Sheep-Associated Malignant Catarrhal Fever: First report in a Calf in Northeastern Turkey

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ABSTRACT

In this report, systemic vasculitis was described in a Brown Swiss calf with sheep-associated malignant catarrhal fever. The calf was referred to the university clinic due to respiratory and nervous symptoms. Nasal discharge, dyspnea, cough, conjunctival hyperemia, bilateral corneal opacity and ulceration, superficial lymph node enlargement, incoordination and muscle tremors were detected on clinical examination. The hematologic profile revealed lymphocytosis and neutrophilia. Grossly, hyperemia of viscera, lymphoid tissue enlargements and swelling in the brain were observed. Fibrinoid necrotic vasculitis and lymphoid cell infiltrations were main histopathologic changes in the brain, liver, spinal cord, heart, lymphoid tissues and upper respiratory tract. Characteristic histopathologic findings were confirmed by PCR, which demonstrated the presence of ovine herpesvirus-2 (OvHV-2) in the lymph nodes and liver samples of the calf.

Keywords: Brown Swiss calf, Malignant catarrhal fever, Histopathology, Ovine Herpesvirus-2, PCR, Vasculitis.

INTRODUCTION

Malignant catarrhal fever (MCF) is a fatal lymphoproliferative disease of cattle and other ungulates caused by the ruminant gamma-herpes viruses alcelaphine herpesvirus type 1 (AIHV-1) and ovine herpesvirus 2 (OvHV-2) (1). The disease is an infectious disease of domestic cattle, wild ruminants (2, 3, 4) and occasionally pigs (5, 6).

Sheep-associated MCF (SA-MCF) is caused by ovine herpes virus type 2 (OvHV-2) and AIHV-1 causes Wildebeest-associated MCF (WA-MCF) (1, 2, 3). Caprine herpes virus type 2 (CpHV-2) causes disease in goats and a fourth type has also been identified which is of unknown origin (7, 8). Sheep are the major source of MCF in cattle and when they are held together with cattle the incidence of infection increases. Cattle are naturally infected by close contact with infected sheep actively shedding the virus (3, 9). A high level of homology between WA-MCF virus and SA-MCF virus was reported in viral DNA sequence analysis of lymphoblastoid cell lines derived from infected cattle with SA-MCF (10).

The most common clinical signs are fever, nasal discharge, depression and bilateral keratoconjuntivitis, lymph node enlargement and corneal opacity. Neurological signs maybe observed in terminal stages of the disease and these include muscle tremors, ataxia, head pressing, nystagmus, and twitching (2, 11-15). Vasculitis, mucosal and epidermal lesions, hyperplasia and necrosis of lymphoid tissues and lymphoid cell infiltration in non-lymphoid tissues are typical changes in MCF (12, 14). MCF in cattle has been reported as sporadic around the world and in Turkey (4, 12, 15-18, 25). Although the diseases was mostly encountered in adult animals, there are few report in young animals around the world (19-23).

This study describes the first identification of the SA-MCF in 2-month old Brown Swiss calf with molecular, histopathological and cytological methods in Northeastern Anatolia, Turkey.

CASE REPORT

A two month old female Brown Swiss calf, treated with variety of antibiotics (penicillin-streptomycin and enrofloxacin) and nonsteroidal anti-inflammatory (flunixin meglumine) drugs for respiratory problem, was referred to the Large Animal Clinic, Faculty of Veterinary Medicine, University of Ataturk as a result of ophthalmological and neurological symptoms (Fig. 1A).

The results of physical examination are presented in (Table 1). Peripheral blood samples were collected for hematologic and molecular analyses. A cell counter (Abacus Junior Vet 5, Hungary), adjustable to animal species, was used (Table 2).

Due to of irreversible clinical signs the calf was euthanized and necropsied upon request of animal owner. Cerebrospinal fluid (CSF) was obtained by fine needle aspiration during the necropsy. Fluid was centrifuged at 4000 rpm for 5 minutes, smeared on clean slides and stained with Giemsa.

