Assessment of Maternal Immunity Against LSD in Calves Born to Cows Immunized with Sheep Pox Vaccine

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ABSTRACT

Lumpy skin disease (LSD) is a poxvirus disease of cattle characterized by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, edema of the skin, and sometimes death. Vaccination of cattle is the most effective option for controlling the spread of lumpy skin disease. All the vaccines used to prevent LSD are currently based on live attenuated viruses. Two types of vaccines against LSD are commercially available; lumpy skin disease vaccine and sheep pox vaccine with the latter is commonly used, but their ability to induce passive immunity is poorly known. Here, we evaluated the passive immunity transferred from dams immunized with sheep pox vaccine to calves. Pregnant cattle were injected with living attenuated sheep pox vaccine Romanian strain via intradermal route. Clinical conditions were monitored throughout the study. The humoral immune response was evaluated in dam and in serum samples collected from newborn calves. The results indicated that sheep pox vaccine vaccines are safe and produce a good humoral immune response in pregnant cattle. Moreover, results showed that, in calf serum, passive immunity persists until three months of age.

Keywords: Lumpy Skin Disease, Sheep Pox vaccine.

INTRODUCTION

Lumpy skin disease virus (LSDV) is an infectious disease of cattle that can have severe economic consequences (Babiuk et al., 2008; Tuppurainen and Oura, 2012). LSD is notifiable disease of cattle (OIE 2010). The disease is caused by Lumpy skin disease virus (LSDV), one of the members of genus Capripoxvirus in the family Poxviridae (Buller et al., 2005; Diallo and Viljoen, 2007). It is antigenically and genetically related to sheep and goat viruses (Gershon and Black, 1988; Kitching et al., 1989). The virus is mainly transmitted mechanically by blood-feeding flying insects (Chihota et al., 2001; Babiuk et al., 2008) and ticks (Tuppurainen and Oura, 2012; Rouby et al., 2017). Outbreaks of LSD are highly associated with seasonal peak of mechanical vectors in wet and warm weather conditions (Chihota et al., 2001). LSD is host-specific, causing natural infection in cattle and Asian water buffalo (Bubalus bubalis), although the morbidity rate is significantly lower in buffalo (1.6 percent) than in cattle (30.8 percent) (House et al., 1990). Lumpy skin disease is an acute infectious disease characterized by pyrexia, generalized skin and internal pox lesions, emaciation, enlarged lymph nodes, edema of the skin, and sometimes death (Radostitis et al.)
2006). Lactating cows appearing to be severely affected and result in a sharp drop in milk production because of high fever caused by viral infection itself and secondary bacterial mastitis (Tuppurainen and Oura, 2011). Young animals are severely affected and clinical symptoms are rapid to appear.

