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Full Length Research Paper

Observations on lumpy skin disease in local Egyptian cows with emphasis on its impact on ovarian function

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The present study monitored reproductive and health aspects of local cows in Egypt following natural infection with Lumpy skin disease virus (LSDV). The study was carried out on 640 mature Egyptian native cows reared at Lower Egypt. Cows were clinically examined and genital organs were scanned by ultrasonography and ovarian function was recorded. Blood samples were collected for virus isolation and propagation on calf Kidney cells, electron microscopy (EM) and for PCR assay. Moreover, progesterone level (ELISA), protein electrophoretic pattern (Polyaccrylamide Gel Electrophoresis), immunoglobulin concentrations (Radial Immune Diffusion) and some trace element values (Atomic Absorption Spectrophotometry) were determined in the serum. Results indicated that LSDV had typical cytopathic effect and plaque. EM showed that size of LSDV size ranges from 300 – 350 nm with crescent or ovoid shape. PCR assay indicated the presence of a band at 192 bp which belonged to viral attachment protein encoding gene. Epidemiological studies revealed that 25.47% of the examined cows were positive for LSDV with obvious year, age and seasonal variations. LSDV infected cows showed fever, become completely of food with skin nodules. Serum progesterone level was low ($P < 0.01$) during both follicular and luteal phases of the estrous cycle and 92.64% of infected cows had inactive ovaries. Infected cows showed obvious ($P < 0.01$) low albumin and high γ globulin values as well as low concentrations of copper and iron ($P < 0.01$) in their serum as compared to non infected cow-mates. It was concluded that PCR is a useful method for rapid diagnosis of LSDV. Cows infected with LSDV mostly suffering from cessation of ovarian activity mainly due to poor body condition.

Key words: LSDV, Cow, Ovary, PCR, infection, Immunoglobulin.

INTRODUCTION

Lumpy skin disease virus (LSDV), a member of the capripoxvirus genus of the Poxviridae, is the etiologic agent of an important disease of cattle in Africa. Capripoxviruses (CaPVs) represent one of eight genera within the chordopoxvirus (ChPV) subfamily of the Poxviridae. The Capripoxvirus genus is currently comprised of LSDV, sheep pox virus (ShPV), and goat pox virus (GPV). These viruses are responsible for some of the most economically significant diseases of domestic ruminants in Africa and Asia (Fenner, 1996). CaPV infections are generally host specific and have specific geographic distributions (Davies 1991; Carn, 1993; Coetzer et al., 1994). CaPVs are, however, serologically indistinguishable from each other, able to induce heterologous cross-protection, and able in some instances for experimentally cross-infection (Davies, 1991; Carn, 1993). Restriction fragment analysis and limited DNA sequence data support a close relationship between CaPVs (Kitching et al., 1989). LSD is characterized by extensive cutaneous lesions and signs typical of generalized poxvirus diseases (Davies 1991; Coetzer et al., 1994). Transmission of LSD between cattle is inefficient, and arthropod-vectored transmission may be significant in epizootic outbreaks and in the spread of LSD into nonenzootic regions (Carn and Kitching, 1995; Yeruham et al., 1995). Attenuated LSDV strains and ShPV have been successfully used as LSD vaccines in enzootic and outbreak areas; however, vaccine failure and restrictions on the use of live virus vaccines create the need for a safe and effective, live attenuated vaccine (Coetzer et al., 1994; Tulman et al.,

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2001). Current molecular data on the LSDV genome consists of restriction endonuclease analysis, cross-hybridization studies, and limited transcriptional and DNA sequence analysis (Tulman et al., 2001).

Ovarian function in farm animals includes two main functions, production of female gametes and secretion of hormones that share in regulation of the reproductive process. Disturbed ovarian activity is the most prominent cause of reproductive failure and economic losses as the result of fewer days in milk and fewer calves produced per year of life as well as high culling rate mainly due to failure of pregnancy (Ahmed, 2007).

To the best of our knowledge, there are no available literature on the effect of LSD on ovarian activity and this is considered the first report on this subject. There-fore, the aim of the current study was to monitor the effect of infection with LSDV on ovarian function and some health aspects in local cows in Egypt. Also, diagnosis of LSDV in blood through PCR technique was another goal of this investigation.

