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RESEARCH ARTICLE

Pathologic and Immunohistochemical Findings of Natural Lumpy Skin Disease in Egyptian Cattle

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ABSTRACT

This study was designed to investigate clinical and pathologic characteristics of acute and subacute lumpy skin disease (LSD) among naturally infected cattle and to study the localization of LSDV capsid antigen within different cells of the skin and regional lymph nodes using immunohistochemistry. Herein, we describe the gross, histologic, and immunohistochemical findings in 13 dairy cattle, 11 beef calves and 2 newly born calves that were naturally infected with LSDV. Prominent gross changes in all cases included numerous 1-6 cm well circumscribed, round cutaneous nodules with severe enlargement of superficial lymph nodes. Histologic changes in all acute cases consisted of severe ballooning degeneration of the epidermis, lymphoplasmacytic dermatitis, folliculitis, furunculosis, with severe vasculitis affecting the dermal capillaries, venules and arterioles. Rare intracytoplasmic inclusions were present in degenerated epidermal cells. Subacute cases showed multifocal areas of pannicular infarction with severe vasculitis affecting the neighboring arterioles and venules. Strong positive immunoreactivity for LSDV was identified primarily within macrophages and in degenerated epidermal cells. However, no viral antigen was present in endothelial cells. It can be concluded that vasculitis is a constant lesion in acute and subacute LSD and is most likely of an immune-mediated mechanism rather than a true tropism of the LSDV to endothelial cells.

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INTRODUCTION

Lumpy skin disease (LSD) is an infectious viral disease of cattle caused by a virus (LSDV) of the family *Poxviridae* and genus *Capripoxvirus* characterized by pyrexia and sudden eruption of skin nodules (Tuppurainen and Oura, 2012). Severe cyclic outbreaks continue to occur in Egypt (Ali *et al*., 1990; House *et al*., 1990; Salib and Osman, 2011) and the disease also spread to Asia and appeared in non-African countries (Yeruham *et al*., 1995; Brenner *et al*., 2006; Body *et al*., 2012). LSDV is thought to be transmitted primarily by biting insects. The virus was detected in mosquitoes of the genera *Aedes* and *Culex* and *Ixodid* ticks during some outbreaks (Chihota *et al*., 2003; Tuppurainen *et al*., 2011). Direct contact between animals is not likely to be a significant source of spread as illustrated by the lack of transmission to or immunity acquired by native cattle which were housed with experimentally infected animals (Carn and Kitching, 1995). The incubation period in natural cases is thought to be between two to five weeks (Carn and Kitching, 1995; Tuppurainen *et al*., 2005).

The disease listed by OIE in "List A" due to its rapid spread and severe economic losses such as hide damage, decrease in milk production and weight gain, mastitis, infertility in males and females, decreased semen quality, and death (Davies, 1991; Irons *et al*., 2005). The disease appears clinically as acute, subacute or subclinical. The acute disease is characterized by pyrexia, lymphadenopathy, skin nodules with subsequent sit-fasts and occasional orchitis and mastitis (Brenner *et al*., 2006). Other lesions observed at post-mortem examination include necrotic plaques in the body mucosa, chiefly of the upper respiratory tract, the oral cavity and rumen.

The pathology of LSD was described in few papers (Ali *et al*., 1990), however, several lesions such as cutaneous vasculitis and pannicular infarction that were

seen recently in some LSD biopsies were under-reported or poorly described. In the meanwhile, the immunohistochemical studies on the distribution of LSDV within affected tissues are rare. Therefore, we have designed this work to investigate fully the clinical and pathologic characteristics of acute and subacute LSD among naturally infected cattle and to study the localization of LSDV capsid antigen within different cells of the skin and regional lymph nodes using immunohistochemistry (IHC).

