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CLINICO-PATHOLOGICAL RESPONSE OF DROMEDARY CAMELS AND SHEEP TO CROSS-EXPERIMENTAL INFECTION WITH TWO VIRULENT ORF VIRUSES ORIGINATING FROM CAMELS AND SHEEP

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ABSTRACT

A comparative study on the clinico-pathological response of camels and sheep to cross-experimental infection with two virulent field orf viruses, originating from camels and sheep, was made. Sheep were completely refractory to infection with the camel orf virus. Equally the camels were resistant to infection with the orf virus originating from sheep. The sheep developed classical clinico-pathological lesions against the orf virus of sheep origin and gave a low level of seroconversion. The camels, which were inoculated with the camel orf virus, also showed classical clinico-pathological signs but no seroconversion was detected. The results were discussed in relation to the epidemiology of the disease in Saudi Arabia.

Key words: Camels and sheep, clinicopathological response, cross-experimental infection, orf viruses

Camel orf or Auzduk, and contagious pustular dermatitis of sheep and goats (Orf) are caused by parapoxviruses of the family poxviridae (Khokhoo, 1982; Anon 2002). Both viruses are morphologically indistinguishable (Munz, 1992) and they cross-react with each other in serological tests (Azawi *et al*, 1995). However, the camel virus shows host-specificity but the sheep and goat orf virus was recorded in other animal species such as rendier (Kummeneje and Krogsrud, 1979), dogs and seals (Hartung 1980).

The genus parapoxvirus also includes other members, which are the bovine papular stomatitis virus (BPSV), the pseudocowpox virus (PCPV) and the parapoxvirus of red deer in New Zealand (PVNZ). Differentiation between these viruses was based on the natural host range, pathology and on restriction endonuclease and DNA/DNA hybridisation analyses (Wernery and Kaaden 2002). Mercer *et al* (1997), found that virus members of the genus parapoxvirus share a good deal of homology between the central regions of their DNAs, however, homology within the genomes' termini, was low.

In the Kingdom of Saudi Arabia (KSA) sheep and goat orf virus infection is very common (Housawi and Abu Elzein 1991; Abu Elzein and Housawi, 1997) while the camel orf infection was only recently recognised (Abu Elzein *et al*, 1998).

Since only little information is available regarding the cross-infection of camels and sheep to either virus, we thought of undertaking the present study. Two virulent field orf virus strains, one from dromedaries and the other from sheep, were used.

Materials and Methods

The viruses

Camel contagious ecthyma virus

This virulent field strain was obtained from a peviously recorded outbreak in camels in Saudi Arabia (Abu Elzein *et al*, 1998). It originated from scab material.

Sheep orf virus strain

This virulent strain originated from sheep scab material and was obtained from an orf outbreak in sheep and goats in the Eastern region of Saudi Arabia (Housawi and Abu Elzein, 1997).

The inocula

Scab material from each virus strain was homogenised in phosphate buffered saline (PBS) pH

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7.4, to make a 10% suspension. Antibiotics were added and was immediately used, without centrifugation, in the experimental infection studies, as described below. Each of these suspensions is referred to as 'the inoculum' in subsequent descriptions.

Experimental animals

Ten, 6 to 12 month old local sheep and six one-humped, 2 to 3 years old Arabian camels (*Camelus dromedarius*) were used in the experiments. All animals were apparently healthy and seronegative for orf virus antibodies using the serum neutralisation test (SNT), against the sheep orf virus.

Experimental infection

The 10 sheep were divided into two groups A and B and each group contained 5 animals. Each group was kept in complete isolation from the other. The six camels were divided into two groups D and E and each group contained 3 camels. The area outside and around the mouth of each animal was shaved for virus inoculation.

Each animal of groups A and D was scarified on the shaved area, then the sheep orf virus inoculum was applied to cover the whole scarified area. The same procedure was repeated to animals of groups B and E using the camel orf virus inoculum.

Each group of animals was placed in an isolation unit and food and water were supplied *ad libidum*. Rectal temperature and score of the mouth lesions were daily recorded.

Sampling

Following inoculation, blood for serum, was weekly collected from each experimental animal for six weeks. The sera were separated, heated at 56°C for 30 min., and stored at -20°C until used. When scabs developed, biopsies were aseptically collected using sterile scissors and forceps, for virus reisolation, identification and for histopathological studies. Those for histopathology were fixed in 10% formal saline. Paraffin sections 4-6 mμ thick were prepared and stained with haematoxylin and eosin (H and E).

Virus reisolation

Twenty percent suspensions of scab material, were prepared as described above, except that the suspensions were centrifuged, in the cold, at 1500g for 15 minutes. After the addition of antibiotics the suspensions were used to inoculate secondary lambs' testicle (SLT) cell culture, which were prepared as described by Housawi *et al* (1991). The samples which gave cytopathic effect (CPE) on the SLT were transferred to vero cell culture. The samples which did not give CPE on SLT were blindly passed twice before scored negative.