MATERIALS AND METHODS

Animals

A total number of 640 mature non-pregnant local cows (2 – 10 years), reared at Lower Egypt was examined during regular weekly field trips throughout a period of 4 consecutive years (2004 - 2007). A complete data on case history, owners complain, clinical examination and reproductive status were recorded for each animal. All cows were subjected to rectal examinations using ultra sound apparatus (Pia Medical Falcs e`Saote, the Netherlands) with an endorectal linear array 6 - 8 MHz transducer and ovarian function was recorded.

Blood sampling

Two kinds of blood samples were collected through the jugular vein puncture from each of examined cows, the first kind of samples kept to clot, centrifuged at x1500 g for 20 min, sera were separated and kept at –20□C until analyzed. The second kinds of blood samples collected on EDTA were kept at -70□C for PCR analysis.

Virological techniques

i.) Virus isolation: LSDV was isolated and propagated on calf Kidney cells according to Vagnozzi et al. (2007) and plaque test was performed according to Burleson et al. (1995).
ii.) Electron microscopy: The harvested positive samples suspension was allowed for electron microscopic examination of model EM 10 Zesiss West Germany of 60 kv and resolution of 10oA in Electron Microscope Unite, National Research Centre. The method was performed according to Madbouly et al. (2005).
iii.) Polymerase Chain Reaction (PCR): The extraction method used was a modification of the method described by Schwartz et al. (1997) and Gubbels et al. (1999).

The PCR primers were developed from the viral attachment protein encoding gene and have the following sequences: forward primer 5'-d TTTCCTGATTTTTCTTACTAT3' and reverse primer 5'-d AAATTATATACGTAAATAAC 3' (Ireland and Binepal 1998). The size of the amplicon was 192 bp (Ireland and Binepal, 1998). A Platinium® Quantitative PCR SuperMix-UDG (2X) reaction mixture (Invitrogen, Life Technologies) was used for amplification of nucleic acid templates. DNA amplification was carried out in a final volume of 25 µl containing 12.5 µl Platinium® Quantitative PCR SuperMix-UDG, 1 µl 0.20 mM each primer, 9.5 µl distilled water and 1 µl DNA sample. The PCR started with one cycle of 42°C for 2 min and 94°C for 10 min. The initial cycle was 94°C for 1 min, 50°C for 30 s and 72°C for 1 min. This was followed by 40 cycles of 94°C for 1 min, 50°C for 30 s, and 72°C for 1 min, and a final elongation step of 72°C for 1 min to complete the extension of the primers (Ireland and Binepal, 1998). Amplified products were analysed using a 100 bp DNA ladder (Whitehead Scientific Ltd) as a molecular marker on 1.5% agarose gels. Gels were stained using ethidium bromide (1 µg/ml) in Tris EDTA (TE) and amplicons were visualized using an UV transilluminator at a wave length of 590 nm. Positive reactions were confirmed according to size.

Biochemical analyses

Progesterone level: Serum samples were analyzed for progesterone level using kits purchased from DIMA, Germany for quantitative determination of progesterone levels by the micro- ELISA well method. The kit had a sensitivity of 2.0 pg/ml with the inter- and intra-run precision coefficient of variations of 2.9 and 4.85, respectively (Hubl et al., 1982).

Protein electrophoresis: Fractionation of serum protein was done using polyacrylamide gel columns electrophoresis (Laemmli, 1970).

Immunoglobulin concentrations: Immunoglobulin concentrations were determined using commercial radio immune diffusion plates (Hassan et al., 1995).

Trace elements: Some trace element values including zinc, copper and iron concentrations were determined in serum using atomic absorption spectrophotometry (Varley et al., 1980).

Statistical analysis

The obtained data were computed and statistically analyzed in relation to LSDV infection (Snedecor and Cochran, 1980).

RESULTS

Virus isolation and propagation

The isolated virus was confirmed to be a typical LSDV and it had cytopathic effect (CPE) whereas intracytoplasmic inclusion bodies were clearly detected. Also typical plaque was found (Figure 1).

Electron microscopy

The virus size was found to range from 300 - 350 nm with crescent or ovoid shape (Figure 2).

PCR

PCR assay indicated that there is a band at 192 bp which belonged to viral attachment protein encoding gene.

