Sheep Pox: Experimental Studies with a West African Isolate

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ABSTRACT

Under conditions of a maximum security laboratory, four cross-bred sheep were inoculated intradermally only or intradermally and intratracheally with a West African isolate of sheep pox virus. All sheep had increased temperature and depression by the fourth or fifth day after infection. Nasal and lacrimal discharge and coughing occurred in all sheep but were more severe in sheep receiving the virus *via* the tracheal route. From the fifth day after infection, numerous papular erythematous skin lesions developed at the inoculation sites. These were 3-7 mm in diameter and gradually became nodular. Some of these lesions healed and others coalesced to form tumor-like masses. In one sheep, euthanized 14 days after intradermal and intratracheal inoculation, nodular lesions were found in the skin around the eyes, nostrils, oral and perianal regions, the mucosa of the rumen and throughout the lungs. Histologically, skin nodules were characterized by ischemic necrosis, vasculitis, microvesiculation, eosinophilic cytoplasmic inclusions in the dermal epithelial cells and vacuolar nuclear degeneration. The pulmonary lesion was that of proliferative alveolitis with occasional cytoplasmic inclusions in the alveolar cells and macrophages. Ultrastructurally, large cuboidal virus particles were found both in the skin lesion and inoculated tissue cultures. The sheep pox virus structure was easily distinguished from contagious ecthyma virus, a parapoxvirus which causes sporadic disease in Canada. Serum neutralizing antibodies developed in all the sheep by 14 days postinfection.

The clinical and pathological characteristics of experimental sheep pox produced with this West African isolate were similar to those caused by Neethling virus of lumpy skin disease in cattle.

Key words: Sheep, experimental pox, Capripoxvirus.

RÉSUMÉ

Variole ovine expérimentale réalisée avec un isolat de l'Afrique occidentale

Cette expérience portait sur quatre moutons croisés, confinés dans un isolement particulièrement strict; elle consistait à leur inoculer par la voie intradermique, seule ou jointe à la voie intratrachéale, un isolat du virus de la variole ovine de l'Afrique occidentale. Au bout de quatre à cinq jours, ils manifestèrent tous de l'hyperthermie et de la dépression, ainsi qu'un écoulement nasal, du larmoiement et de la toux; les trois dernières manifestations s'avérèrent toutefois plus prononcées chez ceux auxquels on avait donné une injection intratrachéale. À compter du cinquième jour, plusieurs papules érythémateuses se développèrent aux sites d'inoculation intradermique; elles mesuraient de 3 à 7 mm de diamètre et devinrent graduellement nodulaires. Certaines guérirent, tandis que d'autres se fusionnèrent et formèrent des masses qui ressemblaient à des tumeurs. Chez un mouton, sacrifié 14 jours après inoculation intradermique et intratrachéale, on constata la présence de lésions nodulaires autour des yeux, de la bouche et de l'anus, ainsi que sur les narines, la muqueuse du rumen et un peu partout dans les poumons. L'histopathologie révéla que les nodules cutanés affichaient de la nécrose ischémique, de la vasculite, de très petites vésicules, des inclusions intracytoplasmiques éosinophiles, dans les cellules de l'épiderme, et une dégénérescence vacuolaire de leur noyau. La lésion pulmonaire consistait en une alvéolite proliférative qui s'accompagnait de quelques inclusions intracytoplasmiques, dans des cellules alvéolaires et des macrophages. La microscopie électronique permit de déceler de grosses particules virales cuboïdes, dans les lésions cutanées et les feuillets cellulaires inoculés avec des échantillons. La morphologie du virus de la variole ovine se distingua facilement de celle du virus de l'ecthyma contagieux, un parapoxvirus qui cause une maladie sporadique, au Canada. Des anticorps sériques neutralisants se développèrent chez tous les moutons, en l'espace de 14 jours après l'infection.

Les caractères cliniques et pathologiques de la variole ovine expérimentale reproduite avec le virus précité ressemblaient à ceux qui résultent de l'infection par le virus Neethling de la maladie nodulaire cutanée des bovins.

Mots clés: moutons, variole expérimentale, capripoxvirus.