Geographically, LSD recently was reported not only in Middle East but also in Europe and Asia (Wainwright et al., 2013; Tageldin et al., 2014; Al-Salihi and Hassan, 2015; Ripani and Pacholek, 2015). Regarding to Egypt, LSD was recognized clinically for the first time in the Suez Governorate in May 1988 (House et al., 1990). A resurgence of LSD was reported in 2006 in several Egyptian governorates after the importation of cattle from Ethiopia (El-sherief, 2010, Rouby, 2010; Awadin et al., 2011) where all age groups and both sex of Egyptian cattle were infected with severe and serious complications (El-sherief, 2010, Rouby, 2010). The disease re-emerged in 2011 and again in 2014 (Awadin et al., 2011, Fayez, 2011, Amin et al., 2015; Rouby and Aboul-Soud, 2016). Currently, LSD is considered as an endemic disease in Egypt and several sporadic cases of the disease were reported in Egypt (Elhaig, 2017 and El-Tholoth & El-Kenawy 2016, Rouby, 2018). Vaccination has been considered to be the cheapest and sustainable means of disease control in the enzootic situation like Egypt. Live attenuated capripoxvirus vaccine strains were used to control LSD, including the homologous LSDV Neethling strain, or the heterologous RM65 SPP, Romanian SPP and Gorgan GTP virus strains (Gari et al., 2011). In Egypt, Romanian sheep pox vaccine is currently used to contain LSD. In vaccinated and naturally infected animals, antibodies remain at levels detectable by traditional serological tools such as serum virus neutralization test (SNT) for three to six months after infection or Vaccination, after which cell-mediated immunity is likely to become the major element in protection (Tuppurainen, 2017). Evaluation of humoral immune response depending mainly on the antibody titers in sera of vaccinated animal by using SNT and ELISA. Serological tests that can be used for LSDV include an indirect fluorescent antibody test (IFAT), virus neutralization, enzyme-linked immunosorbent assays (ELISA) and immune blotting (Western blotting) (Abera et al., 2015). The virus neutralization test is the only validated serological test available. The agar gel immunodiffusion test and indirect immunofluorescent antibody test are less specific than the VNT due to cross-reactions with antibody to other poxviruses (OIE, 2010). A local response to the vaccine is usually correlated with good antibody production (Hunter and Wallace, 2001). Antibodies offer a valuable measure of response to immunization as it remains at the detectable level for three to six months after vaccination. Calves born to immunized cows will have passive immunity that persists for about six months (Weiss, 1968). The present study aimed to investigate the development of the humoral immune response in pregnant cows vaccinated with sheep pox vaccine and to evaluate the passive immunity transferred from vaccinated dams to calves.

**MATERIALS AND METHODS**

i. **Vaccine:** sheep pox virus vaccine Romanian strain, Veterinary Serum and Vaccine Research Institute (VSVRI) abbassia, Cairo, Egypt, was used in vaccination.

ii. **Virus:**

Romanian Sheep pox virus was supplied from Pox Department, Veterinary Serum and Vaccine Research Institute (VSVRI) Abbassia, Cairo, Egypt and used in SNT and ELISA

iii. **Vaccination of animals:**

All animals in the study were from a single dairy herd, located in Beni-suef (coordinates: 29°04′N31°05′E), Egypt. According to the farm records, no recent history of LSD outbreak was registered. Animals were injected intradermally in the tail fold using living attenuated sheep pox vaccine Romanian strain. Pregnant cows were
selected and divided into two group (group one, vaccinated at three months of pregnancy while group two, vaccinated at four months of pregnancy). clinical conditions were monitored throughout the experiment (Fig. 1).

iv. **Sample Collection**

Blood sera were collected without anticoagulant by vein puncture according to (Radostits et al. 2010). Skin over the jugular vein was prepared by clipping and rubbing with a swab soaked in alcohol, then disinfected by tincture of iodine before blood collection. About ten ml of blood were aseptically drawn from jugular vein into a sterile evacuated test tube using a jack. These test tubes were left at room temperature in a sloping position for two hours to allow clotting. The collected samples were labeled, identified and transferred to the laboratory where they held in refrigerator till the next day to give chance for more separation of serum volume. The serum was siphoned off by Pasteur pipette post centrifugation at 3000 r.p.m for ten minutes. Clear sera were stored in cryo-tubes at \(-20^\circ\) C until its use for serological studies. Serum samples were collected from pregnant cows on the day of vaccination (time 0) and weekly after vaccination interval for 36 weeks. Simultaneously, Serum samples were also collected from calves born to vaccinated cows before colostrum intake and monthly after colostrum intake for four months.

