Lumpy Skin Disease in Sharkia and Dakahlia Governorates During The Period 2009-2011

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ABSTRACT

This study was done in the period during 2009 to 2011 on a total number of 929 cattle in 2 Egyptian Governorates "Sharkia and Dakahlia" which were 552 Native breed and 377 imported breed, aged from one month to 9 years old. All of them vaccinated by sheep pox vaccine.

The infected animals with LSD showed severe clinical signs which include skin nodules varied in number from one up to several hundreds, sometimes covered all the body, varied in diameter from 0.5 to 5 cm, regular in shape, rounded with flatted centers. The nodules are hard, farm and painful with erected hairs make scabs detached leaving wounds and make scars when recovery. The affected animals showed body temperature 39.5-41ºC became normal within 1-3 days, enlarged lymph nodes, oedema in one or more leg. There is a nasal and lacrimal discharge on the animals.

Our obtained results revealed that LSD affect 158 out of 552 local bread and 186 out of 377 foreign bread, the morbidity was 28.63% and 49.37% respectively. The morbidity in Sharkia was 26.93% and Dakahlia was 47.52%.

The mortality rate was very low from 0-1.5%.

PCR technique was done for accurate diagnosis of LSD that give 100% of skin samples "samples of choice".

INTRODUCTION

Lumpy skin disease (LSD) is an infectious, eruptive occasionally fatal disease of cattle and is important economically *(1-3)*.

The disease is caused by Neethling pox virus of a capripox viruses that is identical with Kenyan sheep and goat pox viruses *(4)*.

The disease was first distributed in 1929 in Zambia. Its infectious nature was recognized during the 1943 and 1945 disease outbreaks in several regions of Africa. The economic significant of the disease was demonstrated during a panzootic episode in South Africa in the late 1940s. Where it was first detected in 1988 in Egypt and in 1989 *(5)*.

The clinical outset with fever, watery eyes, increased nasal secretion, loss of appetite, reduced milk production, depression and reluctance to move several days later, the appearance of skin nodules can be seen and may cover the whole body but are most numerous on the head, neck, perineum, genitalia and udder and limbs but may cover the entire integment. The nodules may also be found in the respiratory system especially in the nasopharynx, trachea, bronchi and lung in addition to other systems such as the rumen, renal cortex, testicles and uterus *(6-8)*. The morbidity rate in cattle were generally varied between 1-2%, but in few instances reached 100%, while the mortality rate varied between nil to 40% rarely exceeded 3% *(2)*.

The office international des epizooties considered LSD one of the "List A" diseases that had the potential for rapid spread with the ability to cause serious economic losses *(9)*.

A PCR test specifically detecting the LSD virus based on differences between LSDV and sheep and goat pox viruses.

This paper describes the occurrence of LSD in Sharkia and Dakahlia governorate.

MATERIAL AND METHODS

A total of 992 cows representative in 552 local breed and 377 foreign breed, aged from one month to 9 years and 2 Egyptian governorates (Sharkia and Dakahlia) during the period from 2009-2011. All of them vaccinated by sheep pox vaccine.

All suspected animals were clinically examined, regarding physical status, temperature, superficial lymph node and skin lesions *(4)*.

Laboratory diagnosis

Skin scabs: detached skin scabs from recovered animal were collected in clean dry plastic pages and transported in ice box to the laboratory at -20ºC until used for direct diagnosis using PCR.

The LSDV positive and negative controls for diagnostic PCR experiments:

One vial of LSDV Ismaelia propagated on MDBK cells for 10 passages were kindly provided by Dr. Ausama A. Yousif, Department of Virology, Faculty of Veterinary Medicine, Cairo University. A vial of a lyophilized live attenuated (LA) fowlpox virus vaccine produced in the Veterinary Serum and Vaccine Research Institute, Abbasia, Egypt, was used as negative control. The vials were labeled "Fowl Pox Vaccine, 1000 doses, Veterinary Serum and Vaccine Research Institute". The vials were kept at -20°C. Vials were opened in a class II BSL environment. Vials were kept on ice until aliquots were aspirated using nuclease-free filtered tips.