INTRODUCTION

Sheep pox (SP), an acute, contagious and often fatal disease of sheep is caused by a member of the Capripoxvirus group and is characterized by generalized cutaneous eruptions (1-2). Although SP does not exist in the western hemisphere, it still remains an important problem in Asia and Africa. Despite the serological uniformity which exists among SP virus (SPV) isolates from different regions (1), differences in the host response and virulence between various strains of SPV have been noted (1,2). Furthermore, variation in the clinical and pathological manifestation of SP has also been reported (3). A nodular form of SP causing skin lesions not unlike those described for bovine lumpy skin disease (LSD) (4), has been reported

to occur mostly in Africa (5,6). In animals affected by the nodular type of SP, the skin lesions rarely undergo vesiculation and granular lesions of 5 to 30 mm predominate. This type of SP is occasionally called "stone pox" in the literature and spreads rapidly in field epidemics with lower mortality rate than reported for the "vesicular type" of SP (7,8). In an outbreak of nodular form of SP in Nigeria a morbidity rate of 100% and mortality of 30% is recorded among the affected sheep and goats (6).

Since there are few recorded clinical and pathological observations for the natural or experimental nodular form of SP in the literature, we have transmitted a West African isolate of SP virus to Canadian cross-bred sheep and in this report describe the clinical, pathological and virological findings. In addition this communication may assist field and regulatory veterinarians in the diagnosis of exotic SP and its differential diagnosis with contagious ecthyma (CE), a parapoxvirus infection which occurs sporadically in Canada.

MATERIALS AND METHODS

Sheep pox virus

The Nigerian isolate (INPDAM-FD-30.11.77) of West African SPV (6) in the form of lyophilized lamb testis cell culture virus suspension was kindly supplied by Dr. W.P. Taylor of the Animal Virus Research Institute, Pirbright, Woking, Surrey, England and used in this study. The virus was passaged three more times in fetal lamb testis (FLT) cell culture maintained in Hank's minimum essential medium (MEM) containing 5% fetal bovine serum (FBS) and antibiotics. The second and third FLT passage virus suspensions were used for inoculation of sheep in group 1 (S5 and S6) and group 2 (S7 and S8) respectively. The virus titers at the second and third passage levels in FLT cell cultures were $10^{4.5}$ and $10^{5.3}$ median tissue culture infectious doses ($TCID_{50}$) per mL, respectively.

Experimental animals and procedures

In a biocontainment facility, three male (S5,S6,S8) and one female (S7) cross-bred Canadian sheep about four to five months old were divided into two groups, and an area of about 10 x 20 cm was closely clipped on the right or left axilla. The shaved skin was washed with lukewarm water and soap, dried with sterile gauze and then cleaned with ethanol soaked gauze. The dried skin was scarified with a wire brush and 1.0 mL of the virus suspension was applied to the area with a sterile cotton swab. One sheep in each group (S6,S8) also received 1.0 mL of virus suspension intratracheally by injection into the lower part of the trachea. The animals were observed and examined for clinical changes and their rectal temperatures were recorded daily. On the 14th day postinoculation one sheep (S6) was euthanized with barbiturates, exsanguinated and examined for pathological changes. Samples of affected skin and lung were collected and fixed in 10% neutral buffered formalin, routinely processed, sectioned at 5 μ and stained with hematoxylin and eosin (H & E).

Serum neutralization test

Serum samples were tested for neutralizing antibodies according to a microtest procedure described by Mushi and Plowright (9), using serial doubling dilutions. An estimated virus dose of 100 to 300 $TCID_{50}$ per well was added to the serum dilutions and the mixture incubated at room temperature for 90 minutes, after which time one volume (0.025 mL) of bovine fetal kidney (BFK) cell suspension (250,000 cell/mL) in MEM containing 5% fetal bovine serum and antibiotics was added to each well. The microplates were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C for ten days, at which time final observation for cytopathic effect (CPE) was made. The neutralizing antibody titers were calculated by the method of Karber (10).

Electron microscopy procedures

Nodular lesions collected from the experimentally infected sheep were homogenized in distilled water after which the suspension was sonically disrupted with a few five second pulses using a microtip on a soniprep sonicator (Model 105. MSE, Manor Royal, Crawley, Sussex, RH10 X22 England). Crude, infected FLT cell culture lysates were sonically disrupted and diluted fivefold in distilled water. For comparison, scabs from a sheep infected experimentally with contagious ecthyma virus (CEV) were prepared in the same fashion as the SP nodules. All preparations were put on carbon treated, formvar coated, copper grids and stained with 2% phosphotungstic acid. The grids were examined in an Hitachi HU-12 transmission electronmicroscope at 100 kv.

