Electrophoretic characterization of sheep pox virus, Goat pox virus and lumpy skin disease virus

BY
Amal, M. A. Raof,* Shahein, M. A.* and Hoda, Abd El Monem**

Virology Department*- Biotechnology Department**
Animal Health Research Institute-Dokki- Giza

SUMMARY

The counter immuno electrophoresis (CIE) test was used for differentiation between sheep pox virus (SPV), goat pox virus (GPV) and lumpy skin disease virus (LSDV). The viruses were isolated from clinically infected animals from farms at different Governorates of Egypt on chorio- allantoic membrane (CAM) of SPF eggs then adapted on VERO tissue culture cells (3 passages). The isolated viruses were identified by serum neutralization test (SNT) and agar gel precipitation test (AGPT) using reference antisera.

The isolated viruses were used for counter immuno electrophoresis (CIE) in order to differentiate them. The CIE showed the presence of protein of varied electrophoretic mobility and sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) was used to differentiate profiles of different bands of the three isolated viruses.

INTRODUCTION

Sheep pox and goat pox are malignant pox diseases of small ruminants characterized by fever and generalized skin lesions. High mortality rates occur in young animals resulting in significant economic losses. Sheep pox is caused by sheep pox virus (SPV), a member of the genus Capri poxvirus in the family Poxviridae; the other members of the genus are goat pox virus (GPV) and lumpy skin disease virus (LSDV) of cattle Murphy et al., (1995).

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 choradopox virus subfamily of the pox viridae Kitching and Taylor (1985).

Sheep pox virus and goat pox virus cause systemic disease in sheep and goats that is often associated with high morbidity and high mortality Bowden et al., (2007).

Sheep are the moving banks of sheep herds and their economic contribution in terms of meat, wool and skin/hide is immense. The causative agent [sheep pox virus (SPV)] is antigenically and geneti-
cally closely related to goat pox virus (GPV) and lumpy skin disease virus (LSDV), these are members of the genus Capri pox virus Bhanuprakash et al., (2006).

Strains of SPV and GPV are not considered host-specific and although the majority of strains show a host preference, a single strain may cause disease in both sheep and goats. Goats may become mildly infected with sheep strains, which can cause severe disease when transmitted back to sheep. Similarly, sheep may become infected with virulent goat strains. It has been proposed that both sheep pox and goat pox be described as a single disease, called Capri pox (Heine et al., 1999).

Outbreaks of sheep and goat pox occur frequently incurring economic losses to the sheep and goat industry. Mortality in young animals can exceed 50% (Kitching and Taylor, 1985). While, in susceptible lambs and kids less than one month of age, morbidity may approach 100% and mortality may be as high as 95% (Davies and Otema, 1981).

The sheep pox virus (SPV), Goat pox virus (GPV) and Lumpy Skin Disease virus (LSD) are responsible for some of most economically significant for sheep, goat and cattle in Africa and Asia (Kitching et al., 1986). They occur as endemic infections in south western Asia, the Indian, and most parts of Africa except southern Africa (Rao and Bandyopadhyay, 2000).

Although the application of annual vaccination programs in sheep flocks, the sheep pox disease still restricted in its distribution to the middle eastern countries, south western Europe and north Africa (Esposito and Nakano, 1991), as well as China and Russia (OIE, 2000).

All strains of capripoxvirus are antigenically with one strain provides immunity against all other strains. Because of this antigenic homology among all strains, there is the potential to use a single vaccine strain to protect cattle, sheep and goats (Kitching, 2003).

Clinical signs during an outbreak of sheep pox were reported as; the affected animals had high body temperature, pale buccal mucosa with ulcers and edematous inflammation of the mouth and mucous membrane, erosions, laminitis and coronitis (Aruni et al., 2001).

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

Isolation of sheep pox virus was obtained on chorio allantoic
membrane CAM and no apparent pock lesion was observed through five successive passages (Agag et al., 1997).

