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## Characterization of Candidate Seed Orf Viruses to be Used as Vaccine in Sheep and Goats in Saudi Arabia

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### Abstract:

According to the Office International Des Epizooties (OIE) standards for production of poxviruses vaccines, it is a pre-requisite to perform characterization of the candidate seed virus before its incorporation as a seed for the particular vaccine. In the present study, two candidate field orf viruses were subjected to characterization according to the OIE standards. Treatment of the viruses with Chloroform and diethyl ether has resulted in dropping of the titre of each virus by 2 log<sub>10</sub> and 1.3 log<sub>10</sub> respectively. Sensitivity to trypsin and heat inactivation of both viruses resulted in dropping of the titre by 5 log<sub>10</sub> in 120 minutes and complete virus inactivation at 60 °C degree respectively. Either of these viruses is found suitable to be used as vaccine seed for production of future orf vaccine to protect sheep in Saudi Arabia. The obtained results were compared with those reported elsewhere in the literature.

**Key words:** characterization; orf viruses; seeds; vaccine.

### Introduction:

Orf or contagious pustular dermatitis (CPD) is a specific dermal disease of sheep and goats (Haig and Mercer, 1998; Balasso, 1981). The disease is caused by a parapox virus of the family *Poxviridae*. Field infection with orf virus was noted to occur around the mouth, on the mammary gland and feet of the affected animals (Gameel *et al.*, 1995). Severe and mild orf outbreaks have been reported from different parts of the world and annual losses as a result of natural orf infection is estimated to be by millions of dollars (Robinson and Balassu, 1981).

Saudi Arabia (SA) is no exception where, orf infection was found to be widespread among sheep and goats causing great economical damage (Housawi *et al.*, 1991; Gameel *et al.*, 1995; Abu Elzein and Housawi, 1997). In spite of this, no vaccine has been used in the country.

In an attempt to produce a potent vaccine to be used in Saudi Arabia, two local field orf virus isolates were subjected to standard characterization procedures according to OIE specifications (Anon – 2004), so as to be used satisfactorily as vaccine seed.

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**Materials and Methods:****Candidate viruses:**

Two field orf viruses which caused orf infection in sheep and goats in 1995 and 1997 were obtained from our collection. They were designated Hou/1/95 and Zn/1/98, respectively. They were in scab form.

**Preparation of inoculum:**

Scab material, for each virus, was made as 30% suspension (w/v) in phosphate buffered saline (PBS) pH 7.4. The supernatant fluid was collected follow centrifugation at 1500 rpm for 15 minutes.

After adding antibiotics to the collected supernatant (Housawi *et al.*, 1991); it was used to inoculate each of the following cell culture type:

1. Vero cell culture.
2. Primary and secondary lamb's kidney cell culture, (LK).
3. Primary and secondary lamb's testicles cell culture, (LT).

**Preparation and inoculation of cell cultures:**

The primary cell culture types, LK and LT were prepared and inoculated as described by Housawi *et al.*, (1991). Each virus was passaged twice in its respective cell culture type.

**Virus titration:**

Each virus was titrated in its respective cell culture in which it was passaged, as described by Housawi *et al.*, (1991). The titres were calculated as described by Reed & Muench (1938).

**Virus growth curve:**

The growth curve studies for each virus was performed in the vero cell culture types, which was selected according to the results of the virus titration studies. The mothd of Talhouk & Elzein (1986) was followed.

**Trypsin treatment:**

Standard method of trypsin treatment of viruses was followed (Talhouk & Elzein 1986). Following trypsin treatment, the viruses were titrated in vero cell culture monolayers and the drop in titre was calculated as described by Reed & Muench (1938). Virus control, treated with equal volume of PBS pH 7.4, was included in the experiment.

**Chloroform treatment:**

The method of Feldman & Wang (1961) was followed to study the effect of chloroform on the two virus isolates. To 1 ml of virus (titre  $10^6$  TCID<sub>50</sub>/ml) contained in a small glass tube, 0.05 ml of neat chloroform was added. The tube was closed firmly and left for 10 minute at room temperature (22 °C). The tubes were shaken every two minutes. A virus control tube which received PBS pH 7.4 only and treated in the same way as the chloroform treated tube was included in the test. The tubes were centrifugated at 500 rpm for 5 minutes. The upper layer was collected and subjected to titration in vero cells as described by Housawi *et al.*, (1993). The titre was calculated according to Reed & Muench (1938).

**Effect of diethyl ether:**

The standard method of Andrewes & Horstman (1949), was followed in this study. To each virus preparation (titre of  $10^6$  TCID<sub>50</sub>/ml), 20% of analar diethyl ether was added. The glass tubes containing the mixer of virus and ether, were closed firmly and placed for 24 hours at 4°C. During this incubation the tubes were shaken frequently. Control virus, which received PBS, pH 7.4 instead of ether, was incubated in the same manner. The ether was then evaporated from the preparation according to Andrewes & Horstman (1949), titrated in vero cells as described by Housawi *et al.*, (1993) and the titres were calculated as described by Reed & Muench (1938).