The isolated viruses were titrated in vero cell culture as described by Housawi *et al* (1991), and 50% tissue culture infective dose 50 (TCID 50 /ml) was calculated as described by Reed and Muench (1938).

Virus identification

(i) Fluorescent antibody test (FAT)

The indirect FAT method of Leindo and Castro (1981), which was reported to be more sensitive than the direct method, was used to detect the camel orf virus antigen in thin sections from lesions collected from the experimentally-infected camels, when scab material had developed. A rabbit hyperimmune serum, prepared previously against the sheep orf virus (Housawi *et al*, 1993) was employed in the test.

(ii) The agar gel immunodiffusion test

The agar gel immunodiffusion (AGID) test was followed for the identification of the two orf viruses. Two orf antigen preparations were used in the AGID test. One was scab material from the experimental camels. It was made in a 50% homogenate as described above. The other, was the sheep virus isolate at its third passage in Vero cells, at a titre of 10TCID50/ml. This virus was concentrated 25X, using poly-ethylene glycol (PEG) 4000 crystals (BDH Ltd., UK).

A rabbit anti orf antiserum (Housawi *et al*, 1993) was used in the AGID test. The set-up of the AGID test was designed such that, the rabbit hyperimmune serum was added to the central well. The concentrated sheep orf virus was added to two opposite cells. The 50% homogenate of the camel orf virus was added to two other opposite wells. The pH 7.4 was added to the remaining wells PBS.

The serum neutralisation test

The serum neutralisation test (SNT) was used to identify the sheep orf virus, which was isolated in the vero cells.

Antibody detection

To detect seroconversion in the convalescent sheep and camels, the standard serum neutralisation test (SNT) was employed using a known orf virus

Table 1. Score of the clinico - serological and virological results of the experimental camels and sheep.

Virus Strain	Group	Sheep	Signs	CPE on SLT	IFT	SNT	AGID
sheep orf		1	+	+	nd	+	+
sheep orf		2	+	+	nd	+	+
sheep orf	A	3	+	+	nd	+	+
sheep orf		4	+	+	nd	+	+
sheep orf		5	+	+	nd	+	+
camel orf		1	-	nd	nd*	nd	nd
camel orf		2	-	nd	nd	nd	nd
camel orf	B	3	-	nd	nd	nd	nd
camel orf		4	-	nd	nd	nd	nd
camel orf		5	-	nd	nd	nd	nd
Virus Strain	**Group**	**Camel**	**Signs**	**CPE on SLT**	**IFT**	**SNT**	**AGID**
sheep orf		1	-	nd	nd	-	nd
sheep orf	D	2	-	nd	nd	-	nd
sheep orf		3	-	nd	nd	-	nd
camel orf		1	+	-	+	nd	+
camel orf	E	2	+	-	+	nd	+
camel orf		3	+	-	+	nd	+

*nd = not done, + = positive reaction, - = negative reaction

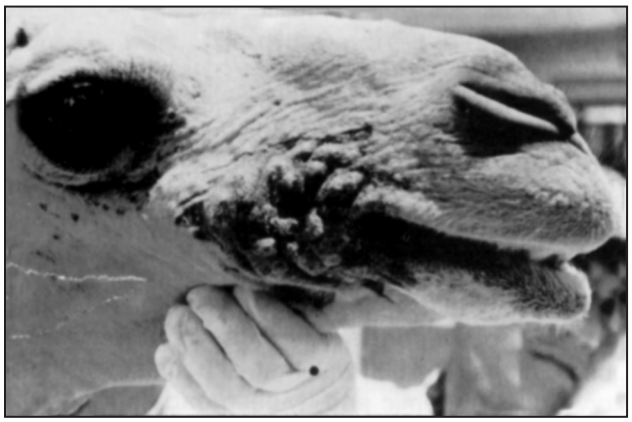

Fig 1. Experimental camel orf infection: Note well defined camel orf nodules in the lips and lip commissures.

originating from sheep and passaged three times in vero cell culture. An equal volume of 100 TCID 50/ml of that virus was added to two fold dilution series of each serum as described by Housawi *et al* (1991). The end-point titres of the sera were calculated as described by Reed and Muench (1938).

Results

Clinical observations

Table 1 summarises the overall results of the clinical signs, CPE on SLT, IFT and the SNT.

By the third day post-inoculation, sheep of group A which had received the sheep orf virus, showed oedema and erythema at the sites of scarification. Within 2 to 3 days, thereafter, nodules appeared which progressed into bigger and pustular lesions of about 1 cm in diameter. The pustules ruptured into yellowish lesions which dried to form scabs which became thicker, darker in colour and had irregular edges. Secondary lesions were also observed which similarly progressed to reach the scab stage. The whole span of infection took 32 to 36 days when complete shed of scab took place and the skin returned to normal.

Camels of group D which received the sheep orf virus did not show any clinical signs until the end of the four months of the experiment.