At necropsy, retropharyngeal, submandibular and prescapular lymph nodes were swollen and congested. There were

Table 1: Clinical signs of a Brown Swiss calf with SA-MCF

Parameter	Results
Body Temperature (°C)	39.2
Respiratory rate (cycle / min.)	44
Heart rate (beats / min.)	108
Dyspnea	Severe
Nasal discharge	Bilateral
Corneal opacity	Bilateral
Corneal ulceration	Bilateral
Photophobia	Bilateral
Blepharospasm	Bilateral
Scleral congestion	Bilateral
Lung auscultation	Harsh vesicular-bronchial sounds audible in cranial lobes
Lymph nodes	Superficial lymph nodes enlargement

Table 2: Hematologic findings of a Brown Swiss calf with SA-MCF

Parameter	Results	Normal range (26)
WBC ($x10^3/\mu L$)	23.95	4.0-12.0
LYM ($x10^3/\mu L$)	12.27	2.5-7.5
MON ($x10^3/\mu L$)	0.38	0. 03-0.8
NEU ($x10^3/\mu L$)	11.01	0.6-4.0
EOS ($x10^3/\mu L$)	0.29	0-2.4
BAS ($x10^3/\mu L$)	0.01	<0.2
RBC ($x10^6/\mu L$)	10.0	5.0-10.0
HGB (g/dL)	11.9	8-15
PCV (%)	36.86	24-46
MCV (fL)	37	37-51
MCH (ρg)	12	13-18
MCHC (g/dL)	33	33-37
RDW (%)	24	16-24
PLT ($x10^3/\mu L$)	236	200-730

WBC: White Blood Cell; LYM: Lymphocyte; MON: Monocyte; NEU: Neutrophil; EOS: Eosinophil; BAS: Basophil; RBC: Red Blood Cell; HGB: Hemoglobin; PCV: Packed Cell Volume; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin quantity; MCHC: Mean Corpuscular Hemoglobin concentration; RDW: Red Cell distribution width; PLT: Thrombocyte.

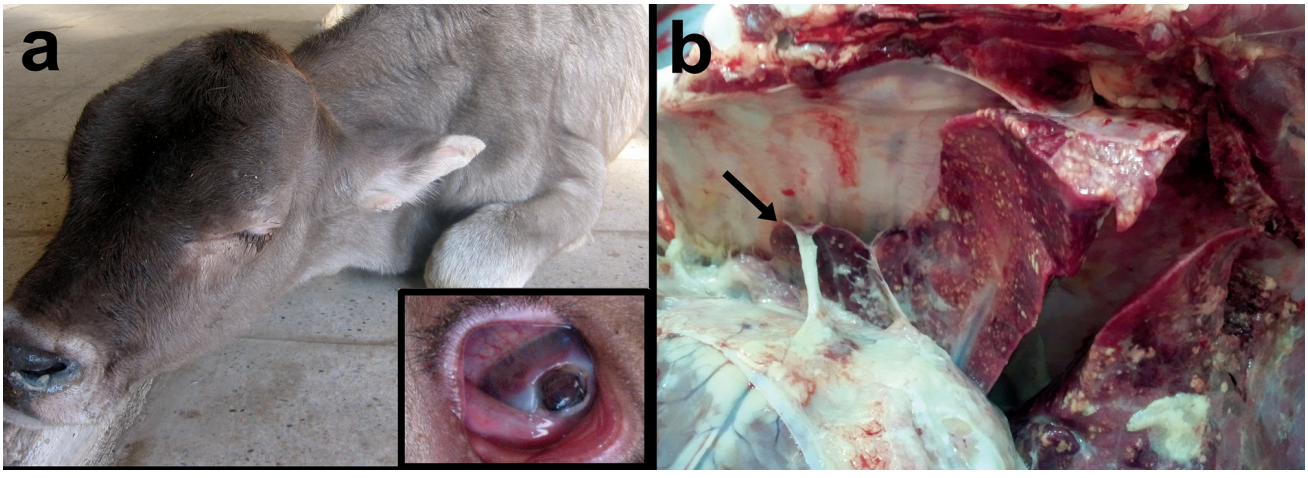

Figure 1: A) Corneal opacity and ulcer; B) Fibrino-purulent and necrotic pneumonia and pleural adhesions (arrow).

erosive lesions on the tongue and upper respiratory mucosa, especially on the nasal conchae. Blood vessels appeared dilated with opaque areas on the cerebral meninges (Fig. 2A).