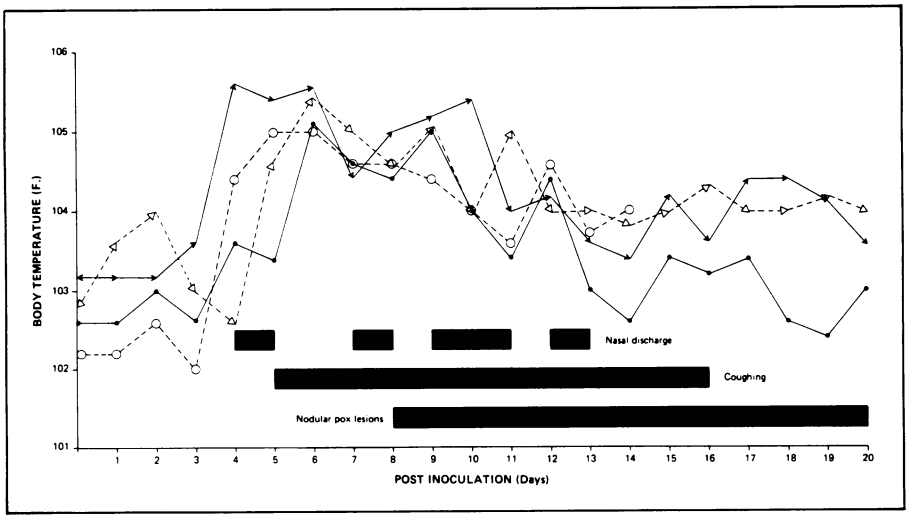

FIGURE 1. Clinical response of Canadian cross-bred sheep to experimental inoculation with a Nigerian strain of sheep pox virus; S5 ●____● and S7 ▲____▲ were inoculated intradermally (ID); S6 ○____○ and S8 △____△ were inoculated ID and intratracheally (IT).