Camels of group E which received the camel orf virus strain showed erythema at the scarification sites between days 4 to 5 post inoculation. This was

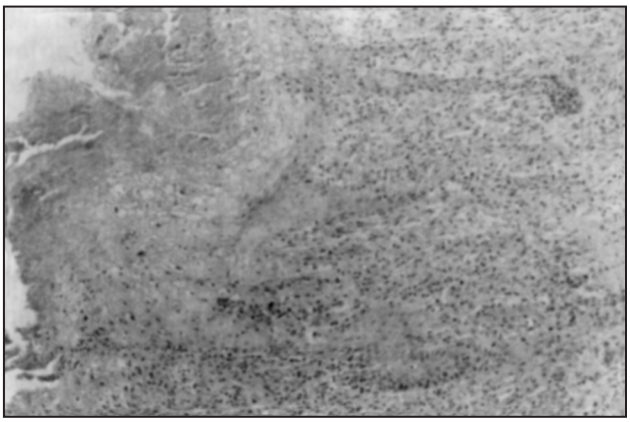

Fig 2. Lip: Section shows epidermal hyperplasia, hyperkeratosis, acanthosis with cell swelling and reticular degeneration, pseudoepitheliomatous hyperplasia (arrow). Note inflammatory cell in dermis. HE X 50.

followed by papule formation which developed into wet-surface pustules and then developed into encrusting nodules of 0.5 - 1.0 cm in diameter. These were well defined and separated from each other (Fig 1). Scabs then formed. The whole span of the disease took 35 days after which complete healing was achieved.

Sheep of group B which received the camel virus isolate did not show any clinical signs until the end of the four months experiment.

The rectal temperature for each of the inoculated sheep and camels was within the normal range.

Histopathological findings

Histopathological investigations of the the lip of the affected camels revealed epidermal hyperplasia characterised by marked hyperkeratosis, acanthosis and pseudoepitheliomatous hyperplasia. Acanthotic cells showed cytoplasmic swelling, reticular degeneration, and eventually epidermal necrosis. Proliferation of dermal tissue and inflammatory cell infiltration of mainly lymphocytes and some neutrophils were observed in the dermis (Fig 2).

Virus isolation

The SLT cell culture inoculated with homogenates from scab material obtained from sheep of group A, which were infected with the sheep orf virus strain, showed cell rounding by day four. The CPE spread to cover the whole cell monolayer in 7 days. Further two passages were performed on SLT and then the virus was inoculated and passaged onto vero cells giving discernible cytopathogenic effect (CPE), (Housawi *et al*, 1993). The virus titre was $10^{6.5}$ $TCID_{50}$/ml.

The SLT cell culture inoculated with the homogenate from scab material obtained from scabs of camels of group E which were inoculated with the camel orf virus strain, did not show any CPE inspite of three blind passages.

Virus identification

(i) The AGID test **:** The sheep virus isolate passaged on vero cells was concentrated 25X and used in the AGID test gave a precipitation line against the rabbit hyper immune serum. This line merged to make a line of complete identity with a line produced between homogenate of the scab material of the 50% homogenate of the camel scab material and the rabbit hyperimmune serum. Another hazy and cloudy line, was produced between the 50% camel scab homogenate and the rabbit hyperimmune serum.

The SNT

The SNT results of the sheep orf virus showed that the virus was inhibited by the hyperimmune serum at a titre of ($-\log_{10}$ 2.1) against 100 TCID50 of the virus.

As the camel orf virus did not grow in cell culture, it was not possible to perform the SNT for its identification.

The FAT

Specific fluorescence was produced when sections from lesions of the infected camels reacted against the rabbit anti sheep orf serum, in the indirect FAT.

Antibody detection

Low level serum neutralisation antibody titres ($0.9 \log_{10}$) were obtained in the convalescent sheep sera inoculated with the sheep orf virus. No serum antibodies were detected in the convalescent camel sera.

Discussion

The present study was inspired by two observations. The first was that, although sheep and goat orf is endemic and widespread in KSA, the incidence of camel orf is very low. The second was that, throughout our studies on the sheep and goats orf in KSA, during the last two decades, we did not see any outbreaks involving camels, sheep and goats, simultaneously. So, it seems that there is host specificity for each disease.

Results of the present study clearly indicated that each orf virus was specific for the animal species from which it was originally isolated. The sheep orf virus did not give any clinical reaction in the inoculated camels; and the camel orf virus did not cause any clinical signs in the inoculated sheep. However, each virus gave classical and dramatic clinical pictures in its respective host. The camel virus was severe in the inoculated camels (Fig 1) and equally, the sheep virus gave severe clinical signs in sheep. The convalescent sheep and camels did not give appreciable serological response as detected by the SNT against the sheep orf virus. This was expected, as the neutralising antibody response in orf infection is either of low titre or undetectable (Robinson and Balassu, 1981).

Our field epidemiological observations in sheep orf in KSA indicated that, camels were never involved in any of the numerous orf outbreaks in sheep during the last two decades. Also we did not see any sheep or goats involvement during camel orf outbreaks.

From our investigations, in KSA, it is obvious that camel orf is a distinct entity from sheep orf. Each disease seems to have its respective epidemiological peculiarities in this country.

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