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DUBDUBA SYNDROME: NON-SUPPURATIVE MENINGOENCEPHALOMYELITIS IN DROMEDARY CAMELS IN SAUDI ARABIA

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ABSTRACT

A neurologic disease in 5 camels in Saudi Arabia is described. These camels of various breeds, Mejaheem, Omani and Wedheh, were 3 to 10 years old. Camels had a history of depression, reduced appetite and separation from the flock. On presentation, they had signs of head shaking, tremor, ataxia, wry neck and lateral recumbency. Postmortem examination showed congestion of the heart, liver and brain. A mild to marked non-suppurative meningoencephalomyelitis was detected histologically. In addition, Purkinje cell necrosis with neuronophagia was present. Immunohistochemistry failed to detect evidence of bovine viral diarrhoea virus, West Nile virus, Rabies virus, Equine herpesvirus, Influenza A virus, Canine distemper virus, Feline herpesvirus, *Toxoplasma gondii*, and *Neospora caninum*. The clinical presentation of these cases indicates a potentially novel camelid disease. The histological findings appear to be most consistent with a viral encephalitis. Future studies are aiming at identifying new cases of this disease. Clinical, epidemiological and postmortem investigation with special attention to serologic and more virologic work appear to be necessary to identify the cause of the disease.

Key words: Camel, dromedary, Dubduba syndrome, meningoencephalomyelitis, Saudi Arabia

Camels in the Kingdom of Saudi Arabia are raised in variable sized herds and usually graze freely. However, feed supplements, mostly barley and hay, are usually added. Herds are moved from the far north to the central and eastern regions of the country in a nomadic fashion following the presence of adequate vegetation for grazing. Vaccination programs are not practised and adequate record system is not usually maintained.

Reports of neurologic diseases are limited in these regions. Most of the disease reports rely on indirect diagnostic approaches rather than detection of the causative agents. *Listeria monocytogenes,* the most common bacterial infection of the nervous system was described in llama (Hamir and Moser 1998). However, a camel case of *L. monocytogenes* has just recently been reported (Al-Dubaib, In Press). Parasitic diseases such as *Toxoplasma gondii* were documented using indirect haemagglutination test (Hussein *et al,* 1988). In addition, *Neospora caninum* was described utilising indirect fluorescent test (Sadrebazzaz *et al,* 2006).

Several neurologic viral diseases have been described in camels. Rabies was reported in the UAE, Jordan and Saudi Arabia (Afzal *et al,* 1993, Al-Rawashdeh *et al,* 2000, Al-Dubaib 2007). Seroconversion against West Nile Virus was described in Nigeria and the United Arab Emirates (Olaleye, 1990; Wernery *et al,* 2007). Alkhurma haemorrhagic virus –a tick borne agent- was reported in ticks obtained from camels (Charrel *et al*, 2007). Finally, Borna disease virus has been documented in llama (Rott and Becht, 1995).

Food toxicity may occur following inappropriate handling and storing of animal feed or due to contamination of feed with botulinum toxin (Provost *et al*, 1975). In addition, spraying of pesticides may result in an increase of cases with neurologic disorders. Lastly, diseases due to nutritional deficiencies are not uncommon despite, supplying animals with hay and concentrates.

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The goal of this paper is to describe a neuorologic disease involving multiple number of camels of various breeds in Saudi Arabia.

Materials and Methods

Five camels were admitted to the Veterinary Teaching Hospital, King Faisal University, Al-Ahsa, Saudi Arabia between May and December, 2007. These cases arrived with a history of depression, reduced appetite and separation from the flock. The camels had a history of grazing at the Dubduba Desert over the past 2 years. On presentation, the camels had signs of head shaking, tremor, ataxia, wry neck and lateral recumbency. The camels were in good body condition, except for one that was severely emaciated. The camels were in terminal stages of the disease with poor prognosis, therefore, treatment was not attempted and all were euthanised within 2 days of admission.

Postmortem examination was performed on each case. Tissue samples from organs such as brain (caudate nucleus cerebral cortex and cerebellum), lung, kidney, liver, intestine, cerebellum and heart were collected. Samples were fixed in 10% buffered formalin, paraffin-embedded, sectioned at 4 µm thickness and stained with haematoxylin and eosin. Immuno-histochemistry using a variety of antibodies and antisera aiming at identifying infectious agents was performed hoping to exploit at least some cross-reactivity with the infectious agent (Table 1).

