
Immunobiochemical profile in cattle infected with lumpy skin disease.

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Abstract

Lumpy skin disease virus (LSDV) was isolated, from naturally infected cattle that have a history of previous vaccination with live attenuated sheep pox virus (SPV) vaccine. Lumpy skin disease (LSD) is a skin disease of cattle caused by a strain of genus Capripox virus which causes an acute, subacute or inapparent infection in all ages and breeds of cattle. The disease causes considerable economic losses due to emaciation, damage of hides, infertility in males and females, mastitis, loss of milk production with morbidity up to 100% in natural outbreaks and mortality rate rarely exceeds 5%. This study aimed to demonstrate the alteration caused by (LSDV) infection on some immunobiochemical parameters as, total protein, Albumin immunoglobulins, Ca^{++} , ph^{++} , sodium, potassium, in addition to liver and kidney functions. This study was carried out on sixty Egyptian cows in a private farm (El Menoufia Governorate- El Sadat city). About 35 animals are monitored and inspected (apparently healthy) and considered as control group. The other 25 animals were infected with apparent clinical signs and considered as infected group. Blood samples were taken from both groups and serum was separated for measuring the total protein, albumin, globulin, Ca^{++} , ph^{++} , mg^{++} , liver function (ALT, AST, and alkaline phosphatase), kidney function (serum creatinine and urea). Our findings revealed that there were significant decreases in total protein, albumin, globulin, Ca^{++} , ph^{++} , sodium and potassium ions. ALT, AST, Alkaline phosphatase, urea, Ig G and creatinine were significant increases. so we can concluded that the LSDV causes severe alteration in different biochemical parameters specially total protein, albumin and globulin and so reduce the immune response in infected cattle to be considered in the treatment.

Key words: LSDV; Cattle; Total proteins; Immune response; Liver; Kidney.

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. The disease is listed in the office international des Epizooties List "A" which identifies diseases with the

potential rapid spread and severe economic losses (Irons et al., 2005). Field observations and supporting evidence indicate that the disease is transmitted by biting flies. There are also indications of transmission in the absence of insect vectors, whereas contact-transmission among animals is extremely inefficient (Carn and Kitching, 1995).

Lumpy skin disease is primarily spread among animals by biting insects (such as mosquitoes and biting flies). Less commonly, the virus may be spread by direct contact to the skin lesions, saliva, nasal discharge, milk, or semen from infected animals. Lumpy skin disease is characterized by rapid eruption of multiple circumscribed skin nodules, and generalized lymphadenitis and fever and result in mastitis and orchitis (Coetzer, 2005). Other lesions visible at post mortem examination include necrotic plaques in the membranes, mainly in the upper respiratory tract, the oral cavity and rumen.

Historically, the stable fly *Stomoxys calcitrans* Linnaeus was the arthropod thought most likely to have a role in the epidemiology of LSD. This was based on virus isolation from flies that had fed on infected cattle (Weiss, 1968). In a literature search, one report claims that the female mosquito, *Aedes aegypti* (Diptera: Culicidae) is capable of the mechanical transmission of LSDV from infected to susceptible cattle, but the clinical disease recorded in the animals experimentally exposed to infected mosquitoes, was mild in nature only (Chil et al., 2001). In addition, an attempt at mechanical transmission of LSDV by biting insects failed (Chihota et al., 2003). The aim of this study was to demonstrate the alteration caused by (LSDV) infection on several immunobiochemical parameters as total protein, immunoglobulins, calcium, potassium, sodium, potassium, in addition to hepatic and renal functions in affected cows.

Materials and methods

This study was carried out on sixty Egyptian cows in a private farm (Mena Governorate, El-Sadat city). About 35 animals were monitored and inspected (apparently healthy) and considered as control group. The other 25 animals were infected with Lumpy skin disease virus (LSDV) and showed apparent clinical signs and considered as infected group. The blood samples were directly taken from jugular vein (without anticoagulant) from both control and infected groups, the samples were left at room temperature for 15 min then centrifuged for serum separation. After separation serum samples were kept at -20°C for performing immunobiochemical analysis.