Total DNA extraction using QI amp Minelute virus spin kits Lot #142326963

Total DNA extraction using commercial silica-based spin columns:

Qiagen DNeasy blood and tissue kit (QIAGEN Sciences, Maryland, USA) was used for the extraction of DNA from scabs, blood and serum from animals showing typical clinical signs and crusted skin from recovered animals. Extractions were performed according to the manufacture's recommendations using about 20 mg of scabs (when available), 20 µl of blood, 200 µl of serum, and 200 µl of control viruses as starting materials. DNA extracts were kept frozen at -20ºC until using PCR.

LSDV PCR protocol

The LSDV amplification reactions were carried out using the Vivantis DNA amplification product (Vivantis Technologies, Selangor Darul Ehsan, Malaysia). Total DNA extracts of individual scabs were tested separately. Each reaction tube contained 1.25 units of Taq DNA polymerase in 1X ViBuffer A, containing 200 µM of each dNTP, 0.2 µM of each of the forward and reverse primers, 2.5 mM MgCl2, and 2 µl of each of the samples, and controls (negative, and positive DNA extract controls). The reaction tubes were centrifuged briefly, and placed in a Techne™ TC512 thermal cycler (BioRad, Hercules, California, USA).

Thermal cycling conditions for amplification of the LSDV genes were done according to the manufacturer's instructions, briefly: an initial denaturation step (95°C for 5 minute), 40 amplification cycles (95°C for 30 seconds, 55°C for 1 minute, and 60°C for 2 minutes), and a final extension step (72 °C for 5 minutes).

After amplification, 15 µl were analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide. DNA bands were visualized by UV irradiation in a UVP Transilluminator (Upland, CA).

Table 1. Data of skin scabs samples used for PCR

Sample no.	Data
C1	Small skin scabs from animals infected and recovered 2 years ago 2 in one age of animal 5 year ♀
C2	Skin nodules scabs from infected natural outbreak infection 2 month old. Age 1 year ♀
C3	Skin nodules scabs from natural infected no vaccine deep infection of skin (infected 2 month old)
C4	Skin scabs from 10 month old calf vaccinated by sheep pox vaccine 3 month before natural infection. Infection one month old.
C5	3 month old calves from vaccinated dams get infection 2 months age.
MW	Virantis 100 bp plus DNA ladder
LS	LSDV positive control
FP	Fowl pox virus negative control
BLK	B lank (No DNA control)

RESULTS

Clinical signs

Grossly, the naturally affected animals showed skin nodules varied in number from one nodule up to several hundred, sometimes covered all the body of the animals. These nodules varied in diameter from 0.5 to 5 cm, regular in shape, rounded with flatted center. Some scars detached leaving wounds, which sometimes contain pus and/or myces. The nodules occur anywhere on the skin including nose, udder and vulva in cows, the scrotum in bulls as well as in the mouth (the gum). The nodules have erected hair. Legs become swollen and developed sores, enlarged lymph nodes beside nasal and lacrimal discharge which is thick to pussy fluid. The affected animals appear dull, difficult to walk and have salivation.

By clinical examination of the affected animals, the temperature ranged from 39.5°C up to 41°C in early infection then become normal within 1-3 days. The nodules raised above the skin surface by 0.1-1 cm. and it is farm, hard and painful. Enlarged lymph nodes were noticed. Scabs is hard, dry, raised, edges have erected hair, thick in appearance and easy detached after 3 days up to 2 months.

Recovery takes 2-3 months. The nodules leave wound, healed leaving scare on the skin.

2009-2011, with no cases were reported in 2010. Severe clinical signs were appeared including skin nodules (rounded with flattened center about 0.5 to 5 cm in diameter and varied in number from one to several hundred. Allover skin of the animals enlarged lymph nodes, nasal, lacrimal discharge, raised above skin surface by 0.1-1 cm, hard, firm and painful ends by scabs which more hard, firm, dry, raised edges have erected hair easily detached after 3 days up to 2 months. After recovery leave wound healed leaving scars on skin. Reported in Egypt for the first time in 1988 then become endemic disease in Egypt. Previous results were recorded ***(10, 12-18)***.

