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Alterations in some epidemiological patterns and virus heterogeneity recently observed in sheeppox outbreaks in the Sudan

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ABSTRACT

Observations including alterations in some epidemiological patterns, host specificity and virus heterogeneity for sheeppox (SP) in the Sudan were recorded in the present study. Field observations, virus isolation, plaque production and pathogenicity tests were employed. Outbreaks of SP occurring during the unusual summer season were observed. Investigations also affirmed the host specificity of the sheeppox virus (SPV), which could neither naturally nor experimentally infect goats, although it was lethal to sheep. Two clinically and serologically distinct types of sheeppox virus were isolated from these field outbreaks. The viruses, which were identified serologically and virologically as SPV, displayed different characteristics and thus, heterogeneity within the virus in Sudan was recognized.

Key words: sheeppox virus, seasonality, specificity, heterogeneity, Sudan

Introduction

Sheeppox virus (SPV), a member of the *Capripox* genus of the family *Poxviridae*, is the etiologic agent of the most economically important and endemic disease of sheep in northern and central Africa, southwest and central Asia, and

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the Indian subcontinent (CARN, 1993; ESPOSITO and FENNER, 2001). The disease manifests itself in pyrexia, cutaneous and lung lesions and lymphadenopathy (MUNZ and DUMBELL, 1994; ESPOSITO and FENNER, 2001). Transmission of SPV was reported to occur via the aerosol and insect vector (KITCHING and TAYLOR, 1985a; KITCHING and MELLOR, 1986). Sheeppox virus is generally considered to be host specific as the disease outbreaks or virus isolates occur or are noted to cause disease in sheep only (MURTY and SINGH, 1971; MUNZ and DUMBELL, 1994; RAO and BANDYOPADHYAY, 2000).

In the Sudan the first scientific investigation on SP was stimulated by BENNET et al. (1944) who affirmed the endemicity of the disease in the country and host specificity of the virus. However, HAJER et al. (1988) have reported non-host-specific SPV which could infect goats. Sheeppox in the Sudan is responsible for dramatic economic losses, particularly in animals prepared for export (LOSOS, 1986). It was also known to be a seasonal disease associated with the cold winter (MUZICHIN and ALI, 1979).

In this communication, we report certain alterations in the disease epidemiology, affirm the host specificity and heterogeneity within SPV isolates in the Sudan.

Materials and methods

Field observations and sampling. Many of the diversified farms in the outskirts of Khartoum were placed under observation for a one-year period in order to assess the occurrence of the disease during the different seasons. Information, including clinical signs, morbidity and mortality rates, were collected. Samples were collected from different farms in different seasons. Investigated winter outbreaks occurred at Rawasi Farm and Bagir Farm. In Rawasi Farm the population was 5200 adult rams and ten goats, including four kids. In Bagir Farm the infected population was a holding of 16 sheep, including five nursing ewes and their 3-4 month-old single lambs, and 13 goats including kids.

The investigated summer outbreaks occurred in Mingif Farm, University Farm and in the Central Quarantine. In the Mingif Trade Company Farm in Muwaleih, west of Omdurman province, the disease observed in 1300 rams. In the University Farm a mixed flock of sheep and goats showed the disease, and a large population of pure sheep at the Central Quarantine in Kadarow (Khartoum) was also affected. Samples were received from the Rawasi, Bagir and Mingif outbreaks, while only

clinical data were available from the other outbreaks. Skin samples were collected as described by ABUSAMRA (1980). Samples were chopped and minced in a pestle and mortar with sterile sand. The paste was centrifuged and the supernatant alliquoted and stored at -20 °C before processing.

Preparation of cell cultures. Lamb testicle (LT), lamb kidney (LK), lamb thyroid (LTY) and ovine foetal lung (OFL) cell cultures were used for the isolation and titration of the viruses. LT and LK were prepared as described by FERRIS and PLOWRIGHT (1958) while LTY and OFL were prepared after NITZSCHKE et al. (1967). Primary cultures and subcultures for these cells were grown in Falcon flasks and Roux bottles using Glasgow Modified Essential Medium (GMEM) (Sigma, St Louis, Mo, U.S.A.) supplemented with antibiotic-antimycotic and anti-PPLO solutions and 10% or 2% calf serum as growth and maintenance medium, respectively.

Virus isolation and titration. Each 0.2 ml of the processed sample was placed in a cell culture tube. The tubes were then incubated at 37 °C rolling at speed of 5 rpm for one hour before 0.8 ml of fresh medium was added to each tube; rolling then resumed for one hour. Thereafter, the content of the tubes was discarded and 1 ml of fresh maintenance medium was added to each tube. They were then incubated stationary in tilted racks at 37 °C. The tubes were examined daily under an inverted microscope for two weeks. Control tubes received 0.2 ml of GMEM medium.