Severe bronchopneumonia and pleural adhesions (Fig. 1B), congestion of liver and kidneys were observed. Examination of CSF revealed an increase in lymphocytes (Fig. 2B).

For histopathological examination, tissue samples of lung, heart, liver, kidney, spleen and brain were obtained and fixed in 10 % formalin buffered saline. After the routine histopathology process, paraffin sections of 5 μm were prepared and stained with hematoxylin and eosin (H&E).

Fibrinoid-necrotic vasculitis was observed on the wall of the blood vessels of concha, central veins of the liver (Fig. 3A), heart (Fig. 3B), meninges, cerebrum (Fig. 4A), cerebellum (Fig. 5A) and spinal cord (Fig. 5B). There was a lymphocytic infiltration on the intima and media of these vessels. Neuronal degeneration and necrosis in the cerebrum, cerebellum, spinal cord with vacuole formations were observed (Fig. 4B).

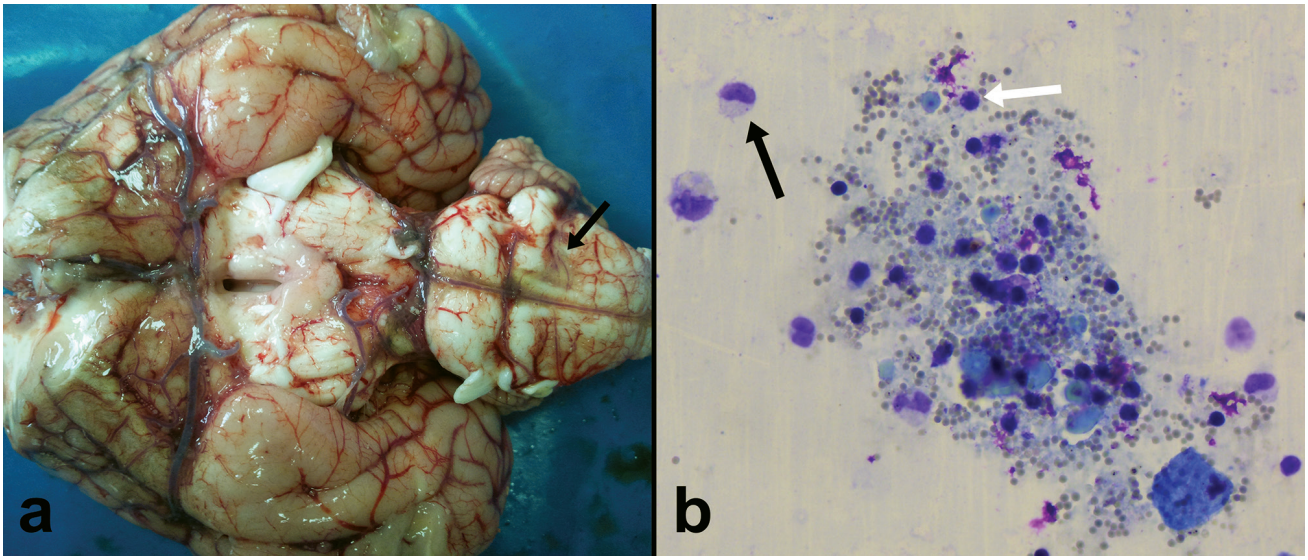

Figure 2: A) Brain. Hyperemic vessels and grey to green-yellow exudate (arrow) on meninges; B) CNS smear. Increase the lymphocytic (white arrow) and monocytic (black arrow) cells. Giemsa stain. ×1000.

