Use of a Polymerase Chain Reaction assay to detect lumpy skin disease virus (LSDV) in skin lesions of cattle

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SUMMARY

Virus isolation, indirect immunofluorescent antibody technique (IFAT), Serum neutralization test (SNT) and polymerase chain reaction (PCR) were used for the diagnosis of lumpy skin disease in clinically infected Friesian dairy cow. Among one hundred seventy clinically examined cattle at Salhea – Ismalia Governorate – Egypt, twenty three animals suffered from skin lesions (13.5% morbidity) and three died (1.7% mortality).

Indirect immunofluorescent antibody technique (IFAT) revealed a positive detection of LSD viral antigen in smears from skin nodules of twelve diseased animals. Virus isolation from these nodules was successfully done on Madin Darby Kidney (MDBK) cell lines for LSDV. Serum neutralization test (SNT) done on fifty serum samples collected from twenty three clinically infected and twenty seven apparently normal cows revealed the presence of LSDV specific antibodies. Polymerase Chain Reaction (PCR) was applied for molecular identification of the recovered isolates. PCR assay found to be rapid, sensitive, specific.

INTRODUCTION

Lumpy skin disease along with sheep pox and goatpox are the most serious poxvirus diseases of livestock, and are caused by viruses that belong to the genus Capripoxvirus within the subfamily Chordopoxvirinae, family Poxviridae (Babiuk et al., 2008).

The genus Capri pox virus is one of the largest viruses (170-260 nm by 300-450 nm). Capri pox virus represented one of eight genera within the chordopox virus subfamily of the poxviridae (Kitching and Taylor, 1985).

Lumpy skin disease (LSD) is a disease of cattle, primarily in Africa and Madagascar and rarely in the Middle East. The disease is of economic importance in endemic areas. Effective control of LSD re-
quires accurate and rapid laboratory techniques to confirm a tentative clinical diagnosis (Tuppurainen et al., 2005).

Clinical findings of lumpy skin disease (Neethling) were eruption of nodules in the skin which might cover the whole animal body with systemic affects included pyrexia, anorexia and pneumonia (Davies, 1991).

The transmission of LSDV between animals by contagion is extremely, inefficient and the inoculation of virus is required to establish infection by intravenously feeding arthropods (Carn and Kitching, 1995).

Lumpy skin disease is listed in Office International des Epizooties ‘List A’ which identifies diseases with the potential for rapid and severe economic losses (OIE, 2006).

The virus was isolated for the first time from cattle in Suez and Ismailia Governorates (House et al., 1990). In Lower Egypt (Ali et al., 1990) found that the incidence of LSD in 10 cattle and buffaloe herds from 6 different farms. Another epizootic of LSD was reported in Egypt in 2005 and 2006 affecting 5 different Governorates (OIE, 2006).

The performance of indirect fluorescence antibody test (IFAT) for serological diagnosis and screening of lumpy skin disease (LSD) was evaluated using methods without gold standard. Virus neutralization test (VNT) was used as the second test (Gari et al., 2007).

Viral isolation, polymerase chain reaction (PCR), dot blot hybridization (DBH), and indirect enzyme-linked immunosorbent assay (iELISA) were used for the diagnosis of lumpy skin disease in clinically infected, fevered, and apparently normal dairy cows (Awad et al., 2009).

The PCR was a fast and sensitive method to demonstrate viral DNA in blood and skin samples. It could detect viral nucleic acid in skin lesions 53 days longer than virus isolation (Tuppurainen et al., 2005).

The aim of this study was to compare the sensitivity and specificity of different techniques in laboratory diagnosis of LSDV.

MATERIALS AND METHODS

1- Animals:
One hundred and seventy cattle from Salhea farm of Ismailia Governorate were clinically examined. The clinical examination includes examining temperature, skin, mucous membrane and superficial lymph nodes.

2- Samples:
a- Tissue samples:
Twelve nodules and lymph nodes were taken from twelve in-
fected animals for virus isolation, identification and molecular detection.

b- Blood samples:

A total of fifty blood samples were collected from twenty three diseased and twenty seven apparently healthy cattle for separation of serum. Samples were stored at -20 ºC until used for serum neutralization test.