To define the titre of the viruses, their ten-fold dilution was prepared and 50 µl of each dilution with 50 µl of cell suspension containing 1.5 million cell per ml, were placed in each of eight wells of a row of microtitre plate. The plates were sealed and incubated in a carbon dioxide incubator. The titres were calculated according to method of Spearman and Karber (FINNEY, 1964).

Virus identification. The viruses were provisionally identified as pox in view of the clinical signs exhibited by the infected animals and the cytopathic effect (CPE) they produced in cell cultures. They were further identified by serological tests, including agar gel immunodiffusion (AGID) which was conducted after SHARMA and DHANDA (1971) and counter-immunoelectrophoresis (CIEP) basically carried out as described by SHARMA et al. (1988). In both tests the infected skin antigen was reacted with reference serum against the 0240 Kenyan strain of the virus. Non-infected sheep skin, phosphate buffer saline (PBS) and GMEM were incorporated as negative controls.

Plaque and pathogenicity tests. Plaque production test was used to follow up when the viruses developed different CPEs. The technique was essentially performed as described by PORTERFIELD and ALISON (1960). Two groups of susceptible lambs and kids (four animals per group) were also inoculated with the cell culture harvest to redevelop the clinical disease. Lesions were observed and temperature recorded.

Results

Field findings. Infections in Rawasi and Bagir Farms were clinically observed as being distinct from the Mingif outbreak cases. The nodular form of SP in which nodules are epidermal and protruding was diagnosed in Rawasi and Bagir farms, while in Mingif the nodules were subcutaneous and unapparent unless palpated, or under the tail where large erythematous patches were visible. Outbreaks in Rawasi and Bagir Farms were reported in winter, whereas the Mingif outbreak was reported in summer. Details of the outbreaks are presented in Table 1. In all outbreaks, infected animals showed respiratory complications. None of the goats in the Rawasi, Bagir and University Farms showed any clinical signs of pox, although they were in intimate contact with the infected sheep.

Table 1. Some epidemiological features of sheeppox outbreaks

Location	Season	Sheep No.	Cases	Dead	Morbidity (%)	Mortality (%)	Pox form
Rawasi	Winter	5200	120	93	2.30	1.70	nodular
Bagir	Winter	16	12	7	75.00	43.00	nodular
Mingif	Summer	1300	-	-	20.00	8.00	s/c

s/c = subcutaneous

Virus isolation and identification. The LT cell culture was proved as the system of choice for SPV as it had advantages over the other systems in that it was easier to produce, more susceptible and gave a faster and better yield of virus (Tables 2 and 3). The OFL cells showed no susceptibility to SPV.

Tubes that were inoculated with Rawasi and Bagir isolates developed visible CPE on the 6^{th}- 8^{th} days post-inoculation (p.i.) (Table 2). The CPE was diffuse and marked by cell rounding and formation of large cells (Fig. 1). The Mingif isolate produced different CPE which started as early as the 4^{th} day PI (Table 2) and marked by cytoplasmic bridges connecting separated cell clusters (Fig. 2). The

individual cells tended to be spindle-shaped and in general the CPE started as a foci of cell distraction from where it spread with predictable progression.

Plaque and pathogenicity tests. In the plaque production test, the CPE produced by Rawasi and Bagir viruses was diffuse, while the Mingif isolate of SPV developed discrete plaques.