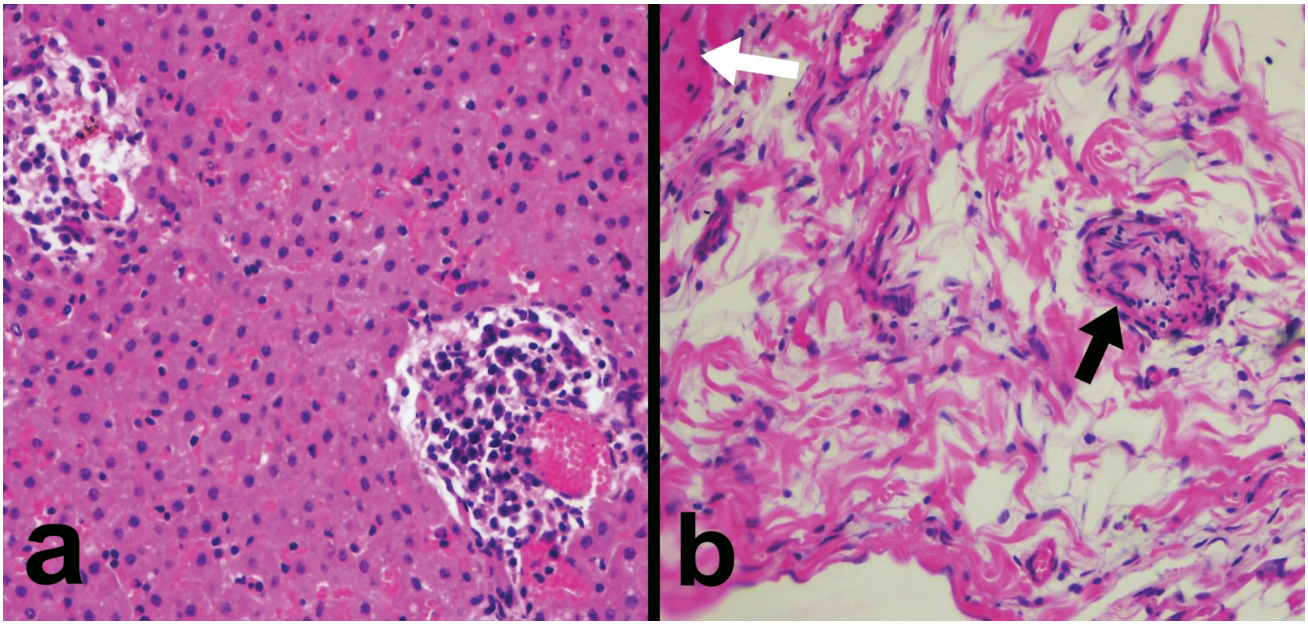

Figure 3: A) Liver. Severe vasculitis in the central veins. H&E. ×400; B) Heart. Epicardial vasculitis (black arrow), myocardium (white arrow). H&E. ×400.

Viral DNA was extracted from lymph node and liver with Qiagen DNeasy tissue DNA extraction kit according to the manufacturers instructions (Qiagen, Hilden, Germany). The hemi-nested PCR for the detection of Ovine herpes virus-2 (OvHV-2) sequences was conducted by two-step amplification reaction cycles (10). Amplification reactions were performed in 50µl volumes containing 1 µl of sample DNA in 10 mM TrisHCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.01% (v/v) gelatine, 10% (v/v) dimethyl sulfoxide (DMSO), 200 µl dATP, dCTP, dGTP and dTTP (Fermentas), 1 µM of each primer and 2 units Taq DNA-polymerase.

The cycling conditions consisted of a precycle at 99°C for 3 minutes, after which dNTP and the enzyme mix were added. This was followed by 25 cycles at 94°C for 20 seconds, 60°C for 30 seconds and 72°C for 30 seconds. A 2 µl aliquot of the primary amplification product, specified by the primers 556 (5-AGTCTGGGGTATATGAATCCAGATGGCTCTC-3) and 775 (5-AAGATAAGCACCAGTTATGCATCTGATAAA-3) was transferred directly to a new reaction mixture and amplified using the primer pairs 556 and 555 (5-TTCTGGGGTAGTGGCGAGCGAAGGCTTC-3) under identical conditions for a further 25 cycles with a final extension at 72°C for 5 minutes (24). Final amplification products (10 µl) were analyzed directly by 2% agarose gel electrophoresis and ethidium bromide staining. MCF positive sample DNA from recent epizooties was used as a positive control and distilled water was used as negative control.