3- Virus Isolation:

0.2 ml from each prepared skin nodules were inoculated for three passages into MDBK tissue culture cells according to the method described by (Carn and Kitching, 1995).

4- Identification of virus by indirect fluorescent antibody technique (IFAT):

Identification of virus was carried directly on smears from the cutaneous nodules for detection of LSD viral antigen according to method described by (Davis, 1991).

5- Detection of LSDV antibodies:

Collected serum samples were used for detection of antibodies against LSDV using serum neutralization test according to (Frey and Liess 1971).

6- Molecular identification of LSDV by PCR:

Molecular identification of LSDV was applied on 3 of 10 positive isolated nodules according to method described by (Gubbels et al., 1999) and (Schwartz et al., 1997). Extraction and purification of DNA were successfully done on 200 µl of prepared nodules as described by (Sambrook et al., 1998). Extracted DNA from each sample was amplified according to protocol published by (Ireland and Binepal 1998). The reaction mixture (50 µl) contained 250 mg of total DNA. 2m M MgCl$_2$ 5P mol of each primer (forward primer was 5' TTTCCCTGATTTTTTCTTACTAT3' and reverse primer was 5' AAATTATATACGGTAAATAAC3'), 200 µM of each dNTP and 2U of DNA polymerase (Biotool, USA) in a reaction buffer containing 75 mM Tris-HCl (ph 9), 2mM MgCl$_2$, 50 mM KCl, 20 Mm (NH$_4$)$_2$SO4 and 0.001% BSA.

Amplification was carried out in a MJ thermal cycler (MJ incorporation USA) programmed to perform a denaturation step at 95 ºC for 5 min. followed by 35 cycles for 1 min. at 94 ºC for denaturation, 1 min at 50 ºC for primer annealing and 1 min at 72 ºC for extension. The last extension step was 10 min. longer.

A 10 µl PCR products were mixed with 2 µl gel lading buffer (Sigma- Aldrich) and electrophoresed in 1.5% agarose gel, containing 1 µg/ml ethidium bromide in Tris- acetate buffer (0.04 M
Tris- acetate and 0.001 M EDTA, ph 8). The resulting DNA fragments were visualized by UV transillumination and photographed (Sambrook et al., 1998). A visible band of appropriate size 192 bp was considered as a positive reaction according to (Gubbels et al., 1999). A reference LSDV propagated on MDBK cell line was obtained from virology department, Animal Health Research Institute, Dokki, Giza and used as positive control.

RESULTS
Clinical findings:
From 170 examined cattle, 23 showed typical animal symptoms of LSDV. It includes salivation, nasal and lacrimal discharges, fever, depression, inappetance and reduction in milk production. Subcutaneous edema of different parts of the body accompanied by swelling of the superficial lymph nodes was observed. Most of the affected animals revealed obvious coetaneous nodules all over the body (Photo 1).

Photo (1): showed nodules on the body of cattle infected with LSDV.
Virological findings:

Twelve prepared skin nodules from 12 animals inoculated on MDBK cells for three successive passages, ten of them showed clear cytopathic effect CPE, which characterized by cell granulation followed by cell rounding and aggregation in a separated manner. This occurred 3-4 days post inoculation (PI), followed by completed sheet detachment after 5-7 days PI (Photo 2), Table (1).

Photo (2): characteristic CPE of LSDV, 72 hours PI on MDBK cells (X 40).

The positive samples were identified with IFAT using specific LSD antiserum. It revealed the presence of intracytoplasmic fluorescence in tissue samples (Photo 3).

Photo (3): Positive nodules showed intracytoplasmic fluorescent granules (x 40).
Serological findings:
Serological investigation revealed presence of LSDV specific antibodies with varied range in 30 cases out of 50 tested samples with an incidence of 60%.

PCR findings:
The results indicated that PCR assay revealed a high incidence of LSDV in 100% of positive samples (Photo 4).

Photo (4): Electrophoretic pattern of PCR amplification: Lane M showing 100 bp marker, Lanes 1, 2, 3 were positive samples (192 bp), Lane 4 was negative sample, Lane 5 was negative control and Lane 6 was positive control LSDV.