Results

The first camel was a 9 year old Omani female that has been sick for 3 weeks. The camel came from a herd of 150 camels that had no history of neurologic disease for the past 2 years. Upon arrival the camel was severely depressed, sternally recumbent with twisted neck. Temperature was 38.4°C (36-39°C), respiratory rate was 14/min (5-12/min), heart rate was 32/min (30-45/min). For humane reasons, the camel was put down. The 2nd case was a 10 year old Mejaheem female that has been sick for 4 weeks. The camel came from a herd of 30 camels. Physical examination showed a temperature of 38.8°C, respiratory rate of 17/min and heart rate was 35/min. Leukocytosis 16 x 10^3/ml (4-12 x 10^3/ml) with marked neutrophilia 13.3 x 10^3/ml was seen. The camel was laterally recumbent and was unable to stand. It could not keep the head upward. The camel was put down the 2nd day due to very poor condition. The 3rd case was a 3 year old Wedheh female that came from a herd of 65 camels. Physical examination revealed slight elevation of temperature 39.6°C. Respiratory rate and heart rate were 34/min, respectively. This camel became sternally recumbent but the head and the neck were upward during the first day. Head shaking and lower lip tremor were obvious. By the 2nd day the camel was laterally recumbent, unable to keep the head and the neck in position. The colour of the mucous membranes was normal. Haematology indicated no significant findings. The 4th camel was a 5 year old Wedheh female that came from a herd of 85 camels. Physical examination revealed temperature to be 37.6°C. Respiratory rate and heart rate were 17/min amd 32/min, respectively. This camel attained sternal recumbency with the head and the neck upward during the 1st day. Head shaking and lower lip tremor were noted. By the 2nd day the camel was laterally recumbent, unable to keep the head and the neck in position. The 5th case was a 7 year old Wedheh female that originated from a herd of 90 camels which were kept in the same geographic location (Dubduba Desert) over the past 5

Table 1. Immuno-histochemistry using a variety of antibodies and antisera aiming at identifying infectious agents.

Antibody	Vendor	Antibody Host Species	Clone/antiserum	Brief Staining Procedure
BVD Virus	(before 11/16/07) Cornell Univ.	Mouse Mono	Clone 15C5	Goat anti-Ms IgG
WNV	BioReliance	Mouse Mono	Clone 7H-2; Protein E	Goat anti-Ms IgG
Rabies	Biodesign	Mouse Mono	Clone RV1C5	Goat anti-Ms EnVision+ polymer/HRP
Equine Herpesvirus	VMRD	Goat Poly	EHV antiserum	Biotinylated Swine anti-Gt IgG
Feline Herpesvirus	Custom Monoclonals	Mouse Mono	Clone FHV7-7C	Goat anti-Ms IgG
Influenza A Virus	Biodesign	Mouse Mono	Clone 1331	Goat anti-Ms IgG
Canine Distemper Virus	VMRD	Mouse Mono	Clone CDV-NP	Goat anti-Ms IgG
Toxoplasma gondii	Lab Vision/ NeoMarkersv	Rabbit Poly	*T. gondii* antiserum	Goat anti-Rb IgG
Neospora caninum	VMRD	Goat Poly	*N. caninum* antiserum	Biotinylated swine anti-goat IgG

years. Clinical manifestation described by the owner included incoordination, head tremor, stiff neck, sternal recumbency with fairly normal appetite. No history of contact with other animal species mainly sheep and no information of use of insecticide in the area. The owner did not use any prophylactic treatment or any vitamins or minerals supplements. Physical examination revealed a rectal temperature of 38.2°C. Respiratory rate and heart rate were 18/min and 30/min, respectively. This camel arrived sternally recumbent with the head and the neck upward during the first day. As described above for previous cases, the animal had head shaking and lower lip tremors. By the 2nd day the camel remained sternally recumbent but was unable to keep the head and the neck in position. Marked leukocytosis 18.1 x 10^3/ml with marked neutrophilia 15.3 x 10^3/ml was seen. Testing of cranial nerves showed no significant findings in all 5 cases.

At necropsy, the 1st case had poor body condition, slight congestion in the mucosa of abomasum, liver and large intestine. The meninges of the brain were congested but there were no significant macroscopic lesions in the brain. On the other hand, the 2nd case had congestion in the brain, heart, liver and kidney. The lungs had multiple abscesses comprising approximately 30% of the lung parenchyma. The most common finding in the last 3 cases was the presence of petechial haemorrhages mainly in the white matter of the brain.