v. **Neutralisation Test**

All collected sera were screened according to method described by (OIE 2012 and House et al. 1990). Sera were inactivated by heating at 56 \(^\circ\) C for 30 minutes in water bath to inhibit heat labile non-specific viral inhibitors that might formed in sera sample and might interfere with SNT. Negative and positive serum samples were diluted at ratio of 1:5 using tissue culture medium (Eagle’s/HEPES (N-2-hydroxyethylpiperazine, N-2-ethanesulphonic acid). Serial tenfold dilution in Eags medium of vaccine strain of sheep and goat pox virus known to grow well in Vero cell were prepared. Equal volumes of sera samples and each dilution of SPPV (10:3 to 10:6) were mixed and incubated at 37 \(^\circ\) C for an hour. In 96 well flat bottomed tissue culture plates 100 ml of serum virus mixture was put in each well of the plate. 100 ml of tissue culture Vero cell suspension in Eagles medium containing antibiotics was added to all wells. Wells containing only cell suspension were considered cell control. Wells containing only Eagles medium serve as control well for medium. Another well containing cell suspension and sheep pox virus was considered virus control. The plates were covered and incubated at 37 \(^\circ\) C up to nine days at CO2 incubator, with daily examination starting at 4\(^{th}\) day (OIE, 2010) for appearance of CPE using an inverted microscope. Recording and calculating results was done when CPE appeared in the virus control wells. The virus in each duplicate titration and calculated according to (Reed and Muench 1938). The NI is the log titer difference between the titer of the virus and the negative serum and tested serum. It was expressed as NI50. The neutralizing index (NI) was calculated according to (Reed and Muench 1938 and Cottral 1978). Neutralization index 1.5 ≥ is protective (Cottral 1978).

vi. **Statistical analysis**

Data were analyzed by the analysis of variance (one-way ANOVA) using general linear model procedures and descriptive statistics were used to quantify levels of antibody titers across each sampling days. A P-value of less than 0.05 was considered significant.

**RESULTS**

**Clinical appraisal of animals employed in this study:**

There are no adverse reactions after vaccination were observed. There is no abortion cases were recorded. All animals were clinically healthy with normal body temperature. All calves were in good condition at birth and receive colostrum adequate amounts between the first 20min to 2 h after birth, with no clinical signs of disease during the study.
i. Appraisal of humoral immune response in vaccinated cattle using serum neutralization test (SNT);
Serum neutralizing antibodies develop on the 2nd Week where the neutralization index was 0.97, 1.58 in group one and two respectively. A significant rise of antibody titer was detected from the three to eight weeks post inoculation. Serum neutralizing antibodies decreases below the protective level at nine months post vaccination (the neutralization index was 1.45, 1.44 in group one and two respectively) (table.1, Fig. 2).

ii. Appraisal of maternal immunity in newly born calves using SNT;
Serum samples were collected from calves born to vaccinated cows before colostrum intake and monthly after colostral intake for 4 months. SNT antibody titers in newly born calves started from the first month after parturition and persisted in protective level until three months of age in calves in group two table 2, Fig. 3.

Fig. (1): Study design and Methodology
Table (1): NI index of cows

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Zero day</th>
<th>Weeks post vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>i</td>
<td>0.32± 0.01a</td>
<td>0.58±</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.17a</td>
</tr>
<tr>
<td>ii</td>
<td>0.44±</td>
<td>0.68±</td>
</tr>
<tr>
<td></td>
<td>0.07b</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Means carry different superscript small letter are significantly different at *p* <0.05

Fig. (2a): Humoral immune response index in vaccinated cows
Fig. (2b): NI index of cows

![Pie chart showing the NI index of cows with different categories and months post vaccination.]

Table (2): Immune response index in newly born calves from vaccinated dams with sheep pox vaccine by using SNT

<table>
<thead>
<tr>
<th>Calves groups</th>
<th>Zero day</th>
<th>SNT Months post vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>i</td>
<td>0.32±</td>
<td>1.71±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01a</td>
</tr>
<tr>
<td>ii</td>
<td>0.32±</td>
<td>1.75±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.02b</td>
</tr>
</tbody>
</table>

Means carry different superscript small letter are significantly different at p <0.05
Fig. (3a): Immune response index in newly born calves from vaccinated dams with sheep pox vaccine by using SNT assay