OvHV-2 genome specific 422 bp and 238 bp PCR am-

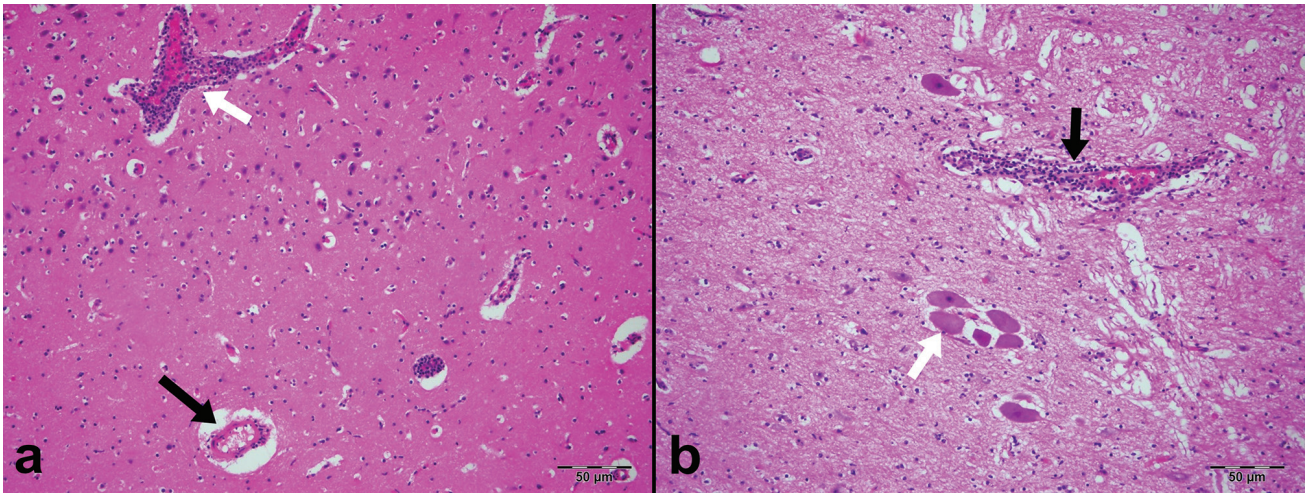

Figure 4: A) Cerebrum. Fibrinoid necrotic vasculitis (black arrow) and severe vasculitis (white arrow). ×200. H&E; B) Cerebrum. Vasculitis (black arrow) and necrotic neurons (white arrow). H&E. ×200.