Histologically, non-suppurative meningoencephalomyelitis was detected in all camels. The cerebellum of 3 animals (case 1, case 2, case 3) had a marked lymphoplasmacytic meningoencephalitis. The meninges were multifocally infiltrated by large aggregates of lymphocytes, plasma cells and fewer macrophages. Multi-layered perivascular infiltrates were present in the gray and white matter of these animals. Lymphocytic infiltration was observed in multiple layers in the Virchow-Robin space, mostly in the cerebellum and spinal cord (case 4). Multiple Purkinje cells were degenerated or became necrotic and were surrounded by lymphocytes, plasma cells and macrophages ("neuronophagia"). There also appeared to be a decreased density of Purkinje cells in several segments of the cerebellar cortex. In addition, the white matter had occasional vacuoles that contained gitter cells ("axonophagia"). The 3rd animal had a focal lymphoplasmacytic cerebellar meningitis. The cerebrum of two animals (case 2 and 3) had few mild perivascular lymphoplasmacytic infiltrates in the white and gray matter. Multiple small clusters of hypereosinophilic and slightly swollen cardiomyocytes were present in the myocardium of one animal (case 3). Few of these necrotic cardiomyocytes were infiltrated or surrounded by few macrophages and neutrophilic granulocytes. There were mild multifocal acute myocardial haemorrhages.

The livers had mild to moderate diffuse hepatocellular lipidosis. Occasional renal tubules of 2 animals (case 1 and 2) contained eosinophilic fine granular material (interpreted as possible myoglobinuria possibly from being recumbent). The lungs of one animal had a mild granulomatous pneumonia with intrahistiocytic crystalline material (interpreted as mild incidental silico-anthracosis). In addition, few peribronchial neutrophilic granulocytes were also present.

The owners of herd 2 and 3 reported that few camels had died of a disease running a similar course in the previous year. In addition, the owner of herd 5 had 8 cases in 2008, 3 cases in 2007 and 6 cases in 2003 that died of similar disease. This herd had no contact with other animal species mainly sheep.

Bovine Viral Diarrhoea virus antigen, West Nile virus antigen, rabies virus antigen, equine herpesvirus antigen, feline herpesvirus antigen, Influenza A virus antigen, canine distemper virus antigen, *T. gondii* antigen or *N. caninum* antigen were not detected in the examined brain sections of 3 camels (case 1, 2 and 3) using IHC.

Discussion

The 5 cases reported in this article originated from different unrelated herds but they spent variable time period in the North Eastern part (Dubduba Desert) of the country over at least the past 2 years. Therefore, the name Dubduba was given to this syndrome. Nonetheless, they share similar clinical and pathologic findings. Similar report of a neurologic disease has been described in three adult camels (*Al-Dubaib,* In Press). The age group seems to be adult animals (3 years and above). Temperature, heart rate and respiratory rate were less significantly affected. This indicates the possibility of a moderately rapidly progressive disease, despite the reports of few weeks of history of clinical course. During a comprehensive survey on camel herds a course of disease ranged between 1 to 2 weeks.

The cause of the meningoencephalomyelitis observed in all 5 animals was a viral infection based on the severity of the inflammatory reaction, composition of the inflammatory cell infiltrates (lymphocytes, plasma cells and macrophages) and evidence of Purkinje cell neuronophagia

Fig 1. Case 1: Severe depression, poor body condition and twisted neck.

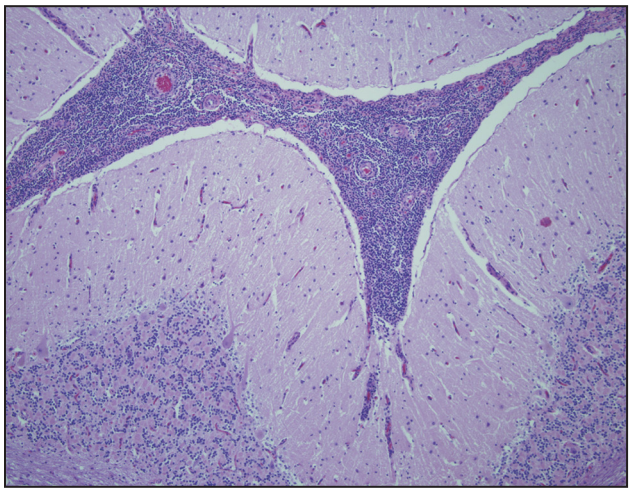

Fig 2. Case 1: Cerebellum with marked non-suppurative meningitis. Haematoxylin and Eosin stain; magnification: 100X.