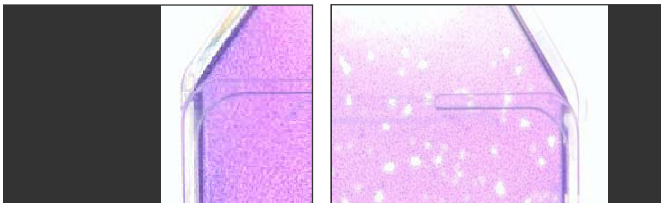

Figure 1. Plaque assay, showing normal cells (left) and infected cells (right) with plaques due to Lumpy skin disease virus cytopathic effect.

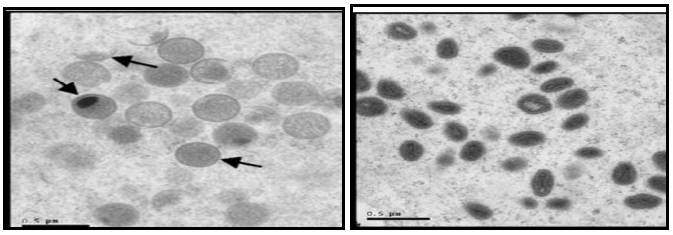

Figure 2. Ultramicrograph of Lumpy skin disease virus showing intracytoplasmic inclusion bodies on the right. On the left the ultramicrograph showing the virus. Magnifications of x 9,300.

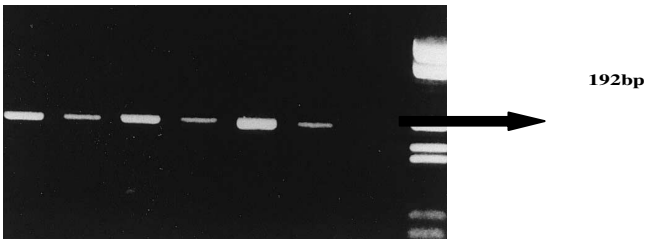

Figure 3. Agrose gel electrophoresis of PCR viral attachment protein encoding gene of Lumpy skin disease virus. Bands at 192 bp showing that the virus is LSDV. Lane 1: DNA arker, Lane 2: negative control, Lane 4: positive control and the rest are tested samples.

Epidemiology

Out of 640 examined, 163(25.47%) were positive for LSDV (Table 1). The disease showed years variation ($P < 0.01$), the highest incidence was recorded during 2006 (41.13%) and the lowest incidence during 2007(13.46%) as shown in table 2. Also age variation (Table 3) was clear, despite it is not significant, but the disease tends to be of high incidence in cows of young age (36.90%) and low in moderate aged cows. Obviously high incidence ($P < 0.01$) of the disease was recorded during winter (36.70%) and autumn (25.60%) seasons of the year (Table 4).

Clinical signs

LSDV infected cows showed fever, very low appetite, severely loss weight and had poor body condition with clear stiff gates or lameness. Some animals revealed arthritis. The most pronounced signs were skin nodules which soon become erupted with clear ulcerations. These ulcers may be infected and become suppurated or showing myiasis. Sloughing of skin from a large area was seen in some cases (Figure 4). The lesions were more pronounced in young cows and may cover the whole body.

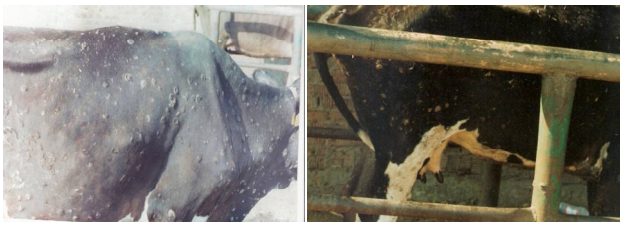

Figure 4. Shows the characteristic skin lesions in local Egyptian cows infected with Lumpy skin disease virus. Note the sloughing of skin at the hind quarter in (Right).

Table 1.Overall incidence of Lumpy skin disease virus infection in local Egyptian cows.

Number of examined animals	LSDV infected cows	
	Number	percentage
640	163	25.47

Table 2. Years variation in the incidence of Lumpy skin disease virus infection in local Egyptian cows.

