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Investigation of lumpy skin disease virus infection in young calves from cows vaccinated with sheep poxvirus vaccine

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ABSTRACT

Lumpy skin disease (LSD) is an important infectious viral skin disease of cattle causing high economic losses. Vaccination is the only effective method to control the disease in endemic countries. In the present study, 10 out of 25 clinically examined calves (40%) in a farm in Dakahlia Governorate, Egypt during summer 2013 showed clinical signs suspected to be lumpy skin disease, despite vaccination of dams 4 months before parturition with sheep poxvirus vaccine (Kenyan strain). The diseased calves showed localized or generalized skin nodules with or without fever. Skin lesions were collected from clinically diseased calves for virus isolation in embryonated chicken eggs for three passages followed by ten successive passages in Madin Darby Bovine Kidney (MDBK) and identification of isolated virus was done by indirect immunofluorescence test. The serum antibody titers were determined by serum neutralization test (SNT) and Enzyme linked immunosorbant assay (ELISA) in calves and also their dams. The results confirmed infection of calves with lumpy skin disease virus (LSDV) and showed that calves older than 3 months old age and those out of first calf heifers are more susceptible for infection. Some calves even with insufficient amount of maternal antibodies could be protected from infection. In conclusion, this study revealed that the maternal immunity may disappear before six months and make the calves vulnerable for infection. So further study should be directed toward identifying the optimal age at which LSD vaccination should be started and also on the role of passively transferred cell mediated immunity in protection of calves against infection with LSDV.

Keywords: ELISA, Immunofluorescence test, Lumpy skin disease, Maternal immunity, Sheep poxvirus vaccine, SNT.

INTRODUCTION

Lumpy skin disease (LSD) is an acute, subacute or inapparent viral disease characterized by pyrexia, localized or generalized skin pox lesions, and generalized lymphadenopathy. The disease caused by a virus (Neethling strain) in the genus Capripoxvirus of the family Poxviridae (Davies, 1991 and Mercer et al., 2007).

The disease was considered a "list A" disease by the Office International des Epizooties (OIE) due to its potential for rapid spread and ability to cause severe economic losses. The disease causes economic losses due to hide damage, loss of milk production,

mastitis, infertility and death (Weiss, 1968). The disease is endemic in Central and southern Africa (Babiuk et al., 2008). In Egypt, the LSD was first appeared in 1988 after cattle importation from Somalia (House et al., 1990). Lastly an outbreak was reported in 2006 having been introduced with foot and mouth disease by cattle imported from Ethiopia (World Animal Health Information Database, OIE).

Diagnosis of LSD is depending initially on clinical signs and laboratory diagnosis is based on virus isolation, histopathology and serological and molecular methods (House et al., 1990, Ireland and Binepal, 1998, Tuppurainen, 2005, Awad et al., 2008 and Lamien et al., 2010).

In Egypt, Protection of cattle against LSD was carried out using a Roumanian sheep pox vaccine strain produced in sheep as reviewed in Davies, 1991 and a Kenyan sheep pox tissue culture vaccine strain produced in Vero cell line (Michael et al., 1994). The live modified lumpy skin disease virus (LSDV) vaccine (Ismailyia strain) was produced and proved to be safe, potent and capable to protect cattle against challenge with virulent LSDV (Daoud et al., 1998). Immunity induced by the live vaccine is considered to last for at least 2 years for Kenyan sheep and goat poxvirus (KSGPV) and 3 years for South African strain of LSDV, they probably produce a lifelong immunity in common with other poxvirus vaccines (Davies, 1991). Following immunization with modified live LSD virus vaccine circulating antibodies appear about the 10th day, reach a high titre by the 30th day and persist for more than 3 years (Buxton and Fraser, 1977).

Calves born to immunized cows will have passive immunity that persists for about 6 months and might interfere with the efficient vaccination of calves less than six months old (Weiss, 1968 and Woods, 1988). For this reason, it is recommended that vaccination be performed after the disappearance of maternal immunity.

The aim of the present work lies in laboratory investigation lumpy skin disease virus infection in young calves from cows vaccinated with sheep poxvirus vaccine in a farm in Dakahlia Governorate, Egypt via virus isolation and identification of isolated virus by indirect immunofluorescence test. Also determination of serum antibody titers in calves and also their dams using serum neutralization test (SNT) and Enzyme linked immunosorbant assay (ELISA).

