Recent Isolation and identification of lumpy skin disease virus from cattle in Egypt

By

Omyma, M. El-Desawy
Dept. of Virology, Animal Health Research Institute, Dokki, Giza, Egypt.

SUMMARY

Lumpy skin disease (LSD) is a serious skin disease of cattle. Accurate and rapid diagnosis of LSD is very important to control the rapid spread of disease in Egypt. In this study 9 skin nodules from infected cattle in private farms in Giza were used for isolation and identification of LSD virus. A total of 40 serum samples were collected during outbreak of LSD and 2nd serum samples from the same animals to detect the neutralizing antibodies for LSD virus by SNT. Isolation of LSD virus was done by inoculation on CAM and MDBK tissue culture. Identification of virus by Transmission electron microscopy (TEM) which was considered a rapid and accurate method for diagnosis of LSD.

INTRODUCTION

Lumpy skin disease is a serious skin disease of cattle caused by a single strain of Capri pox virus which known as Neethling virus, it belongs to family Poxviridae genus Capri and closely related antigenically to sheep and goat pox virus (OIE, 2004).

LSD is characterized by nodular cutaneous eruption, lymphadenitis, edema of one or more limbs, decrease in body weight, infertility and permanent damage to hide (Haig, 1957).

The office international des epizooties (OIE, 1989) considered LSD one of the list A disease due to its rapid spread and ability to cause serious economic loss (Anon, 1985 and Tuppurainen et al., 2005).

The control measures to reduce losses are possible after diagnosis of LSD (Carn, 1993).

Rapid laboratory diagnosis is very important, serological methods are useful for detection LSD antibodies in serum samples but are too time consuming to be used as primary diagnostic methods, serum neutralization test is used for evaluation of antibodies to capripox virus (Tuppurainen, 2004).

Isolation of virus occur by using CAM of 9-11day old fertile eggs and in MDBK cell culture (OIE, 2004), but the diagnosis of LSD can be confirmed by Electron microscopy (EM).
Control of LSD in Egypt depends on application of sheep pox virus vaccine (Romanian Strain, Michael et al., 1991).

A trial for production of inactivated LSD virus vaccine by using (2-bromoethylamine hydrobromide (BEI) and adjuvanted with Nigella sativa oil is safe, sterile and highly immunogenic (Omyma, 2001).

The aim of this study is to throw light on rapid and accurate methods for diagnosis of LSD using ECE, MDBK cell culture and improving the detection LSD virus particles by transmission electron microscopy, this besides using SNT for detection of LSD antibodies in infected animals sera.

MATERIALS AND METHODS
A. Materials:
1. Blood samples:
   40 serum samples were collected during outbreak of LSD from dairy cattle private farms belonged to private farm in Giza governorate and again after 3 weeks from the same animals to be tested for the presence of neutralizing antibodies for LSD virus and collected sera were stored at –20 °C.

2. Nodular skin samples:
   9 skin nodules were collected for virus isolation by storing at -20°C or examined at day of collection with EM.

3. Virus:
   Reference LSD virus was kindly supplied by Virology Department, Faculty of Vet. Med., Cairo University.

4. Antisera:
   Reference antiserum against LSD virus was kindly supplied by Pirbright Laboratory (England).

5. Tissue culture:
   MDBK cells were used for isolation of virus and VNT.

B. Methods:
1. Sample Preparation:
   9 skin nodular samples were collected from diseased cattle and prepared according to Ali and Obeid (1977).

2. Virus isolation:
   a. In Embryonated chicken (ECE):
      0.2 ml from the prepared samples were inoculated via chorioallantoic membrane (CAM) of 9-11 day old, according to Van Rooyen et al. (1969) and the harvested membranes keep at -20 °C for tissue culture inoculation.

   b. Tissue culture:
      The harvested chorioallantoic membranes were prepared for inoculation into confluent sheet of MDBK cell lines and observed daily for the presence of cytopathic effect according to (OIE, 2004).
3. **Virus identification by transmission electron microscope (TEM):**

The skin specimens were ground in a sterile mortar with a small volume of distilled water and centrifuged for 15 min. at 5000 rpm. The supernatant was collected and centrifuged again for 45 min. at 13000 rpm, then the pellet was rinsed with distilled water, then a droplet of 3% phosphotungstic acid mixed with a droplet of sample and a copper grid coated with carbon formvar was dipped into the mixture according to OIE (2004) and Tuppurainen, (2004) and after drying examined with EM.