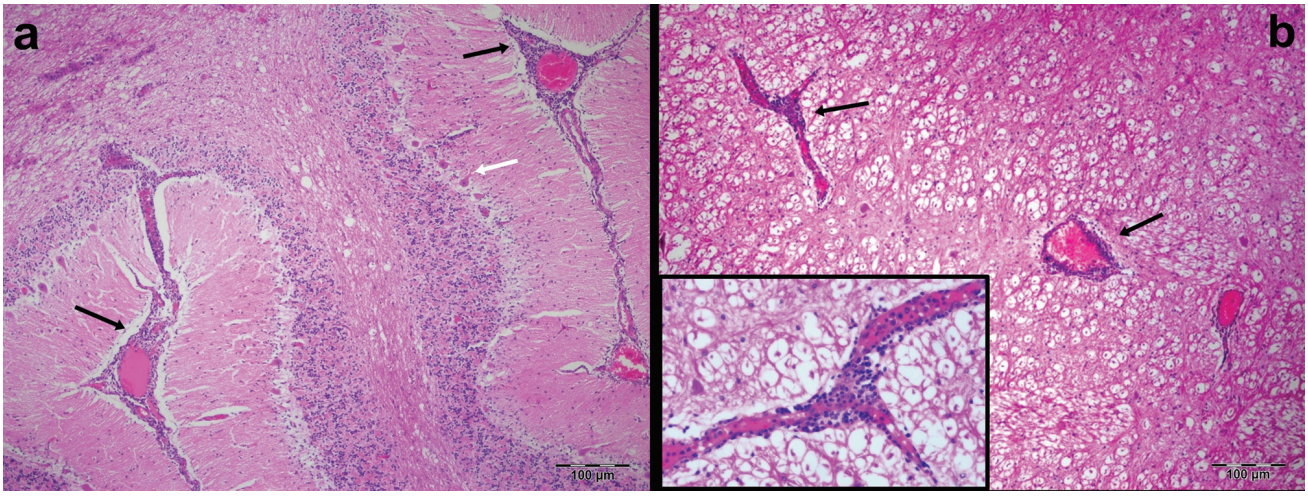

Figure 5: A) Cerebellum. Vasculitis in capillaries of meninges necrotic changes of purkinje cells (white arrow). H&E. ×200; B) Spinal cord. Vasculitis (black arrows). Inset: High magnification of vasculitis in another section. H&E. ×400.

plification was detected from necropsy samples with the 556/755 and 556/555-primer sets, respectively. The same results were also found with positive control samples and no amplification were detected in negative control samples for all PCR reactions.

DISCUSSION

SA-MCF cases have been described around the world in many studies (19-23). Although the disease is known in Turkey for many years only a few cases have been reported until now. In adult cattle, Dabak and Bulut (16), reported an outbreak of MCF in Elazig, east Turkey. Yesilbag (17), studied the disease serologically and reported MCF in cattle, sheep and goats in South Marmara region, west Turkey. Yazici *et al.* (18) using serologogy and nested PCR studied the disease and reported MCF in cattle and sheep in Samsun, northern Turkey.

The present case was observed in a 2 month old Brown-Swiss calf in Erzurum, in northeastern Turkey. Vascular lesions, such as fibrinoid necrosis and lymphoid infiltrations were the main histopathological changes in many tissues. Presence of SA-MCF specific viral nucleic acid was detected with hemi-nested PCR in lymph node and hepatic samples.

Cattle are naturally infected by close contact with infected sheep known to be a major source of MCF, actively shedding the virus, (3, 9). The calf in the present study was reared together with Morkaraman sheep, a local breed in northeast Turkey. No serological or molecular studies were carried out on the sheep as they had been slaughtered.

Clinical findings such as nasal discharge, corneal opacity and ulceration, lymphadenopathy and muscle tremors and incoordination due to nervous involvement were found compatible with previous research (14, 15, 19-23, 25). A similar form of the disease has been described in Turkey (16, 17, 18). In our study, more systemic changes were observed and vascular changes were detected in a variety of tissues. Severe clinical and pathological changes in this young calf may have beeen due to insufficient maternal antibody or lack of immunocompetence. Abuelzen *et al.* (19), reported that similar immunological problems resulted in susceptibility.

Moderate leucocytosis, lymphocytosis and neutrophilia are general hematological findings in MCF (10). Brenner *et al.* (20) reported severe leucopenia, marked lymphopenia and neutrophilia in four 4-7 month old calves with SA-MCF. In the present case lymphocytosis and neutrophylia were detected; the neutrophilia was considered due to the severe fibrinopurulent and necrotic bronchopneumonia caused by the possible secondary bacterial infection.

In conclusion, a case of SA-MCF in a young Brown Swiss calf reared with sheep is presented from Erzurum, northeast of Turkey. The case presented with typical histopathological changes and was confirmed by PCR.