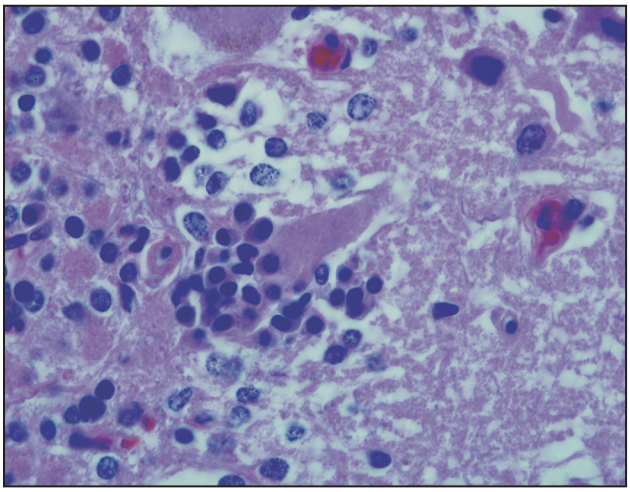

Fig 3. Case 1: Necrotic Purkinje cells with cuff by lymphocytes, plasma cells and macrophages and neuronophagia. Haematoxylin and Eosin stain; magnification: 400X.

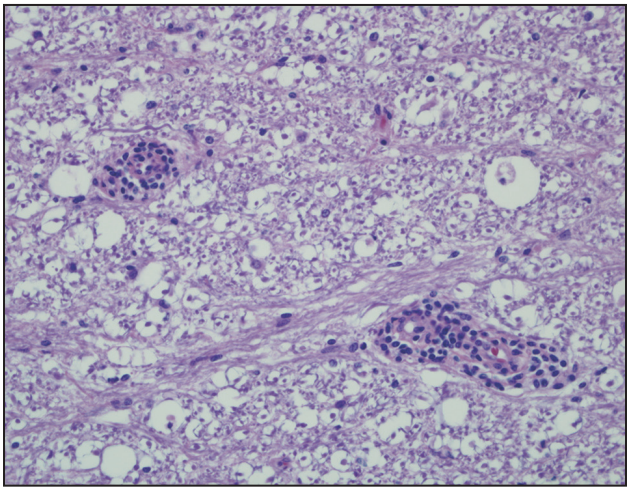

Fig 4. Case 3: Cerebellar white matter with cuffing of capillaries with lymphocytes, plasma cells and macrophages. In addition, there is evidence of axonophagia. Haematoxylin and Eosin stain; magnification: 200X.

(Radostits *et al,* 2000). However, viral intranuclear or intracytoplasmic inclusion bodies were not detected. Serologic titre against West Nile Virus was detected in 2 cases (case 4 and 5) and a limited number of animals from the herd of which case 5 originated. However, this may indicated exposure rather than infection since WNV titre was detected in a significant number of healthy camels exported to the UAE (Wernery *et al*, 2007). The cause of myocardial necrosis is uncertain but this lesion may either be related to the brain lesion or may be secondary (e.g. stress-related). The remaining identified lesions (hepatocellular lipidosis, granulomatous pneumonia and neutrophilic peribronchitis and myoglobinuria) are likely incidental findings that are either unrelated to the meningoencephalitis or may be indirect sequel of the meningoencephalitis. Further, systematic epidemiologic, clinical, clinio-pathologic, pathologic (particular brain and spinal cord) and microbiologic work up (virus isolation and molecular diagnostics such as PCR of affected animals) is needed to elude the pathogenesis of the disease.

Viral diseases impacting the nervous system including rabies, equine herpesvirus, BVDV, and West Nile Virus have been described in camels based largely on serological investigations (Olaleye, 1990; Wernery and Wernery, 1997; Al-Dubaib *et al.* In Press) ruled out rabies, West Nile Fever, Rift Valley Fever, Crimean-Congo Fever, Panflavivirus and Paramyxovirus using immunohistochemistry. In the current study, we utilised IHC to detect infectious agents in brain tissue. The employed antibodies failed to detect antigen. However, immunohistochemistry is notoriously a fairly insensitive method for detection of antigens of

several infectious agents, e.g. West Nile virus, and a negative result does not rule out that the agent caused the inflammation. Antigen presence below the detection level threshold of immunohistochemistry ("false negative results"), or clearance of the infectious agent from brain tissue, e.g. due to a still detectable inflammatory response (truly negative result despite the infectious agent under investigation actually causing the inflammation) are just 2 possibilities. Morbidity of the described syndrome is fairly low (less than 3%) which might explain difficulty in detecting positive cases (unpublished data). Rabies has been described in Saudi Arabia and neighbouring countries and remains a differential diagnosis although the history and clinical presentation appears to be inconsistent with rabies and rabies virus antigen was not detected in the examined brain tissue (Al-Dubaib, 2007; Afzal *et al,* 1993; Al-Rawashdeh *et al,* 2000). Influenza A virus, Canine distemper virus and Feline herpesvirus antigen were among other antigens that were tested for using IHC on brain tissues. None of these antigens were detected; however, these agents may not be typical causes of neurologic diseases in camels. Finally, attempting to grow a virus was not rewarding, probably due to the use of tissues originated from more chronic lesions (Unpublished data).