MATERIALS AND METHODS

Case selection and pathology: At 6 farms in Behera and Alexandria governorates, 570 dairy cattle, 503 beef calves and 82 newly born calves were examined clinically for the presence of LSD lesions during the period of March to September 2010. Out of these, 13 dairy cattle (2.28%), 11 beef calves (2.18%) and 2 newly born calves (2.44%) showed lesions suspected to be LSD. The animals were pyrexic, had enlarged superficial lymph nodes and multiple skin nodules typical of LSD. Selected skin nodules from affected animals were removed surgically, examined grossly and trimmed. In addition, biopsies from the superficial regional lymph nodes (prescapular and precrural lymph nodes) of affected animals were also collected for histopathology and immunohistochemistry. Skin and node specimens collected samples were fixed in 10% neutral buffered formalin and processed using the routine paraffin embedding technique sectioned at 4µm and stained with Mayer's hematoxylin and eosin. Slides were examined microscopically by two pathologists including a board certified pathologist (SAY).

Immunohistochemistry: According to Awadin *et al*. (2011), deparaffinized 4 µm-thick tissue sections (skin and lymph node) were incubated overnight at room temperature with normal goat serum 10% (Sigma, G9023) and the primary LSDV antiserum (1:1000). The primary antibody was visualized with a biotinylated goat anti-rabbit secondary antibody (30 min incubation, room temperature, dilution of 1:500) and peroxidase-conjugated avidin (ABC kit, Vector Laboratories) at room temperature for 30 min by using NovaRED (Vector, SK-4800) as a substrate; counterstaining was with hematoxylin.

RESULTS

Clinical signs, treatment protocol, and gross pathology: Based on the clinical course, the studied animals were classified into those acutely affected (clinical signs appeared from less than 7 days- case Nos. 1-15) or subacutely affected animals (clinical signs appeared from more than 7 days- case Nos. 16-26) as shown in Table 1. Gross lesions are abstracted in Table 2. Acutely affected animals suffered from fever (40-41.5°C), depression, inappetence, salivation, and naso-ocular discharges. Superficial lymph nodes were markedly enlarged especially prescapular and precrural lymph nodes; also edematous swelling of the lower gluteal muscles was recorded in some cases. The most significant clinical signs in acutely affected animals were the presence of well circumscribed, round, slightly raised, firm, and painful (1-6 cm) cutaneous nodules. The distribution of the nodules ranged from an animal to another from the presence of few nodules on the head and neck to the presence of myriad numbers allover the skin and mucocutaneous junctions in severe cases. The skin covering the nodule was either intact with sparse hair or was ulcerated. The cut surfaces were edematous and had multifocal necrohemorrhagic areas in the dermis and subcutis. Subacutely affected animals had enlarged lymph nodes with no fever. The cutaneous nodules in the subacutely affected animals were fewer in number and appeared as ulcerated firm slightly raised nodules or flat alopecic areas that were covered with a scab or with scar tissue (Fig. 1). The cut surface of subacute nodules showed a fibrotic dermis and multifocal grayish necrotic calcified areas in deep dermis and panniculus (Fig. 2). All affected animals were subjected to a symptomatic treatment with Terramycine 20% LA (Pfizer) 1 ml/10 kg b.wt i/m one shot, Diaflam (Pharma Swede) 1ml/10 kg b.wt i/m daily for 3 consecutive days. Animals showing edema in lower limbs was additionally injected with Diurizone (Vetoquinol) 1 ml/20 kg b.wt i/m for 3 consecutive days. Parameters of response to treatment were return to normal appetite, return of increased body temperature to normal level, disappearance of swollen skin nodules and disappearance of limb edema in some cases. Among the 13 studied dairy cows, 4 cows responded to treatment within 7 days, 5 cows responded to treatment within 15 days, 2 cows responded to treatment within 28 days, while 2 cows died after 14 days from onset of signs. One newly born calf responded to treatment within 17 days while the other died after 6 days from onset of signs. Among 11 beef calves, 8 calves responded to treatment within 9 days, 2 calves responded to treatment within 22 days while 1 calf died. No necropsy was performed on dead animals.