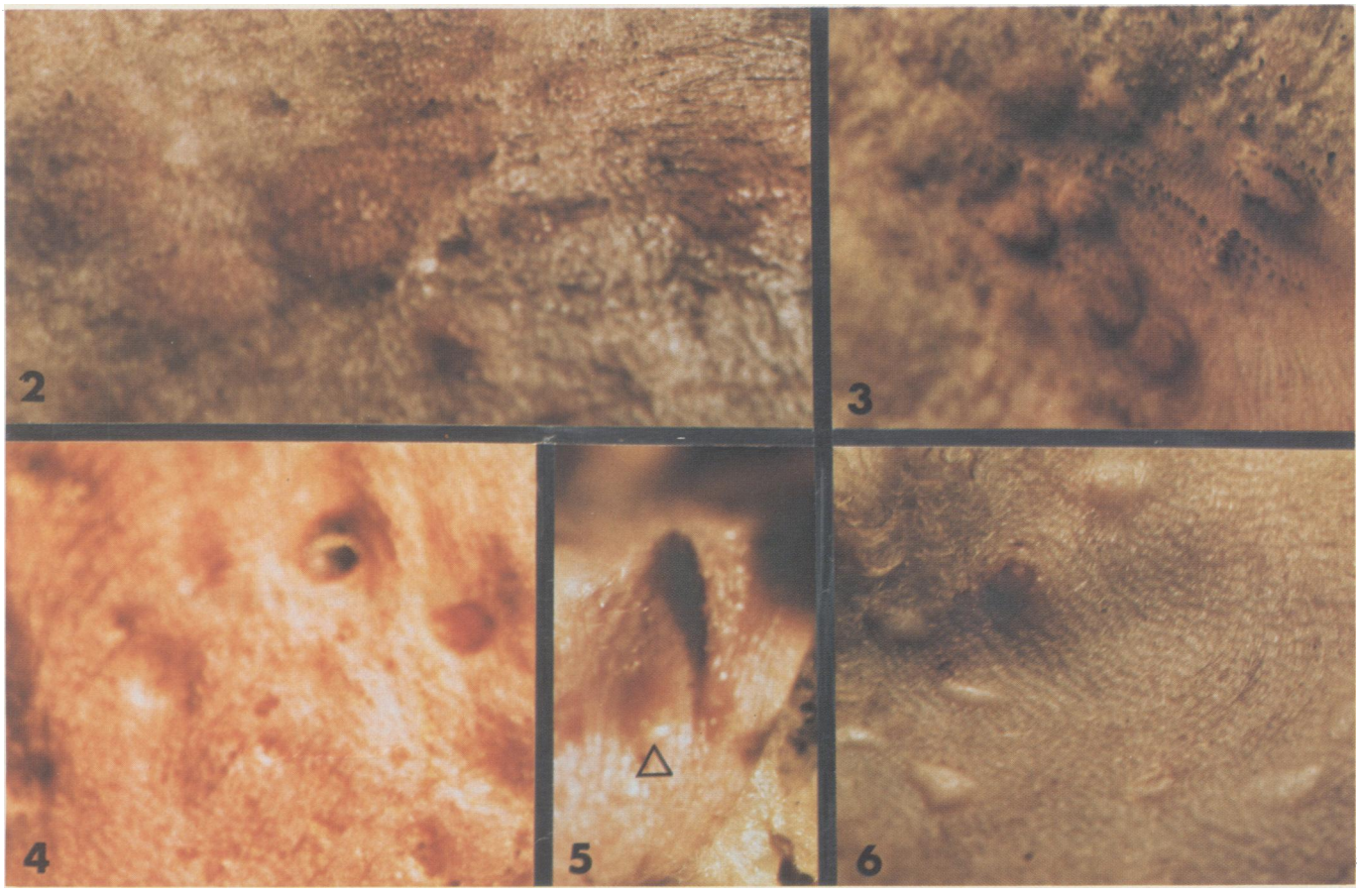

FIGURE 2. Skin lesions produced by a Nigerian strain of sheep pox virus; primary erythematus papule, day 5 postinoculation (PI).

FIGURE 3. Nodular pox lesion, day 8 PI, note circumferential demarcation.

FIGURE 4. Ulcerated appearance of pox lesion after removal of the necrotic centres of the nodules, day 8 PI.

FIGURE 5. Secondary perianal nodule of sheep pox, day 12 PI.

FIGURE 6. Dermal scars of sheep pox lesions, day 20 PI.

RESULTS

Clinical observations

All the sheep showed increased body temperature (104-105° F) beginning four or five days postinfection (PI), lasting two to three days, and then gradually subsiding to normal by the 13th days after infection (Figure 1). During this period all animals were depressed. All the animals had a normal appetite except one (S6) which was anorexic on day 7 PI. During the acute phase of infection, some nasal discharge accompanied by intense coughing, more severe in the two sheep that received the virus intratracheally (S6 and S8), was observed. At day 9 PI one sheep (S6) also had lacrimal discharge. From day 5 PI numerous skin lesions appeared on the site of inoculation. These were erythematous papules, measured 3 to 7 mm in diameter (Figure 2), and gradually became larger and nodular by day 8 PI, some with typical circumferential demarcation (Figure 3). The nodular lesions increased in number and when palpated felt hard and grain-like. No vesicular or pustular changes were observed during the progression of these lesions. When the necrotic centres of the nodules were removed, the lesions appeared ulcerated (Figure 4). From day 12 PI multiple secondary nodules appeared on the other parts of the skin and singularly around the eye, nostril, oral and perianal regions (Figure 5). The secondary skin lesions also appeared as papules and gradually progressed into nodules as described above. Some nodules coalesced and formed hard tumor-like masses in the skin, often partially covered with necrotic crusts. These lesions persisted in three sheep (S5,S7,S8) until day 30 PI when they were euthanized. Some of the individual skin lesions healed, leaving a typical "pock" mark on the skin (Figure 6).

Necropsy and histopathological findings

The dermal nodules of all four sheep and lung lesions of sheep S8 were similar to the finding of S6. At necropsy sheep S6 had numerous dermal and subdermal nodules ranging from 0.5 cm to 3 cm on the thorax and flank (Figure 7). Cut sections of larger nodules were grey, firm and surrounded by edematous connective tissue. Some nodules were hyperemic

