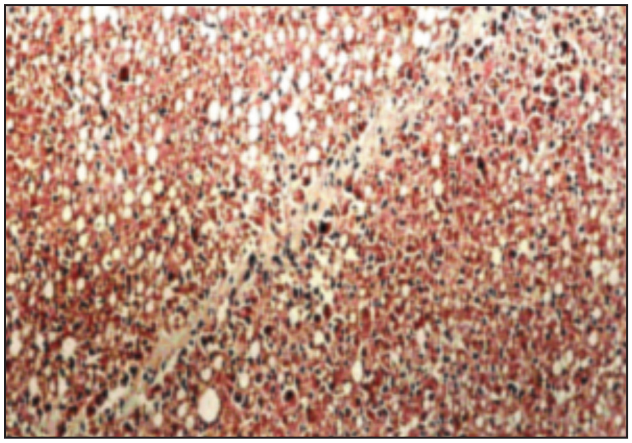

Fig 3. Fatty degeneration and coagulation necrosis of hepatocytes. H&E x 125.

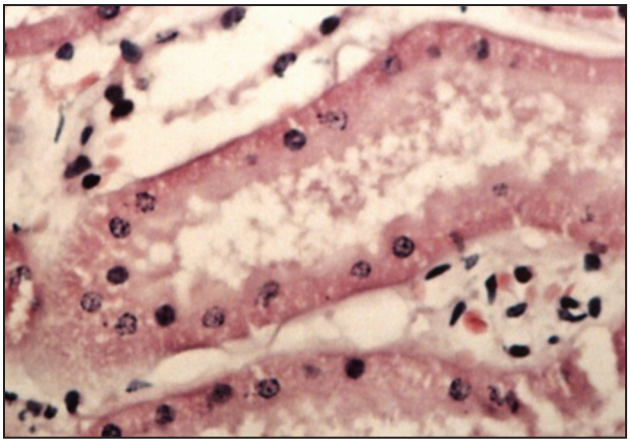

Fig 4a. Coagulation necrosis of renal tubular epithelium. H&E x 250.

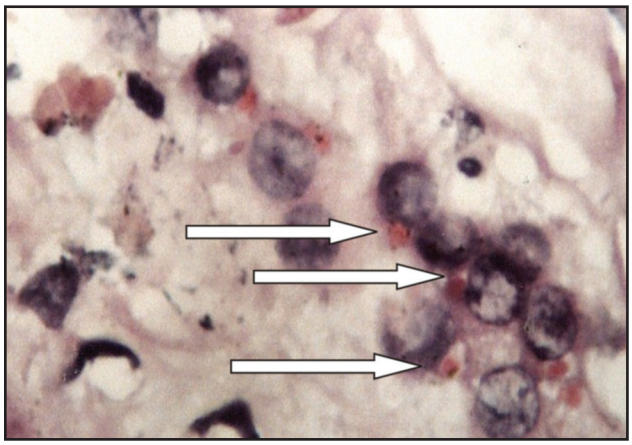

Fig 4b. Intracytoplasmic inclusion bodies in the renal tubular epithelium. H&E x 1000.

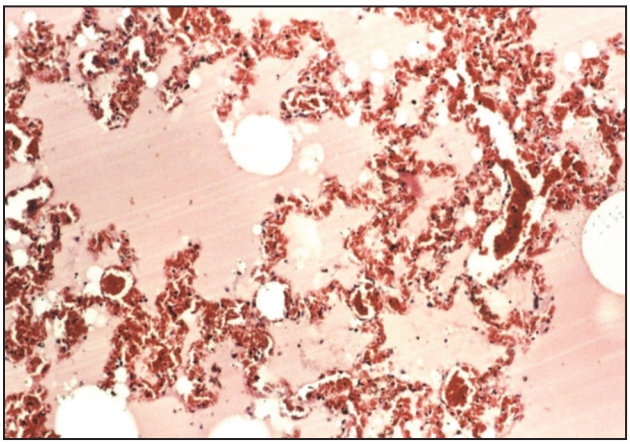

Fig 5. Pulmonary congestion and oedema. H&E x 125.