**Effect of temperature:**

The effect of temperature on the two viruses was studied exactly according to AbuElzein (1983). The temperatures 50°C, 56°C and 60°C were used for various lengths of time. Control, non-heated viruses were included as controls.

Residual virus activity, following heating was titrated by inoculation of the heated virus suspension in vero cells as described by Housawi *et al.*, (1993). The control non-heated virus was also titrated in the same manner. The virus titre was calculated as described by Reed & Muench (1938).

**Pathogenicity:**

To ensure that the viruses were pathogenic to sheep, an experiment was set up as follows:

For each virus, two one-year old, local Arabi sheep (Awasi), which were free from orf antibodies, as judged by ELISA (Housawi *et al.*, 1993), were

inoculated as described by Housawi *et al.*, (1993). The inoculum was passaged in vero cell culture monolayers, at a titre at  $10^6$  TCID<sub>50</sub>/ml.

Each group of sheep was housed in a separate room, and put under close daily observation for clinical signs. *Ad libitum* water & feed were provided.

## **Results:**

### **Virus growth:**

Both viruses started producing discernible cytopathogenic effect (CPE) in each of the three types of cell culture within the first two days. The CPE progressed daily to involve 80% of the monolayers within 4-5 days.

Table (1) shows the titre of the two viruses in the three types of cell culture.

### **Virus growth curves:**

Similar growth curves were obtained with both viruses is a typical growth curve. The titres started to rise from day one, post inoculation (PI), to peak at days four, five, six and seven; then went down from day 8 to reach the minimum by day 13.

### **Sensitivity to trypsin:**

Table (2) and figure (1) show results of the effect of trypsin on the two viruses. Within 10 minutes of exposure of both viruses to trypsin a sharp sudden drop in titre took place  $\log_{10}$  4.4. Then the drop in titre went gradual until the end of the experiment in 120 minutes.

### **Effect of the chloroform:**

Chloroform treatment resulted in dropping of the titre of each virus by 2  $\log_{10}$ .

### **Effect of diethyl ether:**

The treatment of both viruses with diethyl ether resulted in the drop of the titre of both viruses by 1.3  $\log_{10}$ .

### **Effect of temperature:**

Figure (2) shows typical heat inactivation curve for the viruses. Both temperatures of 50°C and 56°C shows slight drop the titre of the viruses after 30 min. However, heating for 30 min. at 60°C completely inactivated both viruses.

**Pathogenicity:**

Both groups of sheep succumbed to the experimental orf infection similar to those of naturally infected sheep (Figure 3). The lesions started as erythema which developed into papule/pustule stage in 3 days post infection (PI) and developed into scab form within 4 days PI. The average length of the scab period until dropped was three weeks.

The scabs were collected from both sheep groups and the contained virus was confirmed to be orf virus by the agar gel immuno-diffusion test (AGID) as described by Sawhney *et al.*, (1973).

**Discussion:**

To select a virus isolate to be used as a vaccine seed, it has to be subject to specific characterization procedures so as to ensure that it is typical of the virus group in question. Accordingly, the present study was undertaken.

Both of the orf virus isolates in the present study (Zn/1/98 and Hou/1/95) gave almost the same results in all the performed tests. On the other hand, the results of these viruses, matched results reported earlier for orf viruses from other countries (Anon 2004, Haig & Mercer 1998, Robinson & Balassu 1981, Trueblood *et al.*, 1963).

From the overall results, one can choose any of the two viruses satisfactorily as a seed for the future orf vaccine. These viruses can constitute good autogenous vaccine, as they are isolates from the Saudi field.

Detail of information about the efficacy of the vaccine on protection of sheep and goats and results of the clinico-pathological response of the vaccinated and control animal are designed to be reported as separate study.

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Table (1)  
 Titres of the two viruses in three cell culture types

Type of Cell culture			
Strain	Lamb Kidney	Lamb Testicle	Vero
Hou/1/95	6.5	6.8	6.5
Zn/1/98	6.4	6.7	6.6

- Log<sub>10</sub>/ml Cell culture virus titre

Table (2)  
 Sensitivity to trypsin (concentration)  
 on the two orf strain viruses

Time (minutes)	Titre of trypsin treated viruses Log 10 TCID <sub>50</sub> /ml	
	Hou/1/95 Strain	Zn/1/98 Strain
0	6,6	6,5
10	4,2	4,4
20	2,00	2,00
30	2,00	2,00
40	1,9	1,9
50	1,9	1,9
60	1,65	1,65
120	1,60	1,6