Morbidity rate was higher in foreign breed than local breed (49.34%) and (28.63%) respectively. This result can be explained using one of two hypotheses. 1- Foreign breeds are kept in tightly controlled environment the chances of low level exposure to the virus are less compared to local breeds where they are usually kept in small-scale farm situation. 2- Local breeds have evolved enhanced immune responses to the virus due to prolonged coexistence with circulating endemic strains of the virus.

Morbidity rate in Dkahlia was higher than Sharkia (47.52%) and (26.94%) respectively. The morbidity rate was higher in summer season than autumn and nil in winter and spring. This can be explained as (1) in summer high insect population (2) high humidity in summer (3) high climate temperature. Seasonal occurrence of LSD outbreak was recorded in summer season. Insects are playing very important role in the transmission of LSD virus ***(11)***.

The morbidity rate of LSD virus is higher in autumn and winter than spring and summer. Their studies occurred on animals arrived to abattoir for slaughtering showing LSD affection ***(19)***. The difference with our obtaining results (1) animals not to be slaughtered except after recovery and recovery lasts at last 2 months (2) the obtained results were applied during the outbreak of disease but the previous investigators was applied their studies on animals showed skin affection after recovery (3) if animal infected in summer after recovery, the affection stay on animals up to autumn. The healing of LSD skin lesions was slow healing and remain for several months might be due to secondary bacterial infection ***(11)***.

Mortality rate was from 0-1.5%. The same results were previously reported ***(20-23)***.

Scabs and skin nodules were more successful in diagnosis of LSDv and have higher number of viruses. The same results were recorded ***(24-26)***, they recorded the virus in blood samples collected from fevered cows in percentage of 33.3%. SNA-PCR detected viral DNA in 100% of skin scabs.

PCR test is a useful method for rapid diagnosis of LSDv during outbreaks. Our obtained results were coincided that those previously reported ***(10, 24 , 27)***.

Vaccinated animals by sheep pox vaccine showed positive result in PCR testing for LSD nodules and scabs during outbreak. From our data there is a need to revise our understanding of LSD immunology and control based on using sheep pox virus vaccines.

Conclusion

There is a need to investigate LSD pathogenesis and immunogenesis.

There is evidence that the use of SPV vaccine to control LSD infection is not sufficient to break the cycle of infection in the field.

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الملخص العربى

التهاب الجلد العنقودى فى الماشية بمحافظتى الشرقية والدقهلية في الفترة من ٢٠٠٩-٢٠١١

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أجريت هذه الدراسة على عدد ٥٥٢ بقرة من السلالة المحلية وعدد ٣٧٧ بقرة من السلالات الأجنبية أعمارها تتراوح من شهر حتى ٩ سنوات محصنة باستخدام تحصين جدرى الأغنام فى محافظتى الشرقية والدقهلية

أظهرت الحيوانات عقد جلدية على معظم الجسم مختلفة الأحجام من ٠,٥ الى ٥ سم وارتفاع درجة حرارة جسم الحيوان وتورم الارجل وتضخم الغدد الليمفاوية السطحية.

الاصابة فى الحيوانات الأجنبية كانت أعلى منها فى المحلية بنسبة ٤٩,٣٪ و ٢٨,٦٣٪ على الترتيب. وكانت نسبة الاصابة بمحافظة الدقهلية أعلى منها بمحافظة الشرقية *بنسبة* ٤٧,٥٢٪ و ٢٦,٩٣٪ على الترتيب. كما بلغت معدلات النفوق من صفر الى ١,٥٪.

وقد تم تشخيص المرض بإجراء اختبار البلمرة المتسلسل الذى أظهر كفاءة عالية تصل الى ١٠٠٪ من عينات الجلد أثناء انتشار المرض.