Results

Three adult she camels of about 5 years of age developed acute neurological signs of head tremors, paralysis of lower lip, paralysis of fore- and hind-legs, recumbency and death within 5 days of appearance of symptoms. When offered food and water, the camels could not control the movement of the neck to take the food. The animals were in good bodily condition when developed the disease and had been grazing Dubduba area of north-eastern Saudi Arabia before 3 months. Camel owners identified the disease (Dubduba disease) as a new emerging camel disease and they believed it to be an outcome of the Gulf war.

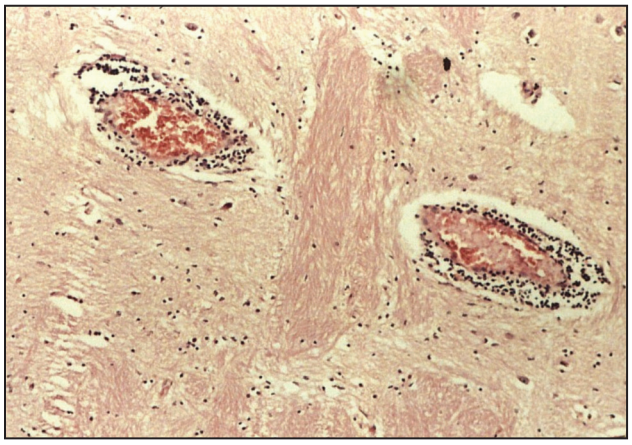

Fig 6a. Acute lymphocytic encephalitis with perivascular cuffing of blood vessels of the brain. H&E x 125.

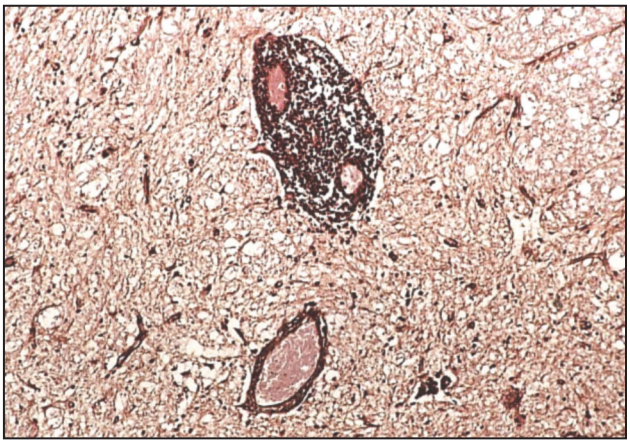

Fig 6b. Acute lymphocytic inflammation of the spinal cord with perivascular cuffing of blood vessels. H&E x 125.

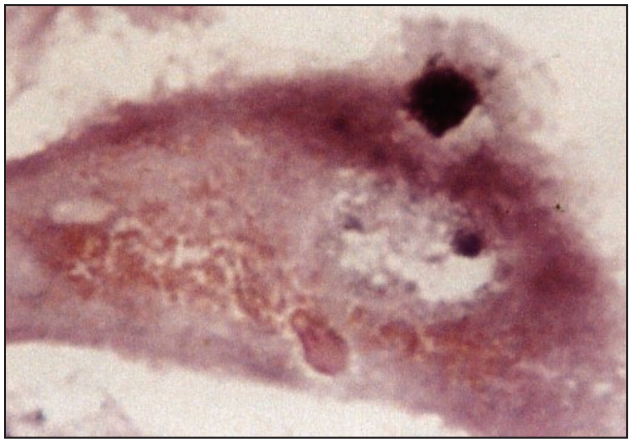

Fig 6c. Nerve cell necrosis with clumping of cytoplasm. H&E x 250.

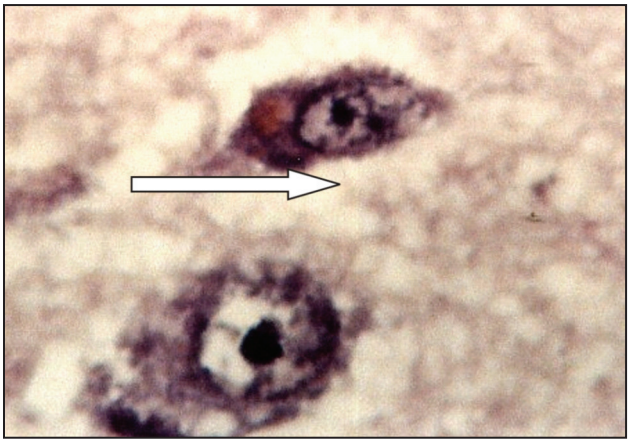

Fig 6d. Inclusion body in the cytoplasm of a neuron. H&E x 1000.