Parasitic conditions such as *N. caninum* and *T. gondii* have been reported with variable morbidity in camels using Indirect Fluorescent Antibody Test (IFAT) and Indirect Haemagglutination Test (IHT), respectively (Hussein *et al,* 1988; Sadrebazzaz *et al,* 2006). Prevalence of toxoplasmosis was nearly 16% in Saudi Arabia but exceeded 30% in the UAE. In this study, protozoal cysts were not detected in the examined brain sections and the use of IHC failed to detect antigens of *N. caninum* and *T. gondii*. However, the use of serologic testing may indicate previous exposure.

The occurrence of food and pesticides toxicity may follow inappropriate handling and storing of animal feed or due to contamination of feed with botulism or chemicals (Provost *et al,* 1975). However, the above mentioned conditions are likely to occur in sporadic and related occasions. In addition, pathologic findings make such possibility more remote.

Future studies are aiming at identifying new cases of this disease. Clinical, epidemiological and post mortem investigation with special attention to serologic and virologic work up including the use of molecular diagnostic methods such as polymerase chain reaction and tissue electron microscopy may be necessary to identify the cause of this potentially newly emerging disease.

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LAUNCH OF A BOOK ON CAMELID PARASITOLOGY IN A NOVEL WAY AT BIKANER

A new book on camels entitled as "Selected Research on Camelid Parasitology" edited by Dr.T.K.Gahlot and M.B.Chhabra was released in a novel manner at the premises of Clinic of College of Veterinary and Animal Science, Rajasthan Agricultural University, Bikaner on 17 July 2009. The editor of book Dr. T.K. Gahlot, alongwith large number of students, guests and faculty assembled at the gate of Clinic. As the first camel man entered in the clinics alongwith his sick camel, he was requested to launch the book. He was Mr.Afzal Khan, a local resident of Bikaner was amazed. He was honoured by a garland, turban and sweets. Mr.Afzal felt highly honoured and expressed his gratitude specially for the gesture of Dr. T.K. Gahlot, Editor of the book to have chosen this way of launching the book. Dr. Gahlot said that camel science developed significantly at Bikaner because of large number of draft camels, Border Security Force camels and those belonged to erstwhile Ganga Risala. There were no books on camels and camels had altogether different anatomy and physiology. Scientists developed treatments of camel diseases over past five decades in this college and we are highly thankful to large number of camel owners who presented their camels for treatment in the Clinics of Veterinary College, Bikaner. The pivotal role of camel owners in furthering camel science, therefore, cannot be forgotten. The present book also points Veterinary College, Bikaner as the second largest centre of camelid parasitology research world over. Dean, Veterinary College- Dr. AK Gahlot and Director of Research- Dr.SBS Yadav appreciated efforts of Dr.TK Gahlot and MB Chhabra who worked dedicatedly to bringout this volume. They also thanked Camel Publishing House for publishing this book

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Selected Research on Camelid Parasitology is most comprehensive guide to Camelid Parasitology. The classic reference book serves as a one stop resource for scientific information on major aspects of Camelid Parasitology. Featuring abundant photographs, illustrations, and data, the text covers camelid protozoa, helminths, and arthropods of dromedary and New World camelids. This hard bound book of 304 pages contains seroepidemiological studies, immunological and other diagnostic procedures, and new treatments of parasitic diseases. There are at least 17 countries involved in camelid parasitology research, viz. Ethiopia, France, India, Iran, Jordan, Kenya, Libya, Mauritania, Nigeria, Sultanate of Oman, Pakistan, Saudi Arabia, Sudan, Sweden, United Arab Emirates, Uganda and U.S.A. As per published papers in Journal of Camel Practice and Research (JCPR), 173 authors have contributed 72 manuscripts which are appropriately placed in 5 sections. Book was edited by T.K. Gahlot and M.B. Chhabra and published by Camel Publishing House.

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