Biochemical assay:

Total protein concentration was determined by Lowry method (Lowry et al., 1951). Serum samples were solubilized in sample buffer containing 50 mM Tris/HCl, pH 7.5, 9% SDS, 15% glycerol, 6% β -mercaptoethanol. Protein samples (20 μ l per lane) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was stained with Commassie Brilliant Blue. Intensities of bands were analyzed densitometrically using densitometer and the resultant values were referred to total protein concentration.

Immunological parameters:

Immunoglobulins (α , β , and γ globulins) concentrations were determined using commercial radio immune diffusion plates (Hassan et al., 1995).

Albumin concentration was performed according to the method described by (Dourmas, 1975) where Calcium, Phosphours, Sodium and Potassium concentrations were determined in serum using spectrophotometer according to methods previously described by (Gindler and King, 1972; Drekh and Jung, 1970; Trinder, 1951; Terri and Sesin; 1958), respectively .

Hepatic and renal function tests:

Liver enzymes as ALT and AST were measured according the method of (Reitman and Frankel, 1957) and alkaline phosphatase according the method of (John, 1982). On the other hand, creatinine and urea concentrations were measured according to the method of (Young et al., 1975; and Putton and Crouch, 1977), respectively.

Statistical analysis:

Data were expressed as means \pm S.E. and the results were considered statistically significant at ($P \leq 0.01$). All data were subjected to analysis of variance (ANOVA) test according to Snedecor and Cochran (1980).

Results

The present results revealed that total proteins and albumin were significantly ($P < 0.01$) decreased in infected group compared to the control group (table 1). However, total immunoglobulin especially γ globulins are significantly ($P < 0.01$) increased in infected group compared to the control group (table 1). Result of immunoglobulins especially IgG were significantly ($P < 0.01$) increased in infected group compared to the control group (1.77 ± 0.13 in control; 3.1 ± 0.14 in infected group) (table 2). Regarding to the level of estimated minerals in the serum of both groups were significant ($P < 0.01$) decreases in calcium, phosphorus, sodium and potassium concentrations in infected compared to control groups (table 3). Concerning the biochemical studies of hepatic and renal functions, there were highly significant ($P < 0.01$) increases in the activity of AST, ALT and alkaline phosphatase, urea and creatinine in infected group compared to control group (table 4).

Discussion

The current study showed that there were many factors affecting the outbreak of the Lumpy skin disease virus (LSDV) in Egypt (2005-2007). 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, might predispose for the infection. However, inferior immune statuses as well as presence of arthropod vectors were the major factors that predispose to infection with LSD (Coetzer et al., 1994).

Concerning the electrophortic pattern, there were significant decreases in both total protein and albumin in infected group however; there was a significant increase in the level of total immunoglobulin specially gamma globulin fraction. These results might be attributed to decreased synthesis and higher catabolic rate as well as damaged liver parenchyma. While, increased gamma globulins, especially Ig G immunoglobulin values were mainly an immune response following infection. These results come in accordance with that obtained by

(Estes et al., 1990; Agag et al., 1989). In general the obtained data indicate the ability of the body defense against these viral infections.

Concerning the biochemical studies of hepatic and renal functions, the highly significant increases in the activity of both AST, ALT and phosphatase of infected cows compared to apparently healthy cows. The effect of LSDV infection on hepatic enzyme activities was so clear. Renal function was also altered due to viral exposure as the data revealed that both urea and creatinine level were significantly increased. These results were in agreement with that recorded by (Abdalla and Gawad, 1992; Aly et al., 2006) who attributed the marked increase of ALT activity to the hepatocellular damage caused by various agents. The highly significant increase in AST activity is rather inclusive with respect to the status of the liver, heart muscle and general tissue breakdown caused by the virus or secondary invaders (Abdalla et al., 1989). The high level of serum urea and creatinine values might be due to degenerative changes in kidney and liver.