Autopsy results showed that the liver was dark in colour and its capsule contained streaky haemorrhages (Fig 1). Similar haemorrhages were found in the kidneys and the heart. The spleen was congested, but otherwise looked normal. Haemorrhages were also seen on the mucous membrane of the stomach. All parts of the brain were congested and haemorrhagic (Fig 2). Severe congestion was evident on the meninges. The lungs were dark blue in colour due to congestion and when sliced open and pressed it passed a lot of frothy fluid.

Histopathological studies showed severe degeneration and coagulation necrosis of the liver (Fig 3). Coagulative necrosis was also seen in the kidney tubules that also contained intracytoplasmic inclusion bodies (Fig 4 a&b). The lungs were severely congested and oedematous (Fig 5). Non-purulent lymphocytic meningo-encephalomyelitis was seen in all parts of the central nervous system (Fig 6 a&b). Some nerve cells were necrotic and some contained acidophilic

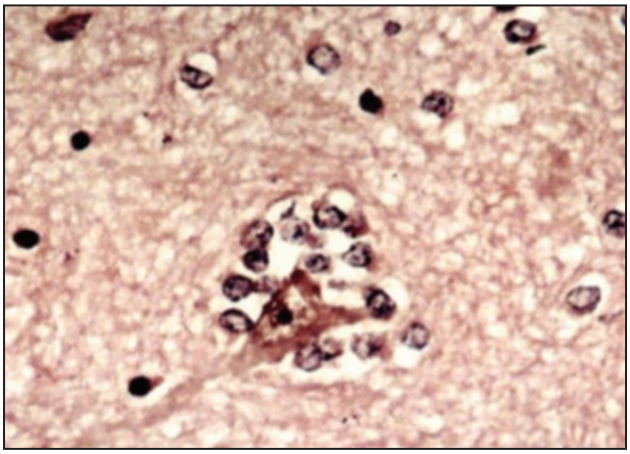

Fig 6e. Microglial cells surrounding a degenerate nerve cell in Gliosis. H&E x 250.

intracytoplasmic inclusion bodies (Fig 6 c&d). Gliosis was seen in many parts of the brain (Fig 6e).

Immunohistochemical studies on brain tissue were negative for: Rabies, West Nile Fever, Rift Valley Fever, Crimean-Congo Fever, Panflavivirus and

Paramyxovirus. Impression smears taken from sliced brain and liver tissues were negative for bacteria by Gram stain.

Discussion

In this paper we report a newly encountered neurological fatal disease in camels which we have called Dubduba syndrome as its etiology remained unidentified. The gross and histopathological lesions of the disease in the brain, liver, kidneys and heart were consistent with the diseases caused by viral infections. The pulmonary oedema was probably due to the severe effect of the etiologic agent on the heart resulting in its failure and therefore, secondary lesions in the lungs (Jones *et al,* 1997).

Considering the geographical location of Saudi Arabia, we investigated viral diseases in the area that are likely to cause Dubduba Syndrome. Rift Valley Fever, Crimean-Congo Fever, West Nile Fever, Rabies, Herpesvirus, Panflavivirus and Paramyxovirus infections have been targeted. None of the investigated viruses were previously known to cause clinical disease in camels but the host-range of viral infections can always change due to mutations that occur in them.

Rift Valley Fever is endemic in sheep farms at the western region of Saudi Arabia (Elfadil, 2005) and antibodies against the virus have been detected in sera of camels (Wernery *et al,* 2007). Even though the lesions we experienced in the autopsied camels were very similar to those seen in calves dying of this disease (Rippy *et al,* 1992), yet no viral antigens were demonstrated in infected tissues.