MATERIALS AND METHODS

Animals and samples:

Ten out of 25 clinically examined calves in a farm in Dakahlia Governorate, Egypt showed clinical signs suspected to be lumpy skin disease (LSD) despite vaccination of dams 4 months before parturition with sheep poxvirus vaccine (Kenyan strain). One of the infected calves severely affected and died 6 days after clinical signs onset. Two dams only showed clinical signs suspected to be LSD. Diseased animals exhibited multiple skin nodules either localized or generalized (Figure 1) with or without systemic signs. Skin lesion were collected from clinically disease animals in sterile dry bottles containing phosphate buffer saline (PBS) with antibiotics then transported in an icebox to the virology laboratory for virus isolation identification. Skin biopsies from three normal calves were included as negative controls. Also, blood samples without anticoagulant were collected from calves and their dams for serum separation by overnight incubation at 4 ºC then storage at – 20 ºC

and used for determination of antibody titers by serum neutralization test (SNT) and Enzyme linked immunosorbant assay (ELISA).

Reference virus strain:

Tissue culture adapted LSDV/Ismailyia88 strain was kindly supplied from Pox Vaccine Production and Research Department, Veterinary Serum and Vaccine Research Institute VSVRI, Abbasia, Cairo, Egypt. It was prepared in Madian Darby Bovine Kidney (MDBK) and has a titre of 104.5 TCID50/ml. This strain was used as a positive control for virus isolation and in serum neutralization test (SNT).

Preparation of skin samples for virus isolation:

Sample preparation was performed as described by (OIE, 2008) as follows: The samples were minced using sterile scissors and forceps and then homogenized in a mortar containing sterile sand with a pestle. Phosphate buffered saline (PBS) containing antibiotics (1000 U/ml penicillin, 1000µg/ml streptomycin and 500 µg/ml gentamycin) was added, making up a 20% (W/V) suspension. The suspension was frozen and thawed three times and centrifuged at 3000 r. p.m. for 10 min. The supernatant was collected and stored at - 20°C till used in virus isolation.

Virus isolation:

Virus isolation was carried out by inoculation of prepared samples on chorioallantoic membranes (CAMs) of 9th days old ECEs according to Van Rooyen et al., 1969. Three passage:s were carried out for each sample. After that 10 % suspension in PBS was prepared from each infected CAM as described before in skin sample preparation. Then 0.2 ml from infected CAM suspension was inoculated into confluent sheet of Madin Darby Bovine Kidney (MDBK) and incubated for one hour with intermittent tilting every 10 minutes for virus adsorption then cells supplemented with Eagle's minimum essential medium (Flow laboratories England) and reincubated for 7 days at 37ºC. The cells examined daily for detecting the cytopathogenic effect (CPE) (OIE, 2008). Normal non-inoculated cells were involved as a control. Ten successive passages were conducted.

Identification of isolated virus by indirect immunofluorescence test:

The test was applied on inoculated MDBK cells to detect LSDV according to Davies et al., 1971 as follow: The cells were fixed with acetone for 10 minutes and then washed with PBS, pH 7.6. To identify LSDV infected cells, viral cytoplasmic antigens were stained with 1: 100 dilution of rabbit hyperimmune serum against LSDV prepared as previously described by Davies, 1982 and incubated for one hour at 37ºC in humidified chamber then the cells were washed with PBS for 15 minutes three times. Then, 1:200 dilution of antirabbit FITC conjugate (fluorescent conjugated goat IgG fraction to rabbit IgG, from ICN pharmaceuticals) was added. After 1 hour of incubation at 37 oC in dark, the slides were thoroughly washed with PBS and mounted with buffered glycerin and examined under fluorescent microscope (Carl Zeiss, Jena, Germany).

Determination of serum antibody titers in calves and also their dams:

Serum neutralization test (SNT):

The technique was carried out as described before by House et al., 1990. Briefly, the collected sera were heated at 56 oC for 30 minutes to destroy any non-specific inhibiting factors. The sera were diluted at a 1/10 dilution. Ten fold serial dilutions of reference LSDV strain were prepared. Then 100 µl of each virus dilution was added to 100 µl of 1/10 diluted serum samples. The mixture allowed reacting for one hour at 37 oC. Then, 100 µl of MDBK cell suspension (105 cells/ml) were added to all plates. The plates were incubated at 37 oC for 7 days. Positive serum control, negative serum control, cell control and virus control were included. The neutralization index (NI) of the serum samples was determined. NI of 1.5 or more was considered as a positive.