In the current study, LSDV infection adversely affected the concentration of serum calcium, phosphorus, sodium and potassium (table 3). On going through the literatures, it seems to be scanty and not sufficient data could be found in the available data 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 (Rostom et al., 1991; Ahmed, 2007). Generally, the significant decrease in the concentrations of these elements could be attributed to two main factors; decreased synthesis and higher catabolic rate as well as damaged liver parenchyma. Changes in the concentration of trace elements, especially sodium and potassium might be related to decreased food consumption or hinder absorption of these elements. Moreover, infection was considered as a sort of stress on animals and is associated with a high level of disturbed oxidant/antioxidant status in the body (Ahmed, 2007). In conclusion, as LSDV affects all immunobiochemical parameters in the body especially protein level. Moreover, animals suffering from LSDV show high temperature with inferior appetite and consequently decreased production and cessation of milking. Animals showed severe symptoms of the disease and even mortalities, so this disease consequently leads to severe economic losses. Proper hygienic measures including combating of arthropods and the use of sheep pox vaccine due to cross reactivity between LSD virus and sheep pox virus must be intensified for controlling of LSD. In addition, this study indicates that the electrophoresis of total protein is a useful method for an accurate diagnosis of LSD infection in vitro.

Table (1): Electrophoretic pattern of serum protein (g/dl) in Egyptian cows with lumpy skin infection. (Means \pm SE).

Protein fraction (gm/dl)	Control group (Total no 35)	Infected group (Total no: 35)
Total protein	6.8 \pm 0.1 ^a	5.48 \pm 0.02 ^b
Albumin	3.9 \pm 0.14 ^a	2.10 \pm 0.20 ^b
α globulins	1.60 \pm 0.03 ^a	1.433 \pm 0.03 ^b
β globulins	1.98 \pm 0.04 ^a	2.01 \pm 0.02 ^b
γ globulins	0.62 \pm 0.066 ^a	1.79 \pm 0.02 ^b

Values with different letters in same raw means were significant ($P < 0.01$) and with the same letters means non significant.

Table (2): Immunoglobulin (Means \pm SE) values (mg/ml) in serum of control and infected groups

Type of Igs	Control group (Total no 35)	Infected group (Total no 25)
Ig G	1.77 ± 0.13^a	3.1 ± 0.14^b
Ig A	0.29 ± 0.01^a	0.31 ± 0.03^b
Ig M	0.41 ± 0.010^a	0.44 ± 0.04^b

Values with different letters in same raw means were significant ($P < 0.01$) and with the same letters means non significant.

Table (3): Serum level of some minerals in both infected and control groups

Type of mineral	Control group (Total no 35)	Infected group (Total no 25)
Calcium (mg/dl)	$9.50 \pm 0.22a$	$8.10 \pm 0.2b$
Phosphorus (mg/dl)	$5.60 \pm 0.36a$	$4.90 \pm 0.03b$
Sodium mEq/L	$137.21 \pm 0.14a$	$115.20 \pm 0.15b$
Potassium mEq/L	$4.8.32 \pm 0.09a$	$4.00 \pm 0.02b$

Values with different letters in same raw means were significant ($P < 0.01$) and with the same letters means non significant.

Table (4): Effect of lumpy skin infection on both kidney and liver functions

Criteria	Control group (Total no 35)	Infected group (Total no 25)
ALT (IU)	$18.3 \pm 0.2a$	$39.01 \pm 0.24b$
AST (IU)	$55.6 \pm 0.5a$	$71.03 \pm 0.021b$
Alkaline phosphatase (IU/L)	$65.22 \pm 0.02a$	$79.02 \pm 0.54b$
Urea (mg/dl)	$21.54 \pm 0.55a$	$36.6 \pm 0.06b$
Creatinine (mg/dl)	$1.4 \pm 0.041a$	$1.9 \pm 0.01b$

Values with different letters in same raw means were significant ($P < 0.01$) and with the same letters means non significant.