Intracytoplasmic inclusion bodies were found in the neurons of the central nervous system. Rabies is the only documented viral disease that infects the brain of camels and is characterised histopathologically by non-purulent encephalitis and presence of intracytoplasmic inclusion bodies in the nerve cells. Immunohistochemical staining of brain tissue plus clinical signs of camels with Dubduba disease eliminated the possibility of rabies virus infection in the camels investigated. Lack of history of bite by a rabid animal as well as lack of signs consistent with rabies virus infection such at profuse salivation, aggressive behaviour, attacking objects and body mutilation (Abbas and Omer, 2005; Al-Dubaib, 2007; El-Mardi and Ali, 2001; Ali *et al,* 2004), were all absent in camels with Dubduba syndrome. The hepato-cellular degeneration and necrosis, as well as the pulmonary and renal lesions described in this report are not known to occur in rabid camels.

Recently, a new fatal haemorrhagic fever virus (called Alkhurma virus) in humans was discovered in the western region of Saudi Arabia. Infection causes severe encephalitis, especially, among people in contact with animals such as butchers (Zaki, 2004). The virus claimed the life of 5 patients and was identified as a flavivirus. Viral genome was demonstrated in ticks collected from sites where camels have been resting which indicated a zoonotic nature of the disease with a possible role of camels in its transmission (Charrell *et al,* 2007). No clinical disease was reported or investigated in camels.

Some herpesvirus infections are known to cause encephalitis in farm animals but clinical infections have never been diagnosed in camels. Belknap *et al* (1994) inoculated cattle calves with bovine herpsevirus 1-5 through the intra-nasal route and was able to induce sever encephalitis in them with minimal respiratory lesions.

Crimean-Congo haemorrhagic fever virus is known to infect camels in countries neighbouring Saudi Arabia such as Iran and United Arab Emirates (Khan *et al,* 1997). Antibodies against the virus in sera of healthy humans and imported animals (including camels) have been reported in Saudi Arabia (Hassanien *et al,* 1997).

Listeria monocytogenes, is known to infect farm animals causing septicaemia and encephalitis (Loeb, 2004; Jones *et al,* 1997). An out break of the disease in a sheep farm at the eastern region of Saudi Arabia has been reported by Al-Dughaym *et al* (2001). The disease has not been reported in camels.

In conclusion, this report describes histo-pathological changes in the central nervous system of camels that died of Dubduba syndrome. Lesions were consistent with viral encephalomyelitis and meningitis but no conventional virus has been identified in infected tissues. A meeting of the research team with consultants from India, Australia, Emirates and Saudi Arabia came to the conclusion that a virus is the most likely cause of the Dubduba disease. More studies are needed targeting virus isolation and identification as well as methods of its transmission.

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References

Abbas B and Omer OH (2005). Review of infectious diseases of the camel. Veterinary Bulletin 75 (8), 1N-16N.

Afzal M, Khan IA and Salman R (1993). Clinical signs and clinical pathology of rabies in camels. Veterinary Record 133:220.

Al-Afaleq AA, AAbu-Elzein EME, Hegazy AA and Al-Naeem A (2007). Serosurveillance of camel (*Camelus dromedarius*) to detect antibodies against viral diseases in Saudi Arabia. Journal of Camel Practice and Research 14(2):91-96.

Al-Afaleq AI, Abu-Elzein EM, Mousa SM and Abbas AM (2003). A retrospective study of Rift Valley Fever in Saudi Arabia. Rev. Sci. Tech 22(3):867-871.

Al-Dubaib MA (2007). Rabies in camels at Qassim region of central Saudi Arabia. Journal of Camel Practice and Research 14(2):101-103.

Al-Dughaym AM, Elmula AF, Mohamed GE, Hegazy AA, Rodwan YA, Housawi FM and Gameel AA (2001). First report of an outbreak of ovine Septicaemic listeriosis in Saudi Arabia. Revue Scientifique et Technique 20:777-783.

Al-Hizab FA and Abdelsalam EB (2007). Non-suppurative meningo-encephalitis in dromedary camels in Saudi Arabia. Journal of Camel Practice and Research 14 (2):105-108.

Ali YH, Saeed IK and Zakia AM (2004). Camel rabies in the Sudan. Sudan Journal of Veterinary Science and Animal Husbandry 43:231-234.

Atta FA, Tageldin MH, Al-Sumry HS and Al-Ismaily SI (1993). Rabies in the Sultanate of Oman. Veterinary Record 132:68-69.