Identification of sheep and goat pox viruses was obtained by IFA technique, NT and AGDT using hyper immune serum against sheep and goat pox viruses Soad et al., (1996).

PCR- RELP method has been developed for genomic identification and differentiation of Capri pox viruses (Hosamani et al., 2004).

The use of counter – immuno electrophoresis (CIE) technique was obtained as rapid diagnosis of pox virus infection by means of 0.7 and 1% agarose solution and sharp lines of precipitations were formed (Mether-Homji et al., 1975).

The counter–immuno electrophoresis (CIE) test was standardized for the detection of goat pox antigen and antibody using inactivated antigen. The precipitinogens of goat pox virus were found to be soluble in nature (Sharma et al., 1988).

Counter–immuno electrophoresis (CIE) was performed to detect pox virus antigens in infected tissues and scab/skin samples collected from diseased animals using sheep pox hyper immune sera during outbreak in Jammu, India (Mondal et al., 2004).

As there was immunologically relation ship between SPV, GPV and LSDV could not be differentiated serologically. So in this work we try to distinguish between these viruses by using Counter – immuno electrophoresis test as a rapid tool for that purpose.

MATERIALS AND METHODS

1- samples:
Scabs, nodules, nasal swabs and lymph nodes were taken from infected animals from farms at different Governorates of upper (Menia Governorate) and Lower Egypt (Kafr EL Sheikh, Monfia, and Do-myate Governorates) for viruses isolation, identification and differentiation they were collected at period November 2006 to October 2007.

The samples collected were represented in table (1).

The lesions were showed in photo (1& 2) for both sheep pox virus and lumpy skin disease virus.
Table (1) showed epizootiological data for SPV, GPV and LSDV.

<table>
<thead>
<tr>
<th>Governorate</th>
<th>Localities</th>
<th>SPV Scabs</th>
<th>SPV Nodules</th>
<th>GPV Scabs</th>
<th>GPV Nodules</th>
<th>LSDV Nodules</th>
<th>LSDV Nasal swabs</th>
<th>LSDV Lymph nodes</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Kafr EL Sheikh</td>
<td>Sakha&amp; Mehl Musa</td>
<td>10</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>13</td>
<td>5</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>2- Monfia</td>
<td>Quesna</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>12</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>3- Domyate</td>
<td>Kafr Saad</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>4- Menia</td>
<td>Malauai</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>18</strong></td>
<td><strong>9</strong></td>
<td><strong>11</strong></td>
<td><strong>8</strong></td>
<td><strong>11</strong></td>
<td><strong>37</strong></td>
<td><strong>17</strong></td>
<td><strong>111</strong></td>
</tr>
</tbody>
</table>
Photo (1): showed pox lesions at the head of sheep infected with SPV.

Photo (2): Showed nodules on the body of cattle infected with LSDV.
2- Anti Sera:
Reference anti sera against SPV, GPV and LSDV were kindly obtained from Sheep pox Department, Serum and Vaccine Research Institute–Abbassia. They were used for identification of the isolated viruses.

3- Isolation:
a- Chorio- allantoic membrane (CAM) of SPF eggs these applied according to Nakano (1979).
b- VERO tissue culture cells according to the method described by and Mangana- Vougiouka et al. (2000).

They were used for isolation and adaptation of viruses isolated from infected lesions (for 3 passages).

4- Identification:
a- Serum neutralization test (SNT): was applied according to Tiwari et al., (1996).
b- Agar gel precipitation test (AGPT) was applied according to Rao and Negi (1997).

They were used for the identification of the isolated viruses using known antisera.