References

- Abdalla, M.A. and Gawad, S.M. (1992): "Characterization of serum lysosomal enzymatic activities." II- Effect of lumpy skin disease in Egypt. Dischterar 2 TL wochenscher Aug- 44 (8) 347 – 349.
- Agag, B.L., Hafiz, M.A.M., Ragab A., Tawfik, A., Mousa, H.L., Shaker, M. and El-Danal, N. (1989): "Changes in serum biochemical component of cattle suffering from LSD in Egypt." Egypt. J. Comp. Pathol. Chin. Path. 2 (2): 9-25.

- Ahmed, W.M. (2007): "Overview on some factors negatively affecting ova activity in large farm animals." *Global Vet.* 1: 53- 66.
- Aly, A.A., Ibtasam, M.A. and Mohamed, M. (2006): "Lumpy skin disease a field program of cattle in El-Sharkia Governorate. Egypt." *J. Comp. F. Clinic. Path.* 14 (1) 162- 173.
- Carn, V.M. (1993): "Control of capripoxvirus infections." *Vaccine*, 11:1275-1278.
- Carn, V.M. and Kitching, R.P. (1995): "An investigation of possible route of transmission of lumpy skin disease virus (Neethling)." *Epidemiol. Inf.* 114: 219-226.
- Chihota, C., Rennie, L.F., Kitching, R.P. and Mellor, P.S. (2001): "Mechanical transmission of lumpy skin disease virus by *Aedes aegypti* (Diptera: Culicidae)." *Epidemiol. Infect.* 126:317-321.
- Chihota, C.M., Rennie, L.F., Kitching, R.P. and Mellor, P.S. (2003): "Attempted mechanical transmission of lumpy skin disease virus by biting insect." *Med. Vet. Entomol.* 17: 294-300.
- Coetzer J.A.W. (2005): "Lumpy Skin Disease. In: Infectious Diseases of Livestock. 2nd Eds. J. A.W. Coetzer and R.C. Tustin. Oxford University Press. 2004: 1268-1276.
- Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (1994): "Poxviridae: Infectious diseases of livestock." Oxford University Press, Cape Town, South Africa. 1: 601-603.
- Davies, F.G. (1991): "Lumpy skin disease, an African capripox virus disease of cattle." *Br. Vet. J.* 147: 489-503.
- Doumas, B.T. (1975): "Colorimetric determination of total proteins." *Clin. Chem.* 21: 1159 - 1166.
- Drekth, A.C. and Jung, D.H. (1970): "Determination of inorganic phosphate in acid filtrate." *Clinical Chemical Acta* 27:373-378.
- Estes, D.N.; Templeton, J.W.; Hunter, D.M. and Adams, L.G. (1991): "Production and use of murine monoclonal antibodies reactive with bovine IgM isotypes and IgG subisotypes (IgG1, IgG2a and IgG2b) in assessing immunoglobulin levels in serum of cattle." *Veterinary Immunology and Immunopathology*, 25: 61-72.
- Fenner, F. (1996): "Poxviruses. In B. N. Fields, D. M. Knipe, and P. M. Howley (Ed.), *Fields Virology*. Lippincott-Raven, Philadelphia, pp: 2673-2702.
- Gindler, E.M. and King, J.D. (1972): "Rapid colorimetric determination of calcium in biological fluids of thymol blue." *Am. J. Clin. Path.* 58: 376-382.
- Hassaan, H.M., Abdel Malak, G. and El-Sheltawi, M.A. (1995): "The possible immunostimulant effect of Ivermectin in rams." *Zag. Vet. J.* 23:48-45.
- Irons, P.C., Tuppurainen, E.S.M. and Venter, E.H., (2005): "Excretion of lumpy skin disease virus in bull semen." *Theriogenology*, 63, 1290-1297.
- John, D.B. (1982): "Clinical lab." *Methods for Determination of Alkaline phosphatase*. 9th Ed., 580-581.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951): "Protein measurement with Folinphenol reagent." *J. Biol. Chem.*, 193: 265-275.
- Putton, C. and Crouch, S. (1977): "Determination of serum blood urea nitrogen." *Anal. Chem.* 49: 464-469.
- Reitman, S. and Frankel, S. (1957): "Colorimetric determination of glutamic oxalacetic and glutamic pyruvic transaminase." *Am. J. Clin. Path.* 28 ; 56-61.