Year	Number of examined animals	LSDV infected cows	
		Number	Percentage
2004	182	55	30.22
2005	178	36	20.22
2006	124	51	41.13
2007	156	21	13.46
χ^2value	17.74**		

**P < 0.01

Ovarian activity

Table 5 reveals that a high percent of LSDV infected cows (92.64%) did not show heat signs as compared to cows that did not reveal the clinical signs of the disease (15.93%). Gynecological examination using ultrasonography revealed that mostly infected cows had bilateral, small sized inactive ovaries with no physiological structures on their surface (Figure 5). Infected cows did not show ovarian activity, not only during the course of the disease, but also, for a longer period after recovery (2 – 6 months). As soon as the skin became intact again and eruptions disappeared, ovarian rebound occurred.

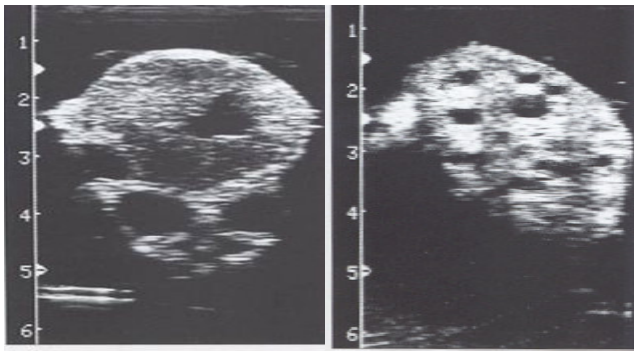

Figure 5. Ultarsonographic scan of active (Right with large follicular growth) and Inactive (Left with small growing follicles) ovaries of local Egyptian cows.

Table 3. Age variation in the incidence of Lumpy skin disease virus infection in local Egyptian cows.

Age (year)	Number of examined animals	LSDV infected cows	
		Number	Percentage
2 - 4	168	62	36.90
4 - 6	199	38	19.10
6 - 8	141	35	24.82
8 -10	132	28	21.21
χ^2value	7.63		

Table 4. Seasonal variations in the incidence of Lumpy skin disease virus infection in local Egyptian cows.

Season of the year	Number of examined animals	LSDV infected cows	
		Number	Percentage
Summer	144	21	14.58
Autumn	168	43	25.60
Winter	188	69	36.70
Spring	140	30	21.43
χ^2value	22.79**		

Blood constituents

Progesterone level: LSDV infected cows having low ($P < 0.01$) serum progesterone level during both follicular and luteal phases of the estrous cycle as compared to non infected cows. The level was undetectable in cows suffering from ovarian inactivity in both LSDV positive and negative cases (Table 6).

Serum proteins electrophoretic pattern: LSDV infected cows revealed obvious ($P < 0.01$) decreased albumin and increased γ globulin values in their serum as compared to non infected cows (Table 7).

Table 5. Incidence of ovarian inactivity in relation to Lumpy skin disease virus infection in local Egyptian cows.

Cows	Number of examined animals	Ovarian status	
		Active No (%)	Inactive No (%)
LSDV non infected	477	401(84.06)	76(15.93)
LSDV infected	163	12 (7.36)	151(92.64)

Table 6. Serum progesterone level in relation to Lumpy skin disease virus infection in local Egyptian cows (ng/ml).

Ovarian activity	Number of examined animals	LSDV infection	
		Negative	Positive
Follicular phase	20	0.49±0.06	0.13±0.02**
Luteal phase	20	2.41± 0.16	1.78±0.03**
Inactive ovaries	40	< 0.02	<0.02

**P < 0.01

Table 7. Serum protein electrophoreses pattern in relation to Lumpy skin disease virus infection in local Egyptian cows (Mean ± SE).

Protein fraction	Non infected cows (No =12)	Cows infected with LSDV (No =12)
Albumin (g/dl)	3.41±0.11	1.87±0.14**
αglobulins (g/dl)	1.07±0.09	1.01±0.04
β globulins (g/dl)	1.58±0.06	1.77±0.05
γ globulins (g/dl)	0.69±0.04	1.66±0.11**

**P < 0.01

Immunoglobulines: LSDV infected cows having significant increase in IgG values ($P < 0.01$) as compared to non infected cows (Table 8).

Trace elements: Serum trace elements concentrations (Table 9) revealed that LSDV infected cows have low concentrations, especially that of copper and iron ($P < 0.01$) if compared to cyclic cows.