5- Counter immuno electrophoresis (CIE):
Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE):

The different protein fraction prepared from Capri pox viruses were subjected to discontinuous SDS- PAGE according to method of Laemmli (1970) as follow:
- Prior to loading on the gel the fractions were heated at 100°C for 4 minutes in sample buffer containing 0.06 M Tris, 12% SDS, 5% B- mercapto ethanol and 11.9% glycerol.
- Sample contained 15 µg of protein in 15 µL of sample buffer was loaded into each lane; the protein content of the sample was determined by modified lowery procedure of Markwell et al., (1978).
- The protein of virus was separated on SDS poly acrylamide lab gel using Heafer mini gel system (SE 250, Mightly small II) with PS 500XT power supply.
- The completed gel used in this study consists of a stacking and separating gel:
  * The stacking gel containing final concentrations of 4% acrylamide/N methylene bisacrylamide (SIGMA), 0.125 M Tris – HCl (pH 6.8) and 10% (W/V) SDS.
  * The separating gel containing 12% acrylamide N- methylene bis-acrylamide, 0.375 M Tris HCl (pH 8.8) and 10% (W/V) SDS.
- Polymerization was achieved by the addition of 0.05 (V/V)
N, N, N, N – Tetra methylene-diamline (TEMED) and 0.05% (W/V) ammonium per sulphate (SIGMA). The electrophoresis buffer (pH 8.3) consists of 0.025M Tris base 0.192 M glycerin and 0.1% SDS.

- Electrophoresis was performed at room temperature at a consistent voltage of 120 V until the bromo phenol blue dye reached 1 cm from the bottom.
- Gels were stained with Coomassie blue R-250.
- Molecular weights were estimated from a linear least-squares fit of the Logarithm of molecular weight versus relative mobilities of the standard.
- The standard error of the estimate for these plots was generally less than or equal to 2 ± KDa.
- A mixture of molecular weight standards (SIGMA) was prepared at a concentration of 4 mg/ml in SDS sample buffer and 10 ml were applied to the gel.
- The standards were bovine serum albumin (64 KDa), ovalbumin (44 KDa), glyceroldehyde-3-phosphate dehydrogenase (36 KDa), carbonic anhydrase (29 KDa), trypsinogen (24 KDa) and lysozime (14 KDa).

RESULTS

1- Isolation:
*Isolation of the viruses from different samples of infected animals was shown in table (2).
Table (2) shown isolated viruses from infected samples:

<table>
<thead>
<tr>
<th>Governorate</th>
<th>Total No. of samples</th>
<th>No. of +ve</th>
<th>SPV Scabs</th>
<th>SPV Nodule</th>
<th>GPV Scabs</th>
<th>GPV Nodule</th>
<th>LSDV Nodules</th>
<th>LSDV Nasal swabs</th>
<th>LSDV Lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Kafr EL Sheikh</td>
<td>34</td>
<td>6</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2- Monfia</td>
<td>31</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3- Domyate</td>
<td>25</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>4- Menia</td>
<td>21</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>22</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

*The viruses were isolated on CAM then adapted on VERO cells.

The normal control Vero cell and infected Vero cells with specific CPE were shown in photo (3&4).
Photo (3): Normal non infected VERO cells

Photo (4): Infected VERO cells with CPE appeared as cell rounding and aggregated together in a separated manner 4-5 days post inoculation.
2. Identification:
By: a- SNT: which revealed that the isolated samples gave positive results for SPV, GPV and LSDV using reference anti sera for each virus.
b- AGPT: this test also shared in identification of isolated samples showed clear precipitated line for each one using reference anti sera for each virus.

3. Counter immuno electrophoresis (CIE):
SDS-PAGE analysis of major poly peptides from viruses was visualized by Coomassie staining. The protein profile of each virus was represented in photo (5).

Photo (5): SDS- PAGE analysis of protein for each virus
Lane (3): Lumpy skin disease virus.       Lane (4): Sheep pox virus
The protein extract for *Goat pox virus* in lane (2) revealed four distinct bands ranged "between" 20-44 KD. On other hand the protein extract for *Lumpy skin disease virus* in lane (3) revealed three distinct bands ranged "between" 29-50 KD and the protein extract for *Sheep pox virus* in lane (4) revealed four distinct bands ranged "between" 20-30 KD.

There was a difference in profiles existence for three viruses, which revealed that sodium dodecyl sulphate- poly acryl amide gel electrophoresis was used to differentiate the profiles of the three isolated viruses SPV, GPV and LSDV.