Belknap EB, Collins JKC, Ayers VK and Schultheiss PC (1994). Experimental infection of neonatal calves with neurovirulent bovine herpesvirus type 1.3. Veterinary Pathology 31(3):358-365.

Charrell RN, Fago Sh, Moureau G, Alqahtani MH, Sarah Temmam and Lamballerie X (2007). Alkhurma haemorrhagic fever virus in *Ornithodorus savignyi* ticks. Emerging Infectious Diseases 13 (1):153-1555.

Coetzer A (1977). The pathology of Rift Valley Fever. Lesions in natural cases in newborn lambs. Ondersteeport Journal of Veterinary Research 44(4):205-214.

Dirie MF and Abdulrahman D (2003). Observations on little known diseases of camels (*Camelus dromedarius*) in the Horn of Africa. Rev. Sci. Tech. Office of International Epizootics 22(3):1043-1049.

Drosten C, Kummerer BM, Schmitz H and Gunther S (2003). Molecular diagnosis of viral haemorrhagic fevers. Antiviral Research 57:61-87.

Elfadil AA, Hasab-Alla KA, Daffallah OM and Elmanea AA (2006). The persistence of rift valley fever in the Jazan region of Saudi Arabia. Revue Scientifique et Technique 25(3):1131-1136.

Ellis AE, Mead DG, Allison AB, Gibbs SEJ, Gottdenker NL Stallknecht DE and Howerth EW (2005). Comparison of Immunohistochemistry and Virus Isolation for Diagnosis of West Nile Virus. Journal of Clinical Microbiology 43(6):2904-2908.

El-Mardi OI and Ali YH (2001). An outbreak of rabies in camels (*Camelus dromedarius)* in Northern Kordofan State. Sudan Journal of Veterinary Research 17:125-127.

Emmons RW and Riggs JL (1977). Application of immuno-fluorescence to diagnosis of viral infections. Journal of Virological Methods 6:1-28.

Hassanein KM, El-Azazy OME and Yousef HM (1997). Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia. Transactions of the Royal Society of Tropical Medicine and Hygiene 91(5):499–504.

Jones TC, Hunt RD and King NW (1997). Viral Diseases. *In:* Veterinary Pathology, 6th Edition. Williams and Wilkins. Baltimore, Philadelphia.

Khan AS, Maupin GO, Rollin PE *et al* (1997). An outbreak of Crimean-Congo haemorrhagic fever in the United Arab Emirates, 1994 – 1995. American Journal of Tropical Medicine and Hygiene 5(3):519-525.

Loeb E (2004). Encephalitic listeriosis in ruminants. Immunohistochemistry as a diagnostic tool. Journal of Veterinary Medicine 51:453-455.

Medani TA (2005). Alkhurma virus infection, a new viral haemorrhagic fever in Saudi Arabia. Journal of Infection 52(6):643-464.

Morrill JC, Soliman AK, Imam IZ, Botros BA, Moussa MI and Watts DM (1990). Serological evidence of Crimean-Congo haemorrhagic fever virus among camels imported into Egypt. Journal of Tropical Medicine and Hygiene 93(3):201-204.

Rippy MK, Topper MJ, Mebus CA and Morrill JC (1992). Rift Valley Fever virus-induced encephalomyelitis and hepatitis in calves. Veterinary Pathology 29(6):496-502.

Tsai TF (1999). Arboviruses in Manual of Clinical Microbiology. ASM Press, Washington D.C. pp 1107-1124.

Wernery U, Thomas G, Syriac R, Raghavan R and Kletzka S (2007). Seroepidemiological studies for the detection of antibodies against nine infectious diseases in dairy dromedaries (part 1). Journal of Camel Practice and Research 14(2):85-90.

Whitehead CE, Anderson DE and Saville WJA (2006). Neurological diseases in llamas and alpacas. A retrospective study of 185 cases (1993–2003). First Conference International Society of Camelids Research and Development (ISOCARD) in Al Ain, 15-17 April, pp 93.

Zaki AM (1997). Isolation of a flavivirus related to the tick-borne encephalitis complex from human cases in Saudi Arabia. Transactions of the Royal Society of Tropical Medicine and Hygiene 91:179-181.

Zaki AM (2004). Alkhurma virus, human death, Saudi Arabia. Pro-Med Archives No. 200403020631: http//www.promedmail.org