Enzyme linked immunosorbant assay (ELISA):

The collected serum samples were tested against LSDV antigen using the ELISA technique as described by House et al., 1990. Briefly, 100 µl of partially purified LSDV antigen that prepared according to Capstic and Coackley, 1962 was diluted 1/10 in carbonate-bicarbonate buffer (pH 9.6) incubated in the plate at 4 ºC overnight and then washed with washing buffer (PBS, pH 7.4 containing 0.05% tween 20). Then removing of the washing solution and the plate was dried on filter paper. The dried pate was blocked with 100 µl of blocking buffer (1% bovine serum albumin in PBS containing 0.05% tween 20) for one hour at 37 ºC. Then, 100 µl of 1/10 diluted sera was added into the coated plate. Each serum sample was run in triplicate. The control positive and negative sera were included in the test. The plate incubated for one hour at 37 ºC then washed three times. The anti-bovine IgG (whole molecule) peroxidase conjugate, Sigma chemical company (100 µl/ well) at a dilution of 1/5000 was added for one hour at 37 ºC then washed three times. Then, 100 µl of substrate (o.Phenylene diamine), Sigma chemical company was added to each well and the plate incubated at room temperature in a dark place for 10 minutes, the reaction was stopped by stopping solution (sulphuric acid 97%). The absorbance of the contents of each well was read at 492 nm using ELISA reader.

RESULTS

Virus isolation and identification:

Inoculation of 10 % suspension of the third passage inoculated CAM into MDBK cell culture for ten successive passage revealed that the causative virus was isolated from 8 (out of 10) skin samples collected from clinically infected calves and isolated in one (out of 2) skin samples collected from clinically infected dams. The inoculated MDBK cell culture showed the characteristic CPE in form of cell rounding and shrinking that form highly retractile discrete foci and appearance of clear irregular spaces in the cell sheet without syncytia formation. Identification of the virus isolated in infected MDBK cells revealed appearance of a bright greenish yellow fluorescent reaction in the cytoplasm of infected cells while control non-infected cells were free from any fluorescence (Figure 2).

Determination of serum antibody titers in calves and also their dams:

The neutralization indices and ELISA readings were determined in both young calves and their dams and the obtained results were presented in table 1 which showed that among infected calves those older than 3 months old age (60%) are more susceptible. Also, those

calves out of first calf heifers (57%) are more susceptible for infection at younger age even with presence of adequate antibodies level in the sera of their dams. Some calves even with insufficient amount of maternal antibodies (e.g. calve no. 5 & 22) could be protected from infection. Dam with sufficient amount of serum antibody titer but suffer from mastitis rendered calve susceptible to the infection (calve no. 19).

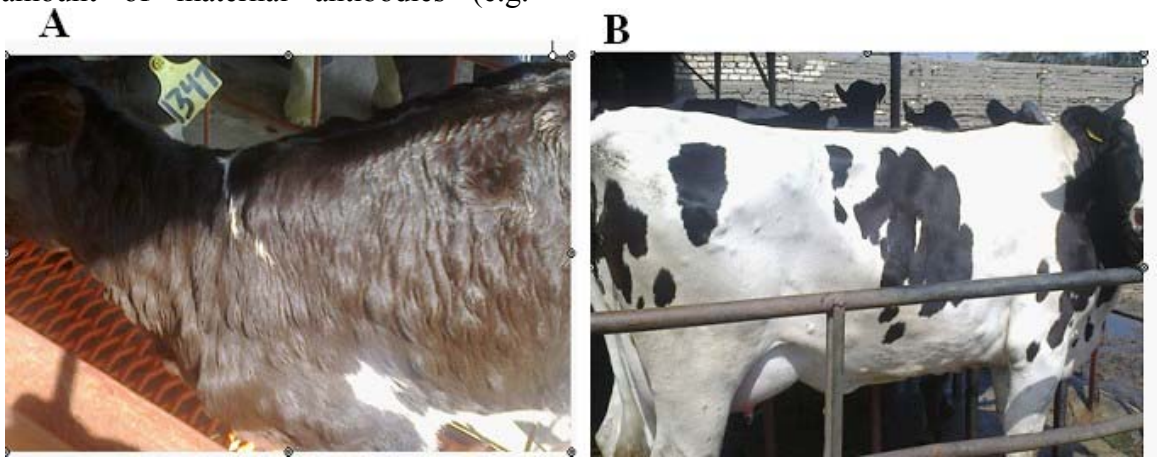

Figure 1: Skin nodules of LSD scattered all over the body of infected calve (A) and adult dam (B).