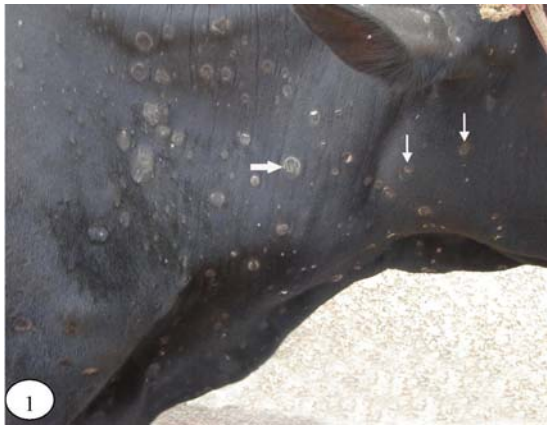

Fig. 1: Dairy cow suffering from subacute LSD. Multifocal flat alopecic cutaenous lesions that are either ulcerated (thin arrow) or covered with a scab (thick arrow).

Table 1: Total animals examined and percentage of acute and subacute cases of LSD

	Examined animal	Diseased animal	Acute cases	Subacute cases
Dairy cattle	570	13 (2.28)	6 (1.05)	7 (1.23)
Beef calves	503	11 (2.18)	7 (1.39)	4 (0.79)
Newly born calves	82	2 (2.44)	2 (2.44)	-
Total	1155	26 (2.25)	15 (1.3)	11 (0.95)

Values in parenthesis indicate percentage.

Histopathology: Histopathological lesions and its incidence in both acute and subacute cases were shown in Table 2.

Table 2: Gross and histopathological results: incidence of both acute and subacute LSD cases

Parameter	Lesions	Acute cases (n=15)	Subacute cases (n=11)
Clinical signs	Fever	15 (100)	0 (0)
	Swollen superficial L.N	9 (60)	5 (45.45)
	Swollen gluteal muscle	2 (13.3)	0 (0)
	Characteristic cutaneous nodules	15 (100)	11 (100)
	Epidermal microviscle	13 (86.6)	0 (0)
	Epidermal intracytoplasmic eosinophilic viral inclusions	3 (20)	0 (0)
	Dermal mononuclear cells infiltration	12 (80)	6 (54.4)
Histopathology	*Cellules claveleuses* of Borrel	4 (26.6)	1 (9)
	Vasculitis	9 (60)	11 (100)
	Furunculosis	6 (40)	3 (27.3)
	Pannicular fat necrosis	2 (13.3)	10 (90.9)
	Sinus histocytosis	12 (80)	9 (81.8)

Values in parenthesis indicate percentage.

Acute form: There was a moderate to severe acanthosis with orthokertatotic hyperkeratosis. The epidermal cells particularly at the stratum spinesum were swollen and had moderate hydropic (ballooning) degeneration. Occasionally, microvesicles were present in the stratum spinosum. Few affected epidermal cells had intracytoplasmic eosinophilic viral inclusions (Fig. 3). The superficial and deep dermis had moderate to severe perivascular and periadnexal infiltrates of lymphocytes, plasma cells, macrophages and rare neutrophils. However, high numbers of neutrophils were present in the dermis beneath the ulcerated areas. Few macrophages had vacuolated nuclei, marginated chromatin, and rare intracytoplasmic inclusions suggestive of sheep-pox cell (*Cellules claveleuses* of Borrel). The superficial dermal capillaries, venules, and small muscular arterioles showed swollen endothelium, necrotic wall, perivascular edema and perivascular to transmural infiltration of lymphocytes, plasma cells, and fewer neutrophils. The hair follicles at affected areas had a hyperplastic and/or degenerated epithelium. Few hair follicles were partially destroyed and replaced with necrotic epithelium, pleocellular infiltrates, and hair debris (furunculosis). The subcutaneous tissue and pannicular fat were spared in most animals, however, in few animals these tissues were infiltrated with few lymphocytes, plasma cells, and macrophages.