Fig. (3b): NI index of calves
**DISCUSSION**

Capripoxviruses (CaPVs) are, serologically indistinguishable from each other so it is able to motivate heterologous cross-protection (Carn, 1993). Therefore, using of vaccine strains of CaPV derived from sheep and goat would be helpful to protect cattle against LSDV (Tuppurainen and Oura, 2012). All the vaccines used to prevent LSD are currently based on live attenuated viruses. These vaccines can be roughly divided into two classes: homologous vaccines and heterologous vaccines. The homologous vaccines are based on attenuated LSD viruses (LSDV), whereas the heterologous vaccines are based on attenuated sheep pox or goat pox viruses (Tuppurainen et al. 2015). During the 2006 outbreak of LSD in Egypt, it was reported that the live attenuated sheep pox vaccine (comprising Kenyan sheep and goat pox virus, O-240 strain) did not provide cattle with complete protection against LSD (Marshall, 2006). Latest molecular studies have described a close relationship between LSDV and the Kenya sheep-1 (KS-1), proposing that KS-1 is actually LSDV (Tulman et al., 2002). The KS-1 strain is obtained from the attenuated Kenyan sheep and goat pox vaccine virus (KSGP) O-240 (Gershon and Black, 1989). Finally, Lamien et al. (2011) and Tuppurainen et al. (2014) confirmed that the commonly used KSGP O-240 is not SPPV but is actually LSDV. The Romanian sheep pox virus strain currently used in Egypt (Tuppurainen et al., 2015) and although it was claimed that it immunized cattle (Davies, 1991) and that it provided protection from challenge. The disease remains endemic in Egypt despite the use of this vaccine, but this might be a result of low vaccine coverage and reduced efficacy (Tuppurainen et al., 2015).

Because of the dermotropic nature of sheep pox virus. Administration of a live virus vaccine by the intradermal route results in more and larger reactions than if it is given subcutaneously. Consequently, the immunity established will be comparatively greater (OIE, 2010). In the current study, all animals were vaccinated with sheep pox vaccine (Romanian strain) intradermally. Clinical conditions were monitored throughout the experiment where there is no adverse reactions after vaccination were obtained. There is no abortion cases were recorded. All animal were clinically healthy with normal body temperature.

The purpose of the present study is to investigate the development of the humoral immune response against sheep pox Romanian strain vaccine in pregnant cows vaccinated at early gestation using Serum Neutralization Test to measure the predicted efficacy of this vaccine in protecting cattle against LSD and to evaluate the passive immunity transferred from vaccinated dams to calves.

Immunity against capripoxviruses is predominantly cell-mediated, but humoral immunity also counteracts and antibody titers are a practical measure of response to vaccination (Weiss 1968; Hunter and Wallace, 2001; Tuppurainen et al., 2017). In the current study the humeral immune response against lumpy skin disease virus was evaluated in six pregnant cattle which vaccinated with live attenuated sheep pox vaccine. Humeral immune response was assessed using SNT after 0, 1, 2,3,4,8,12,20,28, and 36 weeks of vaccination with live attenuated sheep pox viral vaccine. Serum neutralizing antibodies develop on the 2nd Week where the neutralization index was 0.97, 1.58 in group one and two respectively. A significant rise of antibody titer was detected from the three to eight week post inoculation. Serum neutralizing antibodies decrease below the protective level at nine month post vaccination the neutralization index was 1.45, 1.44 in group one and two respectively (table.1). These finding came in accordance with those obtained by Agag et al., (1992). Neutralization is very specific for almost all viruses (OIE 2010). The virus neutralization test (VNT) is the only validated serological test
available. The agar gel immunodiffusion test and indirect immunofluorescent antibody test are less specific than the VNT due to cross-reactions with antibody to other poxviruses. The main disadvantage of using VNT is that it requires live LSDV which is restricted to be used in national reference laboratories operating in high-level bio-containment facilities (Tuppuranen, 2017). The neutralization index is the log titer difference between the titer of the virus in the negative serum and in the test serum. An index of ≥1.5 is positive. According to OIE (2010), Antibodies to capripoxvirus can be detected from day 2 after the onset of clinical signs. These remain detectable for about 7 months, but a significant rise in titer is usually seen between days 21 and 42 and antibodies and remain detectable for about 7 months. Also Buxton and Frazer (1977) reported that cattle recovered from natural infection developed neutralizing antibodies in their sera which persist for years, where the highest titer was reached by the 30th day and persisted for up to six months and their calves' derived passive immunity via colostrums which persisted for up to six months.