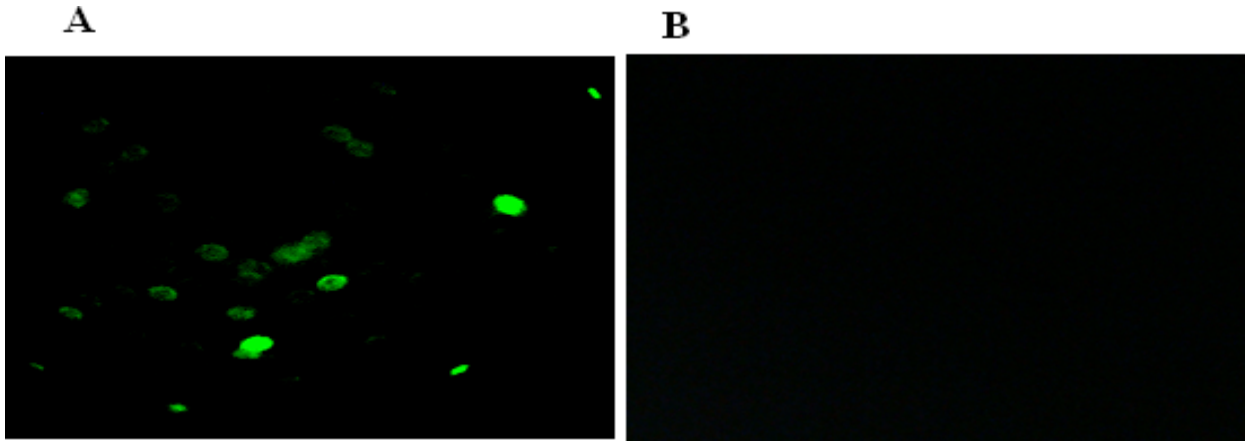

Figure 2: Greenish yellow fluorescent reaction in the cytoplasm of infected MDBK cells with LSDV isolate (A) and control non-infected cells, free from any fluorescence

Table 1: Antibody titers in both young calves and their dams using SNT and ELISA:

No. of animals	Age of calve	Young calves			Calf's dams			
		Appearance of LSD clinical signs	Antibody titers		Appearance of LSD clinical signs	Antibody titers		Parturition seasons
			SNT	ELISA		SNT	ELISA	
1 (1351)	12 days	- ve	**1.8**	**2.120**	- ve	1.5	1.556	
2 (1359)	5 days	- ve	**2.1**	**2.665**	- ve	1.95	2.2344	
3 (1350)	15 days	- ve	**1.5**	**1.554**	- ve	**1.8**	**2.335**	Fifth
4 (1336)	58 days	- ve	**1.95**	**2.234**	- ve	**1.65**	**1.957**	
5 (1340)	43 days	- ve	**1.2**	**0.765**	- ve	**1.8**	**2.113**	
6 (1315)	5 months & 8 days	**+ ve**	**1.05**	**0.593**	+ ve	**1.2**	**0.693**	Second
7 (1324)	4 months & 12 days	- ve	**1.5**	**1.554**	- ve	**1.8**	**2.135**	
8 (1358)	6 days	- ve	**1.65**	**1.934**	- ve	**2.1**	**2.654**	
9 (1328)	3 months & 7 days	**+ ve**	**1.05**	**0.565**	- ve	**1.8**	**2.124**	First
10 (1342)	One month & 2 days	- ve	**1.8**	**2.120**	- ve	**1.8**	**2.123**	
11 (1333)	2 months & 3 days	**+ ve**	**1.2**	**0.675**	- ve	**1.95**	**2.231**	
12 (1349)	16 days		**2.1**	**2.556**	- ve	**1.65**	**1.954**	
13 (1347)	20 days	**+ ve**	**1.05**	**0.663**	- ve	**1.5**	**1.566**	Second
14 (1312)	5 months & 12 days	- ve	**2.1**	**2.752**	- ve	**1.8**	**2.126**	
15 (1320)	5 months	- ve	**1.8**	**2.135**	- ve	**2.1**	**2.642**	
16 (1317)	5 months & 7 days	**+ ve**	**0.6**	**0.482**	- ve	**1.5**	**1.548**	
17 (1332)	2 months and 12 days	**+ ve**	**0.3**	**0.396**	- ve	**2.1**	**2.675**	
18 (1348)	18 days	- ve	**2.1**	**2.335**	- ve	**1.8**	**2.134**	
19 (1329)	2 months & 20 days	**+ ve**	**1.05**	**0.567**	- ve (but suffer from mastitis)	**2.1**	**2.645**	Third
20 (1325)	3months & 23days	- ve	**1.8**	**2.678**	- ve	**1.95**	**2.234**	
21 (1326)	3 months & 23 days	- ve	**1.8**	**1.155**	- ve	**1.65**	**1.964**	
22 (1322)	4 months & 28 days	- ve	**1.2**	**0.744**	- ve	**2.1**	**2.661**	
23 (1310)	5 months & 26 days	**+ ve**	**1.05**	**0.693**	- ve	**1.8**	**2.669**	
24 (1318)	5 months & 6 days	**+ ve**	**1.25**	**0.682**	- ve	**1.5**	**1.511**	First
25 (1282)	10 months	**+ ve**	**0.3**	**0.282**	- ve	**1.65**	**1.934**	First