Subacute form: The epidermis was mildly hyperplastic. The superficial and deep dermis had fewer adnexa, mild pleocellular infiltrates and multifocal areas of fibrosis with no significant vascular lesions. The subcutaneous tissue and panniculus had fairly circumscribed multifocal infarcts. The infracted areas were composed of homogenous eosinophilic necrotic tissue and were encircled by hemorrhages, macrophages, lymphocytes, plasma cells, and fewer degenerated neutrophils (Fig. 4). The pannicular arterioles and venules within and around the infracted areas were necrotic and had severe transmural lymphoplasmacytic and histiocytic inflammation. The lymph nodes either in acute or subacute cases had marked lymphoid hyperplasia and interstitial edema with marked filling of the medullary and subcapsular sinuses with foamy macrophages (sinus histiocytosis), plasma cells, small lymphocytes, and few neutrophils (Fig. 5).

Immunohistochemistry: LSDV capsid antigen positive staining was signaled by the presence of brown intracellular granularity. In acute cases, sparse positive staining was observed in the cytoplasm of few epidermal cells, hair follicular epithelium, apocrine glands, and rarely in macrophages (Fig. 6). In subacute cases, the epidermis and the adnexal epithelium were negative; however, few macrophages showed sparse positive staining. Blood vessels, pannicular fat, fibroblasts, lymphocytes, plasma cells, and neutrophils did not show any positive staining in all studied acute or subacute cases. Strong positive intracytoplasmic staining was present in the macrophages present in the examined lymph nodes of the acute and subacute cases (Fig. 7).

DISCUSSION

In this study, we describe the gross and histopathologic lesions of the skin and lymph nodes in cattle naturally infected with LSDV, thus expanding the descriptions of lesions reported by others. Furthermore, we assessed the localization of LSDV within the affected skin and lymph nodes using immunohistochemistry.

LSDV has spread from Africa to the Middle East and Asia and there is a major concern about further spreading into Europe and other parts of the world. The most characteristic gross lesion in the current study was the presence of multifocal firm cutaneous and mucocutaneous nodules typical of LSD with an enlargement of the regional lymph nodes. Degeneration of epidermal and adnexal epithelium with vasculitis and the presence of few intracytoplasmic inclusions were the most characteristic lesions in the present acute cases. These results are in general agreement with previous studies (Ali *et al*., 1990; House *et al*., 1990). LSD lesions can occasionally extend to internal organs (Ali *et al*., 1990), however, the current cattle were not necropsied, and therefore, we cannot confirm or rule out the presence of systemic LSD lesions. The microscopic picture associated with subacute form of LSD was poorly documented in previous studies. The Main microscopic lesions of the current subacute cases were vasculitis affecting the venules and arterioles of the deep dermis with a resultant pannicular infarction. The epidermis and superficial dermis of these cases were generally spared or had minimal non-specific lesions.

In cattle with LSD, vasculitis/vasculopathy has been rarely observed in histological and ultrastructural sections of the skin (House *et al*., 1990), but the pathogenesis and types of blood vessels targeted by LSDV have not been determined. In this study, small venules, capillaries, and small muscular arterioles were damaged and had severe necrotizing and lymphoplasmacytic inflammation. The damaged vessels stained negative for the LSDV antigen using IHC, which suggests that the vascular effects may be resulting from the release of cytokines by inflammatory cells (immune-mediated vasculopathy) and not by a direct endothelial damage. In general, vasculitis develops either due to direct injury by infectious agents or

Fig. 2: A cross section in subacute LSD skin nodule. Focal area of necrosis and calcification in the subcutaenous tissue.