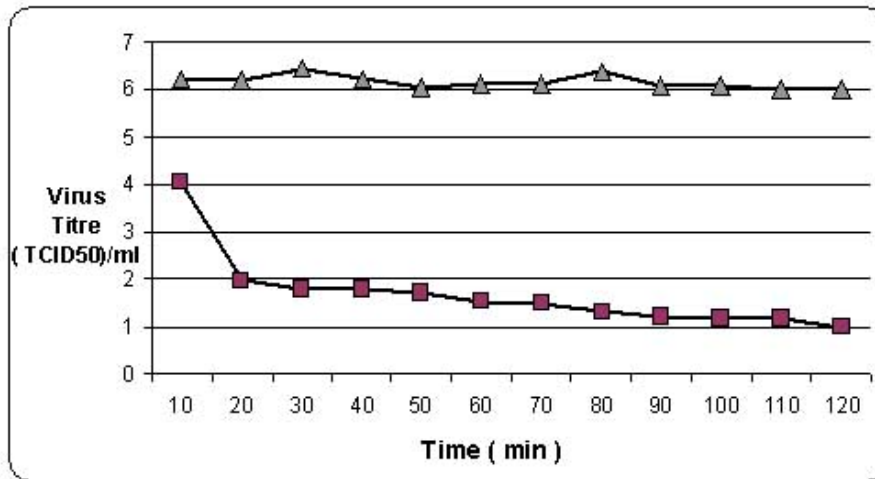


Fig. 1 Trypsin treatment

- ▲ Control – non-trypsin treated Virus
- Trypsin – treated Virus of Hou/1/95

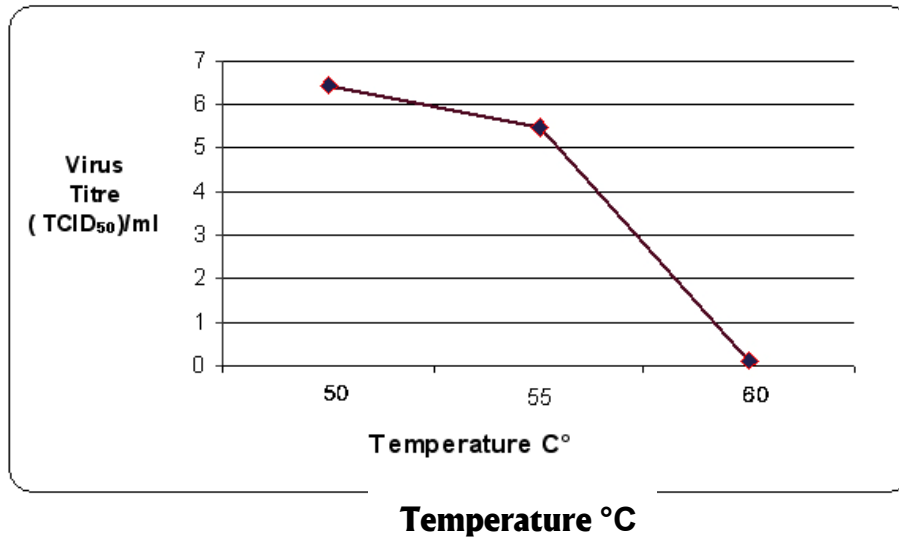


Fig. 2 Heat inactivation.

- Heating for 30 minute of Hou/1/95





Fig. 3: Orf lesions, around the mouth and lips of naturally infected Swakni sheep.

## السمات الخواصية لإختبار عترة فيروس الأورف المرشحة للاستخدام كلقاح للضان في المملكة العربية السعودية

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### الملخص :

تمشيا مع مواصفات OIE لإنتاج اللقاحات الخاصة بعائلة الجدري . يتطلب الأمر إجراء دراسة للخواص الطبيعية للفيروس (العترة) المرشحة للاستخدام كلقاح. ضمن الدراسة الحالية تمت دراسة عتريتين من فيروس الأورف الحقلية. حيث تمت دراسة سماتها وخواصها حسب مواصفات OIE .

حيث لوحظ حدوث إنخفاض في المعيارية الفيروسيية لكلا العتريتين بمقدار  $2 \text{ Log}_{10}$  و  $1.3 \text{ Log}_{10}$  عندما عولجت بالكلورفورم والذي إيثال على الترتيب.

كما تتضمن الورقة دراسة تأثير كل من الترسين والحرارة على العتريتين. حيث تم تسجيل انخفاض في المعيارية الفيروسيية بمقدار  $5 \text{ Log}_{10}$  عندما تم تعرضهما للترسين لمدة ١٢٠ دقيقة. بينما سجل إخماد كامل لنشاط العتريتين عن تعرضها لدرجة حرارة ٦٠ درجة مئوية.

وعليه فقد بينت تلك النتائج بأن أيا من العتريتين مناسبة لأن تكون بذرة لإنتاج لقاح يحمي الأغنام من المرض في المملكة العربية السعودية. كما قورنت النتائج التي تم الحصول عليها مع تلك النتائج المنشورة في مناطق أخرى من العالم.