DISCUSSION

LSD can be confused with skin lesions of pseudo-lumpy skin disease (caused by bovine herpes virus-2), insect bite, Demodex infection, onchocerciosis, besnoitiosis and dermatophilosis. So LSD needs a rapid and specific laboratory diagnosis after being suspected for rapid performing of control measures (Carn, 1993 and Esposito and Fenner, 2001). Protection of cattle against LSD in Egypt was carried out using sheep poxvirus vaccination strategy (Davies, 1991 and Michael et al., 1994). LSD needs a rapid laboratory diagnosis after being suspected. Following diagnosis of the disease, rapid performing of control measures is required to limit losses (Carn, 1993).

In the present study, virus isolation and indirect immunofluorecence were used for confirmative identification of capripoxvirus in skin lesions collected from LSD suspected calves come from cows vaccinated with sheep poxvirus vaccine (Kenyan strain) and also in LSD suspected dams. The causative virus was isolated in MDBK and showed the characteristic CPE without synctia formation. These findings are in agreement with the earlier reports of Buxton and Fraser, 1977 and Daoud et al., 1998 while in disagreement with Haig, 1957 who found that CPE was characterized by syncytia formation. Indirect imunofluorescence was used as a reiable test for identification of the virus in infected MDBK as described before by Ismael, 2000, Tuppurainen, 2005 and El-Kenawy and El-Tholoth, 2010 and 2011.

Regarding investigation of serum antibody titers in calves and also their dams, the results showed that serum neutralization test (SNT) and Enzyme linked immunosorbant assay (ELISA) showed that among infected calves those older than 3 months old age that indicate that the maternal immunity disappeared before 6 months and calves should be vaccinated before this age. This finding is in harmony with Fahmy, 2000 who stated that LSDV maternal antibodies via colostrum maintained in calves till 4.5 month of age then disappeared. On the other hand, this result disagreed with Woods, 1988 that mentioned that passive immunity from vaccinated dams might interfere with the efficient vaccination of calves less than 6 months old. So further study should be directed toward identifying the optimal age at which LSD vaccination should be started.

Higher susceptibility of calves from first calf heifers could be explained by presence of less passively transferred maternal immunity via colostrum than in older cows that have more immunological experience (Divers and Peek, 2008). Dam with sufficient amount of serum antibody titer but suffer from mastitis rendered calve susceptible to the infection as the mastitis may interfere with transferring of maternal immunity via colostrum to calve.

Presence of calves that contain sufficient amount of passively transferred antibody titers and infected with the virus and calves even with insufficient amount of maternal antibodies and protected from infection could be explained by the important role of passively transferred cell mediated immunity in protection of young calves against infection (Capstick and coackely

and 1962, Kitching et al., 1986). The result was supported by Fahmy, 2000 who confirmed transfer of maternal lymphocytes from dams vaccinated with sheep poxvirus vaccine to the newly born calves via colostrums. Also, Presence of antibodies may enhance infectivity of some viruses like foot and mouth disease virus (FMDV) (Baxt and Mason, 1995) but this explanation need further work to prove that in case of LSDV.

REFERENCES

Awad, S., Ibrahim, A. K., Mahran, K., Fararh, K. M., and Abdel Moniem, M. I. (2009): Evaluation of different diagnostic methods for diagnosis of lumpy skin disease in cows. Trop Anim Health Prod. 42 (4):777-783.