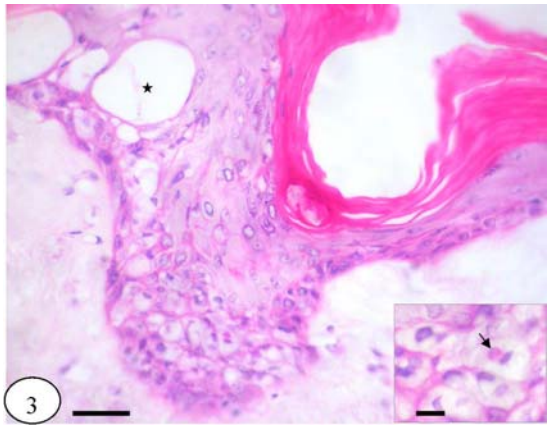

Fig. 3: Photomicrograph of skin of Dairy cow showing severe ballooning degeneration of the epidermis with formation of microvesicles (asterisk). H&E; Bar=20 µm. Inset, degenerated epidermal cell with an intracytoplasmic inclusion (arrow). H&E; Bar=5 µm.

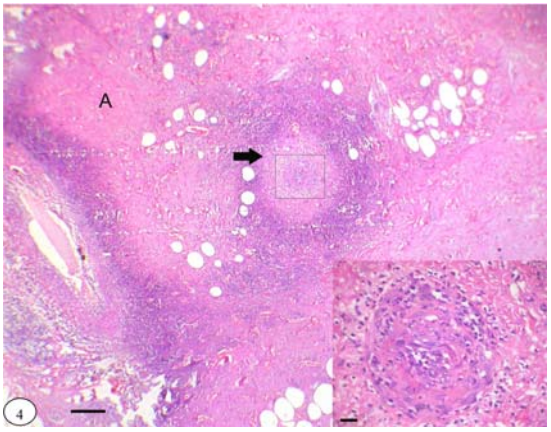

Fig. 4: Skin of dairy cow showing pannicular infarction (A) and severe vasculitis (arrow; inset). HE. Bar=100 µm, inset Bar=20 µm

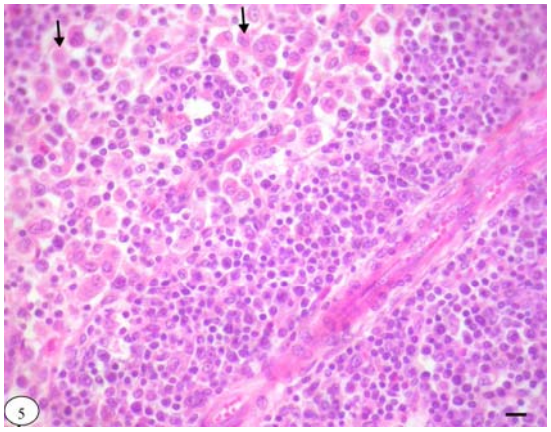

Fig. 5: Prescapular lymph node of beef calf showing severe sinus histiocytosis (arrows). HE. Bar=20 µm.

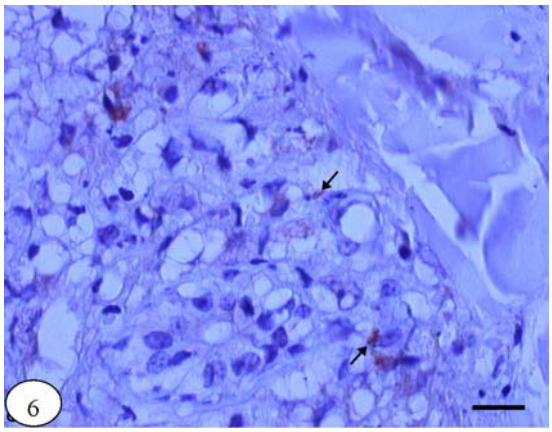

Fig. 6: Skin of beef calf showing positive intracytoplasmic immunoreactivity for LSDV within the degenerated follicular epithelium. Immunoperoxidase labeling for LSDV. Bar=20 µm