**DISCUSSION**

Sheep and goat pox are notifiable disease in most countries of the world, and any suspicion of diseases should be reported to appropriate authorities. During an outbreak of sheep pox the virus is probably transmitted between sheep by aerosol and there is evidence that mechanical transmission of the virus by biting arthropods such as stable flies may also occur (Fenner et al., 1996).

Lumpy skin disease virus was 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). The disease could spread through Asia to each Europe through geographical location (House et al. 1990).

There was immunological relationship between SPV, GPV and LSDV. Cattle can be protected against challenge with LSDV by immunization using SPV vaccine (Burdin, 1970).

There was a common major precipitating antigen by poly acrylamid gel electrophoresis of LSDV, SPV and GVP. Its molecular weight was 67 KD, and located on outer membrane of virus particle (Kitching, 1986).

The viruses of lumpy skin disease and Kenyan sheep and goat pox proved to be identical by direct and indirect fluorescent antibody and serum neutralization tests and with sheep and goat virus strains from the Middle East (Davies and Otema, 1981).

The present study was planned for differentiation the sheep pox virus, goat pox virus and lumpy skin disease virus isolated from infected sheep, goat and cattle (respectively) through collection of skin lesions, nodules, lymph nodes and nasal swabs from farms at different Governorates of upper and lower Egypt. Isolation of three viruses was followed by identification by serum neutralization test (SNT).
and agar gel precipitation test (AGPT). The differentiation of the isolates was applied by using counter immuno electrophoresis (CIE).

Table (1) showed that 111 samples (29 scabs, 28 nodules, 37 nasal swabs and 17 lymph nodes) were collected from infected animals from different Governorates. And the photo (1) showed lesion of sheep pox at the head of infected sheep while the photo (2) showed lesions of lumpy skin disease at body of infected cattle. Similar clinical findings reported in the last outbreak of sheep pox in Jordan (Daoud, 1997) and Algeria (Achour et al., 2000). On other hand, 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).

Table (2) show out of 111 samples, 22 samples (8 samples SPV, 4 GPV and 10 LSDV) were isolated on CAM of SPF eggs and gave slight edematous thickening of CAM through out 3 successive passages. This agreed with Soad et al., (1996) and Tawfik et al., (2001). The isolated viruses then adapted on VERO cells and gave CPE at 3 successive passages, the CPE appeared as granulation of cells followed by cell rounding and aggregated together in a separate manner 4-5 days post inoculation photo (4). This agreed with Rizkallah (1994).

Identification of the isolated viruses was done by SNT and AGPT by using reference antisera for each virus. The findings were similar to that mentioned by Soad et al., (1996) and Tawfik et al., (2001).

In our study, differentiation of the three isolated viruses was done by using counter immuno electrophoresis (CIE). The soluble antigens of SPV, GPV and LSDV were reacted in CIE in the presence of sodium dedocyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE).

The results shown in photo (5) revealed that GPV in lane (2) contains 4 bands 20-44 KD, LSDV in lane (3) contains 3 bands 29-50 KD and SPV in lane (4) contains 4 bands 20-30 KD in SDS-PAGE this result agreed with the results of Singh and Rai (1991) who used SDS-PAGE to identify the immunogenic polypeptides of sheep pox virus Jaipur strain and with Rao et al., (1996) that reported SPV specific proteins were of a higher molecular mass nature as well as Rao et al., (1997) that showed the presence of proteins of varied electrophoretic mobility and sharing of antigenic determinants among a few soluble antigens.
The results of this study showed that counter immuno electrophoresis test was quick as well as more sensitive than agar gel precipitation test that agreed with Sharma et al., (1988) when used this test for detection of GPV antigen/antibody. In the same time it was successful test for differentiation the SPV, GPV and LSDV as there was immunological relationship between them and could not be differentiated serologically. Although this branch need more studies in the future.

REFERENCES