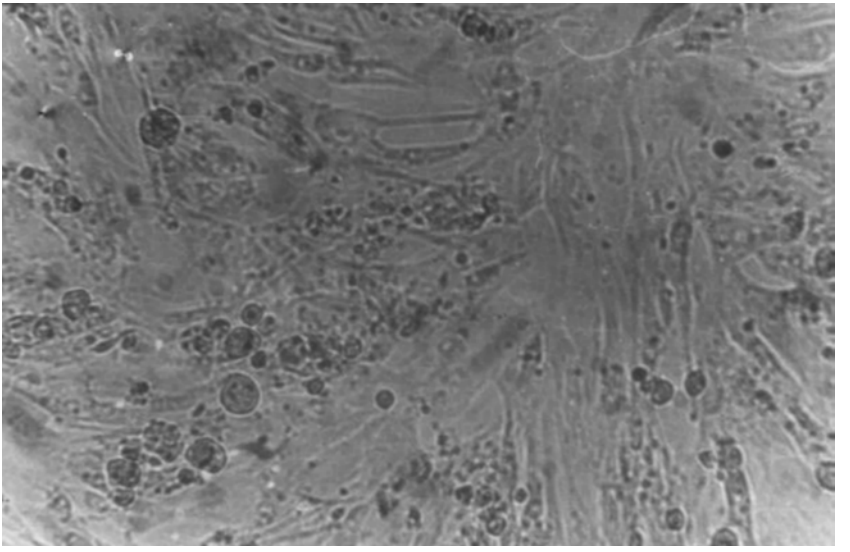

Fig. 1. Lamb testicle cell culture infected with Rawasi sheeppox virus isolate

Table 2. Growth of sheeppox virus isolates in different cell cultures

Virus isolate	Cell culture type	First CPE appear (days p.i.)	80% cell destruction (days p.i.)
Rawasi and Bagir	lamb testicle	6	13-15
	lamb kidney	8	14-16
	lamb thyroid	7	15-16
	ovine foetal lung	Nil	Nil
Mingif	lamb testicle	4	11-12
	lamb kidney	7	13-15
	lamb thyroid	7	ND
	ovine foetal lung	Nil	Nil

ND = not done, Nil = no CPE of cells destruction observed.
Rawasi and Bagir isolates of SPV showed similar growth pattern in various cell cultures.

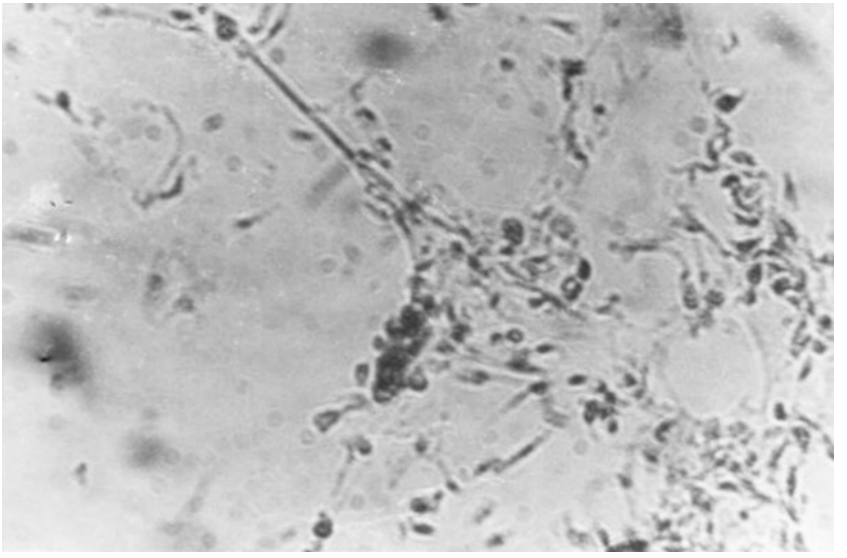

Figure 2. Lamb testicle cell culture infected with Mingif sheeppox virus isolate

Table 3. Titres of sheeppox virus isolates propagated in different cells and titrated in lamb testicle cells

Virus isolate	Cell culture type	Titre ($TCID_{50}$)/ml
Rawasi	lamb testicle	$10^{5.2}$
	lamb kidney	$10^{4.4}$
	lamb thyroid	$10^{4.0}$
Mingif	ovine foetal lung	$10^{3.7}$

The susceptible lambs inoculated with the cell culture harvest of the SPV developed clinical signs of the disease, including pox lesions, an obvious rise in temperature and respiratory distress after ten days of inoculation; 50% mortality was recorded. In contrast the kids which had received the same dose showed no signs of illness, apart from transient elevation in temperature (Table 4).

Discussion

One of the most important features noted during field observations was a change in the epidemiology of SP in the Sudan. The disease, which was known to be

Table 4. Average temperature of lambs and kids inoculated with sheeppox virus

Days p.i.	Lambs	Kids
0	38.0 ± 0.16*	38.4 ± 0.13
2	39.1 ± 0.11	38.6 ± 0.20
4	41.4 ± 0.15	38.9 ± 0.17
6	40.5 ± 0.10	38.7 ± 0.11
8	38.8 ± 0.10	38.3 ± 0.12
10	37.9 ± 0.12	38.2 ± 0.14
12	37.5 ± 0.11	37.8 ± 0.16
14	37.4 ± 0.14	38.2 ± 0.10
16	37.1 ± 0.12	38.4 ± 0.11
18	37.2 ± 0.16	38.2 ± 0.16
20	37.2 ± 0.13	38.3 ± 0.19