Table (1): Showed the results of collected samples for V. isolation, SNT.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Nodules</th>
<th>Serum of infected animals</th>
<th>Serum of apparently</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>12</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Number of positive</td>
<td>10</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

DISCUSSION
Lumpy skin disease virus is a serious skin disease of cattle caused by a strain of Capri pox virus known as Neethling virus, and the disease became endemic in many parts of Africa and regional outbreaks occurred periodically (Woods, 1988).

Clinical findings in eight lumpy skin diseased (LSD) cows and same number of healthy ones were reported in Tal-El Baker village and Tal Alkabir centre, Ismailia province, Egypt. LSD began with fever, anorexia, skin lesions in a form of nodules all over the body, which disappeared spontaneously or gathered to form large
lumps. It was complicated with respiratory manifestation, corneal opacity, mastitis, dehydration and later on recumbence (Abdalla and Gawad, 1992).

The same lesions were recorded in cattle infected with LSD that showed nasal discharge, lacrimation with oedema in dewlap and enlargement of lymph nodes (Michael et al., 1994).

The present study showed that most of the affected animals revealed obvious cutaneous nodules all over the body (photo1), in advanced cases the nodules converted to scabs and then leaving scars. The associated clinical findings were similar to those described by (Blood and Radostits, 1989), (Kenawy, 1991), (Barnard et al., 1994) and (Ibrahim et al., 2006).

Virus isolation from blood and skin samples was sensitive and reliable, but as a single test it may be too time-consuming to use although this depends on how rapidly the diagnosis must be confirmed. In conclusion, this study showed the PCR is superior in detecting LSD virus from blood and skin samples. However, virus isolation is still required when the infectivity of the LSD virus is to be determined (Tuppurainen et al., 2005).

In the current work, LSDV was isolated by inoculation prepared skin nodules on MDBK for three successive passages and produced clear CPE at 3-4 days PI (photo2), this agreed with (House et al., 1990) and (Hassanein et al., 2008). Identification of the samples by IFAT using specific LSD antiserum (photo 3), was agreed with (Kitching and Hammond, 1992), (Ibrahim et al., 2006) and (Hassanein et al., 2008).

Serological investigation was done by SNT using reference antiserum virus, revealed presence of LSDV specific antibodies with an incidence 60%, similar results of high incidence were reported by (Davis, 1991), (Carn et al., 1994) and (Carn and Kitching, 1995).

The results of this study indicated that PCR assay found to be rapid, sensitive, specific and could be applied on tissue samples (photo 4), the same conclusion was previously reported by (Kitching and Hammond, 1992), (Ireland and Binepal 1998) and (Heine et al., 1999).

On conclusion PCR assay should be applied beside classical techniques of diagnosis for any cases with skin lesions as early as possible to apply adequate control measures.
From the previously discussed results, we can conclude that Lumpy Skin Disease is one of the major cattle diseases in Egypt. So that the animals should be quarantined and preventive measured must be done.

REFERENCES


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استخدام اختبار أنزيم البلمرة المتسلسل في تشخيص مرض الجلد العقدى

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الملخص العربي
العزل الفيروموي ، الاختبار الفلورسنتى المشع الغير مباشر ، اختبار السيرم المتعادل واختبار أنزيم البلمرة قد استخدمت لتشخيص مرض الجلد العقدى من حيوانات حلاية مصابة. 32 حيوان مصاب تم اختبارهم من اجمالى 170 حيوان بنسبة اصابة (13.6%) و 3 حالات وفيات (7.1%) لابقار بمزرعة بالصالتين في محافظة الاسماعيلية.

اختبر الفلورسنتى المشع الغير مباشر كشف عن وجود حالات ايجابية لانونيجين فيروس الجلد العقدى من عينات جلدية لحيوانات مصابة. تم عزل الفيروموى من هذه العينات على خلايا الزرع النسيجى MDBK.

تم اختيار عينات السيرم لوجود الأجسام المناعية لعدد 50 حيوان وقد اظهرت وجود أجسام مناعية ضد الفيروموى ثم عمل اختبار أنزيم البلمرة للكشف عن الفيروموى في الحالات الايجابية. واظهرت النتائج أن اختبار أنزيم البلمرة اختبار تأكيدي للاختبارات التقليدية الآخرى.

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