ACKNOWLEDGEMENTS

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REFERENCES

1. Russell, G.C., Stewart, J.P. and Haig, D.M.: Malignant catarrhal fever: A review. Vet. J. 179, 324–335, 2009.
2. O'Toole, D. and Li, H.: Malignant catarrhal fever. In: Brown C. and Torres, A. (Eds.): Foreign Animal Diseases. 7th edit., Boca Publications Group, Inc. Boca Raton, pp. 325-334, 2008.
3. Plowright, W.: Malignant catarrhal fever. Rev. Sci. Off. Int. Epiz. 5, 897-918,1986.
4. Tham, K.M.: Molecular and clinicopathological diagnosis of malignant catarrhal fever in cattle, deer, and buffalo in New Zealand. Vet. Rec. 141, 303-306, 1997.
5. Alcaraz, A., Warren, A., Jackson C., McCoy M., Cheongh, S.H., Kimbal, S., Sells, S., Taus, N.S., Divers, T. and Li, H.: Naturally occurring sheep-associated malignant catarrhal fever in North American pigs. J. Vet. Diagn. Invest. 21: 250-253, 2009.
6. Gauger, P.C., Patterson, A.R., Kim, W.I., Stecker, K.A., Madson, D.M. and Loynochan, A.T.: An outbreak of porcine malignant catarrhal fever in a farrow-to-finish swine farm in the United States. J. Swine Health Prod.18, 244-248, 2010.
7. Li, H., Keller, J., Knowles, D.P., Taus , N.S., Oaks, J.L. and Crawford,T.B.: Transmission of caprine herpesvirus 2 in domestic goats. Vet. Microbiol. 107, 23-29, 2005.
8. Keel, M.K., Patterson, J.G., Noon, T.H., Bradley, G.A. and Collins, J.K.: Caprine herpesvirus-2 in association with naturally occurring malignant catarrhal fever in captive sika deer *(Cervus nippon)*. J. Vet. Diagn. Invest. 15,179–183, 2003.
9. Li, H., Snowder, G., O'Toole, D. and Crawford, T.B.: Transmission of ovine herpes virus 2 among adult sheep. Vet. Microbiol. 71, 27-35, 2000.
10. Baxter, S.I.F., Pow, I., Bridgen, A. and Reid, H.W.: PCR detection of the sheep-associated agent of malignant catarrhal fever. Arch. Virol. 132,145-149, 1993.
11. Callan, R.J. and Van Metre, D.C.: Viral diseases of the ruminant nervous system. Vet. Clin. Food Anim. 20, 327-362, 2004.
12. Jubb, K.V.F., Kennedy, P.C. and Palmer, N.: Pathology of Domestic Animals, 4th ed., Academic Press, San Diego.1993.
13. Mitchell, E.S.E. and Scholes, S.F.E.: Unusual presentation of malignant catarrhal fever involving neurological disease in young

calves. Vet. Rec. 164, 240-242, 2009.
14. Radostits, O.M., Gay, C.C., Hinchcliff, K.W and Constable, P.D.: Disease associated with viruses and Chlamydia-I. In:Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (Eds.): Veterinary Medicine 10th ed., Saunders, Elsevier, Edinburg, London, New York, Philadelphia, St. Louis, Sydney, Toronto, pp. 1245-1248, 2008.
15. Zemljic, T.Z., Pot, S.A,. Haessig, M and Spiess, B.M.: Clinical ocular findings in cows with malignant catarrhal fever: ocular disease progression and outcome in 25 cases (2007–2010). Vet. Ophthalmol. 15, 46-52, 2011.
16. Dabak, M. and Bulut, H.: Outbreak of malignant catarrhal fever in cattle in Turkey. Vet. Rec. 152, 240-241, 2003.
17. Yazici, Z., Arslan, H.H., Gumusova, S.O., Meral, Y. and Albayrak, H.: Occurrence of ovine herpesvirus type-2 infection in sheep and cattle in Samsun Province, Turkey. Dtsch. Tierärztl. Wschr. 113, 348-350, 2006.
18. Yesilbag, K.: Seroprevalence of malignant catarrhal fever-related gamma herpes viruses in domestic ruminants in Turkey. Trop. Anim. Health Prod. 39, 363–368, 2007.
19. Abuelzen, E.M.E., Housawi, F.M.T., Gameel, A.A., Al-Afaleq, A.I. and El-Bashir, A.M.: Sheep-associated Malignant catarrhal fever involving 3-5-week-old calves in Saudi Arabia. J. Vet. Med. B. 50, 53-59, 2003.
20. Brenner, J., Perl, S., Lahav, D., Garaz, I., Oved, Z., Shlosberg, A. and David, D.: An usual outbreak of malignant catarrhal fever in a beef herd in Israel. J. Vet. Med. B. 49, 304–307, 2002.
21. Carno, P.M.S., Oliviera, K.D., Barioni, G., Oliveira-Filho, J.C. and Souza, T.D.: Malignant catarrhal fever in a calf in Espirito Santo State, Brazil: Report of the first case. Brazil J. Vet. Pathol. 4, 44-46, 2011.
22. Luvizotto, M.C.R., Ferari, H.F. and Cardoso, T.C.: Malignant catarrhal fever-like lesions associated with ovine herpesvirus-2 infection in young calves (*Bos indicus*): a case report. J. Venom. Anim. Toxins. Incl. Trop. Dis. 16, 178-185, 2010.
23. Reid, S.W. and Robinson, B.N.: Malignant catarrhal fever in a five-month-old calf. Can. Vet. J. 28, 489, 1987.
24. Office International Des Epizooties (OIE).: Malignant catarrhal fever. In: Manual of Standards for Diagnostic Tests and Vaccines, pp.779-788, 2008.
25. O'Toole, D., Li, H., Miller, D., Williams, W.R and Crawford, T.B.: Chronic and recovered cases of sheep-associated malignant catarrhal fever in cattle. Vet. Rec. 140, 519-524,1997.
26. Meyer, D.J. and Harvey, J.W.: Veterinary Laboratory Medicine Interpretation and Diagnosis. 2nd edition, WB Saunders, Philadelphia, pp. 345,1998.