4. **Serological examination:**

Virus neutralization test (VNT), the serum samples were tested by VNT according to OIE (2004).

**RESULTS**

The tested animals had fever (40-41 °C) and skin nodules cover the body as Fig. (1).

1. **Virus Isolation:**

The results of LSD virus isolation were successfully done through cultivation of prepared samples on CAM. The typical pock lesion were found on harvested CAM 4 days post inoculation in 5 samples out of 9 as shown in Fig. (2).

2. **Tissue Culture:**

2 out of positive samples on CAM give the cytopathic effect on MDBK cell after three passage in the form of rounded or oval cells and finally cell detachment leaving large irregular holls in cell sheet after 3 passages as shown in Fig. (3).

3. **The Result of Virus identification by E.M:**

Out of 9 skin specimen, 7 were positive for LSD viral particles as in Figs. (4 & 5). The viral particles appeared by negative staining roughly brick shaped particles with ridges covering them.

4. **Results of SNT:**

The titers of antibodies in first serum samples collected at the presence of the clinical signs, showed a lower titer, while the 2nd serum samples showed higher titers of antibodies, this indicates natural infection with LSD virus not from vaccination as shown in table (1).
Table (1): Virus neutralizing antibodies titres in serum samples.

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Antibodies titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st serum samples</td>
</tr>
<tr>
<td>15</td>
<td>Less than 4</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>Less than 4</td>
</tr>
</tbody>
</table>

Fig. (1): Skin nodules cover the body of infected cattle.
Fig. (2): Typical pock lesion on the harvested CAM.

Fig. (3): Typical CPE of LSD virus (Left) in comparison with normal control MDBK (right).

C.P.E of LSD in MDBK

Control MDBK cell

Fig. (3): Typical CPE of LSD virus (Left) in comparison with normal control MDBK (right).
Fig. (4): Ultrastructure of LSD virions shown by –ve staining roughly brick shaped with ridges converging them (x 70 000).

Fig. (5): Ultrastructure of LSD virions shown by –ve staining on the surface of cells (release) (x 70 000).
DISCUSSION

LSD is an infectious disease characterized by rapid spread and sudden appearance of lumps in skin after fever. The control of the disease to decrease the economic loss is depending on rapid and accurate diagnosis (Cran, 1993). The first record of LSD in Egypt was in 1988, since it is an arthropod born disease so, the outbreak mostly occurred in summer months. Finally LSD outbreak occur during 2006-2007 in Egypt. The vaccination in Egypt depending on uses of sheep pox vaccine, it gives sufficient cross-protective immunity against LSD virus challenge (Saber et al., 1993).

In this study the harvested CAM revealed the presence of pox lesion 4 days post inoculation in 5 samples out of 9 skin samples. These results agreed with Abd El-Rahim et al. (2002). The cytoplastic pathogenicity on MDBK cell culture revealed rounding or oval cells and finally cell detachment in 2 samples out of the 5 samples on CAM. These findings were coincided with Woods (1988); Hanan (2000) and OIE (2004). But the isolation of LSD virus in tissue culture needs long time until the clear appearance of C.P.E because capripox virus grow slowly in cell culture (Prydie and Coackley, 1959).

The electron microscopy was used as a rapid accurate diagnostic methods. It allows rapid morphological identification and differential diagnosis with other different agents (Hazelton and Gelderblom, 2003; Iman et al., 2007). Because of the large size and distinctive structure of pox virus virions, EM allowed their ready identification, so it is the preferred method for laboratory diagnosis (Fenner et al., 1987). Also TEM detected large numbers of viral particles in the form of roughly brick shaped pox virus particles (Fig., 4) in 7 out of 9 skin biopsies. These results were in agreement with Woods (1988) and Brooks et al. (1998). Also some viral particles were surrounded by multi layered envelope derived from the host cell (Fig., 5) as previously mentioned by OIE (2004).

The results of SNT revealed increased in antibody titre in all 40 serum samples after 3 weeks. It ranged from 32- to more than 64, this comparing with the first titres range from less than – 4. This results agree with Tuppurainen (2004) who found significant increase in titre between 21-24 days. SNT is a rapid serological test for diagnosis. Also it can be used for measuring the antibodies titre of animals to determine the efficacy of vaccination.

REFERENCES


OIE (2004): "Manual of diagnostic tests and vaccines for terrestrial animals."