- Rosby, R.J., Wettemann, R.D., Geisert, J.J., Wagner, J.J. and Lusby, K.S. (1991): "Influence of nutrition and body condition on pituitary, ovarian and thyroid function of non – lactating buffalo cows." *J. Anim. Sci.*, 29: 2073-2080.
- Snedecor, G.W. and Cochran, W.G. (1980): "Statistical Methods." 7th ed., Ames: Iowa state university press.
- Terri, A.E. and Sesin, P.G. (1958): "Colorimetric determination of potassium in serum and plasma." *Am. J. Clin. Path.*: (29): 86.
- Trinder, P. (1951): "Colorimetric determination of sodium in serum and plasma." *Analyst.* (7): 596.
- Weiss, K.E. (1968): "Lumpy Skin Disease Virus." *Virology Monographs* Vol. 3 New York: Springer Verlag.
- Young, D., Postaner, L. and Gibberman, V. (1975): "Colorimetric determination of serum creatinine." *Clin Chem.*, 21:112.

العربي

المناعية في الماشية المصابة بفيروس الجلد العقدي
 يوسف حسن: قسم الأمراض الباطنة، كلية الطب البيطري بالسادات-جامعة المنوفية
 فرج الكرداسي: قسم الكيمياء الحيوية وكيمياء التغذية، كلية الطب البيطري بالسادات-جامعة المنوفية
 لى: معهد بحوث صحة الحيوان (شبين الكوم)

مرض الجلد العقدي من الأمراض التي تسبب خسارة اقتصادية كبيرة في الماشية مثل
 العام والتأثير البالغ على جودة الجلود والعقم في الذكور والتهاب الضرع في الإناث
 في كمية الحليب المنتجة يوميا. والسبب في مرض الجلد العقدي يرجع إلى الإصابة
 الجلد العقدي. التي تصل نسبة الإصابة به وسط قطعان الماشية إلى ١٠٠% ونسبة
 إلى ٥%.

ريت هذه الدراسة على عدد ٦٠ حيوان من الماشية بمزرعة خاصة بمدينة السادات
 المنوفية-مصر. قسمت هذه الحيوانات إلى مجموعتين أساسيتين، المجموعة الأولى
 على ٣٥ حيوان بالفحص الظاهري لا يعانون من أي إصابة وقد اعتبرت هذه هي
 الضابطة (control) والمجموعة الثانية اشتملت على ٢٥ حيوان مصاب
 (وضوح اعراض الإصابة) واعتبرت هذه المجموعة هي المجموعة المصابة بالمرض.
 تهدفت هذه الدراسة إلى توضيح التأثير الناتج عن الإصابة بهذا الفيروس على مستوى كل
 روتين الكلى والالبومين والاميونوجلوبين وكذلك تأثيره على مستوى بعد المعادن مثل
 وم والفسفور والبوتاسيوم والصوديوم. كما تمت دراسة تأثير الإصابة على نشاط الانزيمات
 بوظائف الكبد (ALT, AST & Alk. Phosphatase) في أمصال
 وكذلك تمت دراسة تأثير الإصابة بالمرض على مستوى اليوريا والكرياتنين.

هت النتائج وجود نقص معنوي في مستوى البروتين الكلى والالبومين والجلوبولين
 نقص معنوي في مستوى الكالسيوم والفسفور والصوديوم والبوتاسيوم.

بزيادة معنوية في نشاط انزيمات (ALT, AST & Alk. Phosphatase). كما
 أيضا وجود زيادة معنوية في مستوى (IgG) و اليوريا والكرياتنين.

نتائج السابقة نستخلص أن الإصابة بمرض الجلد العقدي تؤدي إلى تغيرات بيوكيميائية
 في أمصال الحيوانات المصابة خصوصا على مستوى البروتين الكلى والالبومين
 بولين الذي يؤدي إلى نقص الاستجابة المناعية للحيوانات المصابة.