Babiuk, S., Bowden, T. R., Boyle, D.B., Wallace, D. B. and Kitching, R. P. (2008): Capripoxviruses: An Emerging Worldwide Threat to sheep, Goats and Cattle. Transboundary and Emerging Diseases. 55: 263-272.

Baxt, B. and Mason, P. W. (1995): Foot-and-mouth disease virus undergoes restricted replication in macrophage cell cultures following Fc receptor-mediated adsorption. Virology. 207(2):503-509.

Buxton, A. and Fraser, G. (1977): Animal microbiology 1st. Published. Vol. II Rickettsias and viruses. Distributed in USA by J. E. Lippincott Company, Philadelphia and in Canada by J. E. Lippincott Company of Canada Ltd. Toronto.

Capstick, P. B. and Coackley, W. (1962): Lumpy skin disease:the determination of the immune state of cattle by an intradermal test. Res. Vet. Sci., 3 (3): 287-291.

Carn, V. M. (1993): Control of capripoxvirus infections. Vaccine. 11 (13): 1275-1279.

Daoud, A. M.; Michael, A.; Soad, M. S.; Samir, S. S. and Aboul Soud, E. A. (1998): Production of lumpy skin vaccine in Egypt. 4th Vet. Med. Zag. Cong., 26-28 August, In Hurghada, pp. 117-124.

Davies, F. G. (1982): Observations on the epidemiology of LSD in Kenya . J. Hyg. Camb., 88 : 95-102.

Davies, F. G. (1991): LSD- An African Capripox virus disease of cattle. Br. Vet. J. 147: 489-503.

Davies, F. G., Krauss, H., Lund, J., Taylor, M. (1971): The laboratory diagnosis of LSD. Res. Vet. Sci. 12, 123-127.

Divers, T. J. and Peek, S. (2008): Rebhun's Disease of dairy cattle. St. Louis, Mo.: Saunders/Elsevier.

El-Kenawy, A. A and El-Tholoth, M. S. (2010): Sequence Analysis of Attachment Gene of Lumpy Skin Disease and Sheep Poxviruses. Virol. Sin., 25 (6):409-416.

El-Kenawy and El-Tholoth. (2011): Lumpy Skin Disease Virus Identification in Different Tissues of Naturally Infected Cattle and Chorioallantoic Membrane (CAMs) of Emberyonated Chicken Eggs Using Immuno fluorescence, Immuno peroxidase techniques and Polymerase Chain Reaction. Int. J. Virol., 7(4): 158-166.

Esposito, J. J. and Fenner, F. (2001): Poxviruses. In Field, B. N.; Knipe, D. M.; and Howley, P. M.; Chanock, R. M.; Melnick, J. L. Monathy, T. P.; Roizman, B. and Straus, S. E. Fields Virology. Lippincott, Williams and Wilkins, Philadelphia, Pa. P. 2885-2921.

Fahmy, H. A. (2000): Studies on intradermal allergic test of lumpy skin disease in cattle. Ph. D. Thesis, Fac. Vet. Med. Cairo Univ.

Haig, D. A. (1957): Lumpy skin disease. Bull. Epiz. Dis. Afr., 5 : 421-430.

House, J. A.; Wilson, T. M.; El-Nakashly , S.; Karim, I. A.; Ismail, I.; El-Danaf, N.; Moussa, A. M. and Ayoub, N. N. (1990): The isolation of LSD- virus and bovine herpes virus -4 from cattle in Egypt. J. Vet. Diagn. Invest., 2:111-115.

Ireland, D. C. and Binepal, Y. S. (1998): Improved detection of capripoxvirus in biopsy samples by PCR. J. Virol. Methods., 74: 1-7.

Ismael, A. B. I. (2000): Some studies on lumpy skin disease in cow and buffaloes. Department of Animal Medicine. M. V. Sc. Thesis, Fac. Vet. Med. Zagazig Univ.

Kitching, R. P.; Hammond, J. M. and Black, D. N. (1986): Studies on the major common precipitating antigen of capripox virus . J. Gen. Virol., 67:139-148.

Lamien, C. E., Lelenta, M., Goger, W., Silber, R., Tuppurainen, E., Matijevic,M., George, A., Luckins, A. G. and Diallo, A. (2010): Real time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses. J. Virol. Methods., 171 (1): 134-140

Mercer, A. A.; Schmidt, A.; and Weber, O. (2007): Poxviruses. Birkhuser Verlag, P.O. Box 133, CH-4010 Basel, Switzerland.