*Average temperature (°C) ± standard deviation, n = 4; except for lambs after day 10 p.i. where n = 2 due to death of two lambs.
p.i. = post-inoculation

associated with the cold winter season (MUZICHIN and ALI, 1979), appears to have lost this peculiar property and an outbreak of SP can be seen at any time of the year. This is probably because of the change in the rearing system. The intensive production system, where large numbers of animals are perpetuated and fattened in closed fences, has recently been practiced in the country. As the virus is hardy and resistant to desiccation, the particles surviving in the scabs which had fallen during the previous outbreak cause a new epidemic to occur whenever fresh animals were introduced to the farm. The high animal density in the farms is also a predisposing factor. This hypothesis was supported by the fact that the disease incidence was reduced to a negligible figure when the herd immunity to SP was raised by vaccination before introduction to the farm.

In accordance with LOSOS (1986), the nodular form of SP was prevalent in the Sudan as seen in the Rawasi, Bagir and University farm SP outbreaks. However, a rare subcutaneous form of SP was also diagnosed in Mingif farm.

Another important phenomenon noted during field observation was the strict host specificity of SP. In outbreaks in Rawasi, Bagir and in the University farm in Shambat only sheep were affected, although goats, including kids, were in close association with infected sheep. The goats have never been infected with pox or vaccinated against the disease and their blood samples were also negative for precipitating antibodies. In addition, the SPV isolated from the Rawasi outbreak

did not experimentally infect kids, although the virus was highly virulent to lambs. These results coincide with those obtained by BENNET et al. (1944) for SP infections in the Sudan. It also coincides with reports in other parts of the world which indicate that SPV is host specific as the disease outbreaks or virus isolates occur or are noted to cause disease in sheep only (MURTY and SINGH, 1971; MUNZ and DUMBELL, 1994; RAO and BANDYOPADHYAY, 2000). However, natural or experimental infection of the virus to goats was previously described in various reports (DAVIES, 1976; KITCHING and MELLOR, 1986; KITCHING and TAYLOR, 1985b) and the virus isolated from flocks in which both sheep and goats were concurrently affected. Unlike SPV, goatpox virus was confirmed to infect both species in the Sudan (BENNET et al., 1944; MOHAMED et al., 1982). However, a later study by KITCHING and TAYLOR (1985b) proved the virus was more lethal to goats regardless of whether it was derived from sheep or goats. This lack of cross-pathogenicity between SPV and goatpox virus was observed despite the close genetic relationship among them recently published by TULMAN et al. (2002).

Sheeppox viruses were isolated by the first passage in lamb testicle (LT) cell cultures. It is therefore LT cell culture that was the most suitable system for the studied viruses as it was easy to produce and was more susceptible to the virus infection compared to LK, LTY and OFL systems. Moreover, the LT gave a better yield of the virus. This substantiates previous reports by HOUSAWI et al. (1991). The variant CPE produced by the Mingif isolate, compared to the Rawasi one, in which cytoplasmic processes formed a net-like appearance, was similar to that previously reported by SRIVASTAVA and SINGH (1980) and RAO and MALIK (1982).

Although the results reported herein reveal some heterogeneity in the cultural and other biological behaviours within the SPVs in the Sudan, the viruses must, however, be further compared at the genomic and proteomic levels. To our knowledge this is the first suspicion of variations in SPV in the Sudan.

In conclusion, the epidemiology, namely seasonality, of SP already known in the Sudan was not absolute, where some changes have been observed in the way that the disease can occur at any time of the year. The species-specificity and heterogeneity of the SPV isolated were also confirmed.

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SAŽETAK

Iznijeta su zapažanja o nekim epidemiološkim čimbenicima, specifičnosti domaćina i raznolikosti virusa ovčjih boginja (VOB) u Sudanu. Promatrana je klinička slika, specifičnosti izdvajanja virusa, proizvodnja plakova i test patogenosti. Razmatrana je pojava ovčjih boginja za vrijeme neuobičajene ljetne sezone. Istraživanja su također potvrdila specifičnost domaćina za virus ovčjih boginja. Njime se koze nisu mogle zaraziti ni prirodno ni pokusno, iako je bio letalan za ovce. Dva klinički i serološki različita tipa virusa ovčjih boginja bila su izdvojena iz prirodno oboljelih ovaca. Izolati koji su serološki i virološki identificirani kao VOB pokazali su različite osobine pa je time potvrđena raznolikost među sojevima toga virusa u Sudanu.

Ključne riječi: virus ovčjih boginja, specifičnost, raznolikost, Sudan