CORRECTION

Budd-Chiari-Like Syndrome Associated with a Pheochromocytoma Invading the Right Atrium in a Dog (Israel Journal of Veterinary Medicine Volume 67(3); 180-185, 2012).

In Figure 2 of the article an error was detected and is now replaced by the Figure below and its legend. The figure now contains three images from the same study.

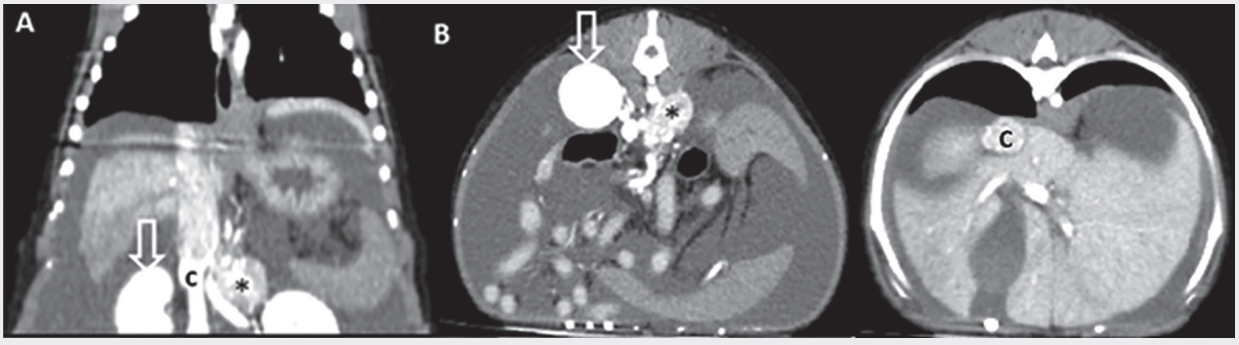

Figure 2: Computed tomography angiography (arterial phase) soft tissue window, demonstrating a contrast enhanced enlarged left adrenal gland (*) invading the caudal vena cava (c). Right kidney (white open arrow). All images are orientated with the right side of the patient on the left side of the image. (A) Dorsal plane demonstrating the enlarged non-homogeneously enhancing left adrenal gland invading the caudal vena cava. Note the dilatation and the non-homogenous filling defect/mass perfusion within the caudal vena cava. (B) Transverse view at the level of the right kidney cranial pole, demonstrating the left adrenal mass. (C) Transverse view at the level of the liver demonstrating the dilated caudal vena cava, due to the infiltrating adrenal mass.