DISCUSSION

In many areas of the world, especially in Africa and Asia, LSD is a subacute to acute cattle disease which is characterized by extensive cutaneous lesions and signs typical of generalized poxvirus diseases (Davies, 1982; Coetzer et al., 1994). However, the transmission of the disease among cattle is inefficient and arthropod-vector

Table 8. Serum immunoglobulin values in relation to Lumpy skin disease virus infection in local Egyptian cows (Mean ± SE).

Parameters	Non infected cows (No =12)	Cows infected with LSD (No =12)
IgG (mg/ml)	1.69±0.21	2.54 ± 0.12**
IgA (mg/ml)	0.24±0.01	0.25±0.01
IgM (mg/ml)	0.33±0.022[A]	0.35±0.02

**P < 0.01

Table 9. Some trace element values in relation to Lumpy skin disease virus infection in local Egyptian cows (ME ± SE)

Parameters	Non infected cows (No =12)	Cows infected with LSDV (No =12)
Zinc (µg/dl)	141.33±7.12	131.24±4.17
Copper (µg/dl)	92.35±2.55	78.71±2.34**
Iron (µg/dl)	191.13±6.30	124.85±5.88**

**P < 0.01

(Coetzer et al., 1994). The importance of this disease increase gradually as the way of eradication and control is very difficult.

Herein, LSDV was isolated from local Egyptian cows and was confirmed by PCR. Whereas, it was reported that PCR is the very sensitive way to detect LSDV in blood and tissues of infected animals (Ireland and Binepal, 1998).

The current study revealed that a considerable percent (25.47%) of local cows in Egypt get infected by LSD during the years 2004 - 2007 with marked year, age and seasonal variations in the incidence of infection. There are many factors affecting the outbreak of the virus in Egypt. It is assumed that many arthropods like stable flies may take part in the outbreak of the disease. Exposure of animals to adverse stressful conditions during seasonal climatic changes, stressful management conditions and importation of animals from other areas, especially from Africa, may predispose for the infection. However, inferior immune statuses as well as presence of arthropod vectors are the major factor that predispose to infection with LSD (Coetzer et al., 1994).

In the current study, LSDV infection adversely affected the general health condition of animals, with low progesterone level, decreased albumin, increased globulins and decreased copper and iron concentrations in the serum. No sufficient data could be traced in the available literature about such topics. However, infected animals are generally suffering from malnutrition and low energy status following loss of appetite and fever with subsequent disturbance of all the metabolic processes includeing ovarian and corpus luteum functions and in tern progesterone level (Rosby et al., 1991; Ahmed, 2007). On the other hand, the hypoalbuminemea could be attributed to two main factors; decreased synthesis and higher catabolic rate as well as damaged liver parenchma. While, increased γ globulins, especially IgG immunoglobulin values were mainly an immune response following infection and there is a genetic background (Estes et al., 1990). Changes in trace elements in the se-rum, especially in copper and iron and copper may be related to decrease food consumption or to hypoproteinaemia, which hinder absorption of these elements. Moreover, infection was considered as a sort of stress on animals and is associated with increased level of prolac-tin and disturbed oxidant/antioxidant status in the body (Ahmed, 2007).

In this study, markedly high percent (92.64%) of infected cows suffering from ovarian inactivity as monitored by gynecological examination aided by ultrasonography as well as by the very low serum progesterone level.

No direct relation between LSDV and ovarian activity could be traced in the available literature, however, ovarian inactivity in LSDV infected cows could be indirectly attributed to the poor body condition and negative energy balance that inhibits ovarian function following fever and depressed appetite with consequent decreased LH pulse frequency (Ahmed, 2007). Moreover, it was reported that stress plays a number of significant roles in female reproductive biology; mainly it influences ovarian function by affecting the growth of Graafian follicles and oocyte maturation (Tompakin et al., 1990).

Conclusion

In conclusion, from the economic point of view, animals suffering from LSDV showed high temperature with inferior appetite and consequently decreased productivity and cessation of ovarian activity. Young animals showed severe symptoms of the disease and even mortalities, so this disease consequently leads to great economic losses.

Proper hygienic measures includes combating of arthropods and the use of sheep pox vaccine due cross reactivity between LSD virus and sheep pox virus must be intensified for controlling of LSD. This study revealed that the PCR is a useful method for a rapid diagnosis of LSD. Virus isolation is still needed to detect the infectivity of the virus. The PCR could demonstrate LSD virus nucleic acid in blood and skin samples.

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