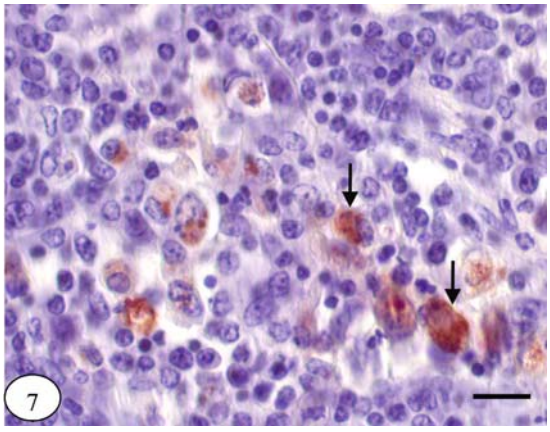

Fig. 7: Prescapular lymph node of beef calf showing positive intracytoplasmic intrahistiocytic immunoreactivity for LSDV. Immunoperoxidase labeling for LSDV. Bar=20 µm.

by an immune- mediated inflammation. Although the pathogenesis and exact etiology of immune-mediated vasculitis are unknown, advances in molecular research have revealed that an imbalance in inflammatory cytokines is central to the pathogenesis of this condition (Kuek *et al*., 2007). To the best of our knowledge, there are no available reports on the cytokines expressions associated with LSD.

In cattle, vasculitis can be caused by BVDV, blue tongue, malignant catarrhal fever (MCF), and septicemic bacterial diseases (Maxie and Robison, 2007). Blue tongue and MCF do not occur in Egypt. In BVD, clinical aspects (disease most commonly associated with diarrhea, gastroenteritis, and/or abortion) and preferential distribution of vascular lesions (small and medium sized-arterioles of GIT, mesentery, and heart) are different from

the lesions and clinical signs observed in this study (Brown *et al*., 2007). The clinical examination of the animals of this study did not indicate any internal or multisystemic lesions that are usually associated with septicemic bacterial diseases.

Cellules claveleuses of Borrel or sheeppox cells (SPCs) are a specialized phenotype of cells that are characteristic to capripoxvirus infection. Typical SPCs should have a vacuolated nucleus, marginated chromatin and granular intracytoplasmic inclusions (Gulbahar *et al*., 2006). Recent studies revealed that SPCs are monocytes, macrophages, or fibroblasts infected with virus (Gulbahar *et al*., 2006). The presence of SPCs in natural LSD is controversial and was only reported once (House *et al*., 1990). In the present study, few macrophages were reminiscent morphologically to SPCs but lacked the presence of the inclusions.

Similar to a previous study in LSDV-naturally infected cattle (Awadin *et al*., 2011), immunolabeling for LSDV antigen indicated infection of epithelial cells in widespread areas of the epidermis and adnexa. The current lesions were not associated with viral antigen in endothelial cells, smooth muscle cells, fibroblasts, lymphocytes, plasma cells and infiltrating neutrophils. LSDV antigen was commonly found in dermal macrophages and the circulating macrophages in regional lymph nodes. It is not clear if the LSDV get phagocytized by these macrophages or if it has a true tropism to these cells. To the best of our knowledge, there are no available studies about the probable tropism of LSDV to the cells of the monocyte–macrophage series.

Conclusion: Based on the result of current study, it was concluded that vasculitis was a constant lesion in the present acute and subacute cases and we speculated that, this vasculitis is of an immune-mediated origin. Future studies on the cellular tropism of LSDV and the nature of viral receptors on target cell membranes might be useful for better understanding to the pathogenesis of this important disease.

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REFERENCES

Ali A, M Esmat, A Attia and Y Abdel-Hamid, 1990. Clinical and pathological studies on lumpy skin disease in Egypt. Vet Rec, 127: 549-550.