All calves born to vaccinated pregnant cattle were in good condition at birth and receive colostrum in adequate amounts between the first 20 min to 2 h after birth, with no clinical signs of disease during the study. The calf must rely on colostrum from its mother until its own immune system is developed at 1 to 2 months of age. Colostrum contains antibodies or immunoglobulins (essential proteins) necessary to provide the calf with protection from disease. This immunity that the calf receives is known as passive immunity. Certainly one of the major challenges in developing an active immune response in young calves has been interference from maternal immunity (Hunter and Wallace 2001). This problem has been demonstrated with several pathogens, including rotavirus, BVDV, bovine respiratory syncytial virus (BRSV), bovine herpesvirus-1 (BHV-1), and Mannheimia (Pasturella) haemolytica. The timing of many vaccines administrated by the parenteral route involves estimating when the level of maternal antibody is low enough for an active immune response to progress sufficiently to provide vaccine immunity. Current vaccination guidelines are based on previous assumptions and field observations that passive immunity against LSDV lasts for approximately six months (Hunter and Wallace 2001; Davies 1991; Weiss 1968). Thus, it is necessary to determine the duration of maternally-derived antibodies against LSDV in calves, in order to establish effective guidelines for the immunization scheme and optimal vaccination strategies in affected countries.

Serum samples were collected from calves born to vaccinated cows before colostrum intake monthly after colostral intake for 4 months. SNT antibody titers in newly born calves started from the first month after parturition and persisted in protective level until three months of age in calves. According to the vaccine producer, vaccination of calves derived from vaccinated mothers should be done at the age of 6 months because of maternally derived antibodies however, in the current study maternally derived antibodies persist only for three months. These finding came in contact with that of Daoud et al. (1998) that detected maternal antibodies against LSD in young and newborn calves and found that there was poor maternal immunity within calves especially in those aging three-six months due to low level of colostral antibodies produced by their dams. This condition facilitates the possibility of infection with lumpy skin disease virus. In contrast, Hanan (2000) determined the maternal immunity of newly born calves from vaccinated dams with sheep pox vaccine by I/D test and also by SNT and ELISA. She found that both humoral and cell mediated immunity transferred via colostrums and still up to five months of age then disappeared. Further study is required to determine the relationship between the longevity of maternally derived antibodies in
calves and the time at which their dams receive the vaccine.

Conclusion

Based on the results of this study, it can be concluded that the duration of capripoxvirus-specific antibodies nearly nine months post vaccination (36 weeks) in all cattle. Vaccination of calves derived from vaccinated mothers should be done at the age below than 6 months because of maternally derived antibodies does not persist till six months of age. Increased herd immunity and reduced susceptibility of offspring born to vaccinated mothers. The timing of vaccination are important in controlling the disease. For effective control and prevention of disease, long term vaccination with 100% coverage should be made mandatory as LSD virus being stable survives in environment for long time. Before introducing new animals to the affected farm, they should be immunized. Calves should be immunized at the age of 3 to 4 months raised from mothers, who are vaccinated or naturally infected. Pregnant cows, breeding bulls can be vaccinated annually. Administration of a live virus vaccine by the intradermal route results in good immune response. Sheep pox vaccine Romanian strain is safe for animals as there is no adverse reactions after vaccination were obtained. There is no abortion cases were recorded.

REFERENCES


https://doi.org/10.1016.j.antiviral.2014.06.009
S., Frost, L., Henstock, M. R., Lamien, C.