Awadin W, H Hussein, Y Elseady, S Babiuk and H Furuoka, 2011. Detection of lumpy skin disease virus antigen and genomic DNA in formalin-fixed paraffin-embedded tissues from an Egyptian outbreak in 2006. Transbound Emerg Dis, 58: 451-457.

Body M, KP Singh, MH Hussain, A Al-Rawahi, M Al-Maawali, K Al-Lamki and S Al-Habsy, 2012. Clinico-histopathological findings and PCR based diagnosis of lumpy skin disease in the Sultanate of Oman. Pak Vet J, 32: 206-210.

Brenner J, M Haimovitz, E Oron, Y Stram, O Fridgut, V Bumbarov, L Kuznetzova, Z Oved, A Waserman, S Garazzi, S Perl, D Lahav, N Edery and H Yadin, 2006. Lumpy skin disease (LSD) in a large dairy herd in Isreal. Israel J Vet Med, 61: 73-77.

Brown CC, DC Baker and IK Barker, 2007. Alimentary system. In: Maxie MG, Ed, Jubb, Kennedy and Palmer's Pathology of Domestic Animals, 5th Ed. Saunders, Elsevier, Philadelphia.

Carn VM and RP Kitching 1995. The clinical response of cattle experimentally infected with lumpy skin disease (Neethling) virus. Arch Virol, 140: 503-513.

Chihota CM, LF Rennie, RP Kitching and PS Mellor, 2003. Attempted mechanical transmission of lumpy skin disease virus by biting insects. Med Vet Entomol, 17: 294-300.

Davies FG, 1991. Lumpy skin disease of cattle. A growing problem in Africa and the Near East. In: Branckaert RDS, R Tucker, N Roland, T Gumprecht, M Criscuolo, B Temple-Benvenuti, EP Cunningham, V Kouba, AW Qureshi, J Phelan, E Lynnerup and Richmond K (eds). World Anim Rev, 68: 37-42.

Gulbahar MY, WC Davis, H Yuksel and M Cabalar, 2006. Immunohistochemical evaluation of inflammatory infiltrate in the skin and lung of lambs naturally infected with sheeppox virus. Vet Pathol, 43: 67-75.

House JA, TM Wilson, S El Nakashly, IA Karim, I Ismail, N El Danaf, AM Moussa and NN Ayoub, 1990. The isolation of lumpy skin disease virus and bovine herpesvirus-4 from cattle in Egypt. J Vet Diagn Invest, 2: 111-115.

Irons PC, EM Tuppurainen and EH Venter, 2005. Excretion of lumpy skin disease virus in bull semen. Theriogenology, 63: 1290-1297.

Kuek A, BL Hazleman and AK Östör, 2007. Immune-mediated inflammatory diseases (IMIDs) and biologic therapy: a medical revolution. Postgrad Med J, 83: 251-260.

Maxie MG and WF Robinson, 2007. Cardiovascular system. In: Maxie MG, Ed, Pathology of Domestic Animals, 5th Ed. Elsevier Limited, Edinburgh, UK.

Salib FA and AH Osman, 2011. Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. Vet World, 4: 162-167.

Tuppurainen E and C Oura, 2012. Review: Lumpy skin disease: An emerging threat to Europe, the Middle East and Asia. Transbound Emerg Dis, 59: 40-48.

Tuppurainen S, E Venter and J Coetzer, 2005. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. Onderstepoort J Vet Res, 72:153–164.

Tuppurainen E, W Stoltsz, M Troskie, DB Wallace, C Oura, PS Mellor, J Coetzer and EH Venter, 2011. A potential role for ixodid (hard) tick vectors in the transmission of lumpy skin disease virus in cattle. Transbound Emerg Dis, 58: 93-104.

Yeruham I, O Nir, Y Braverman, M Davidson, H Grinstein and O Zamir, 1995. Spread of lumpy skin disease in Israel dairy herds. Vet Rec, 137: 91-93.