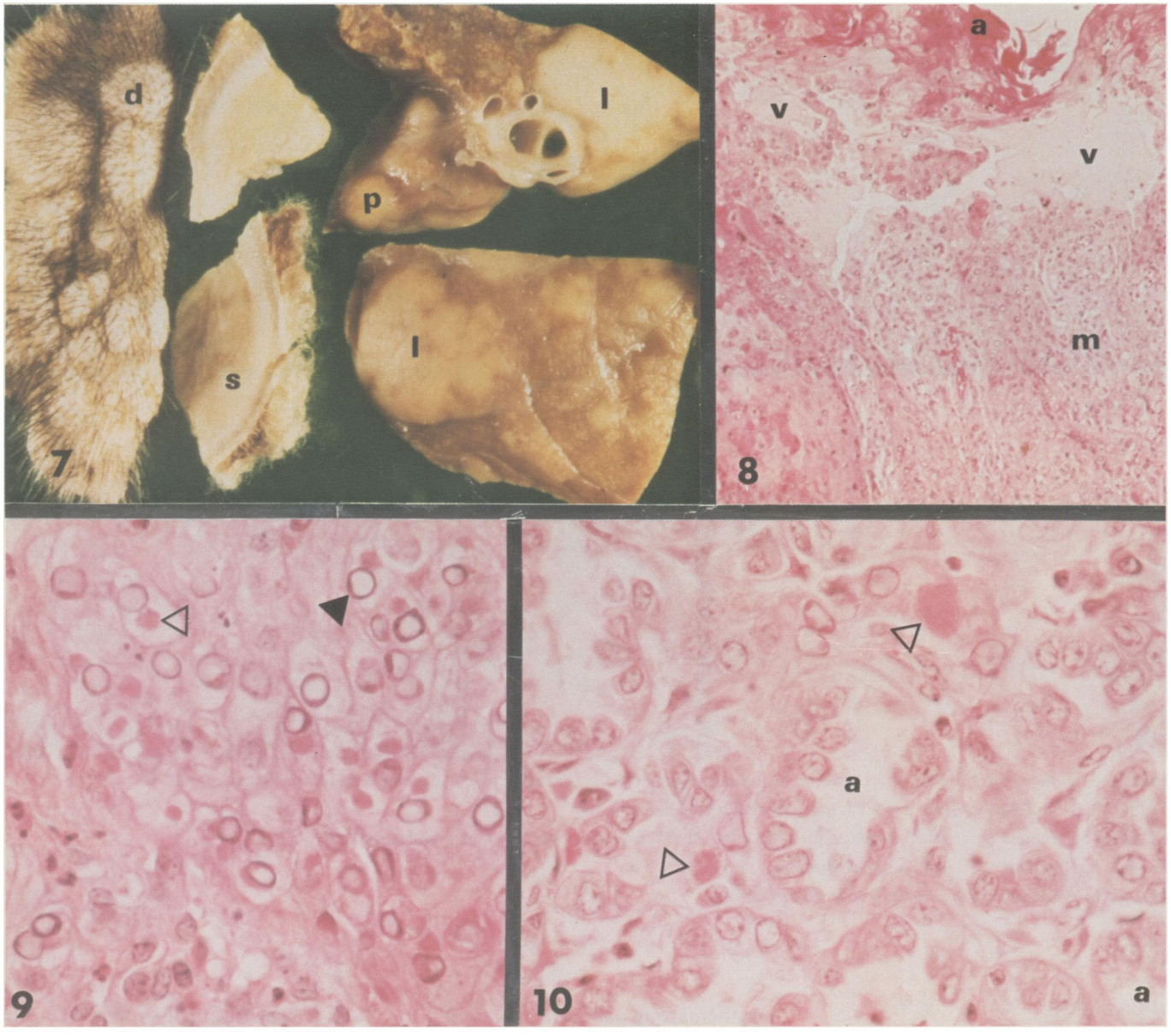

FIGURE 7. Formalin fixed tissue of dermal (d), subdermal (s) sheep pox nodules; single pulmonary (p) or coalescing (l) lymphosarcoma-like lesions, sheep 6, day 14 PI. Bar = 1 cm.

FIGURE 8. Hyperkeratosis (a), variable in size microvesicles (v) and dermal infiltration of macrophages and fibroblasts (m) in the dermal sheep pox nodule. H & E. X100.

FIGURE 9. Typical sheep pox cells with many eosinophilic cytoplasmic inclusions (empty triangle) and vacuolated nuclei (full triangle) of the skin nodule. H & E. X480.

FIGURE 10. Nonsuppurative proliferative (a) pneumonia with cytoplasmic eosinophilic inclusions (triangles). H & E. X480.

and others had necrotic or ulcerated centres. Small secondary nodules were found in the upper eyelid, vulva and mucosa of the rumen. Similar nodules were found throughout the lungs. The subpleural nodules were round or coalescing, forming lymphosarcoma-like lesions in the apical and diaphragmatic lobes (Figure 7). Regional lymph nodes were enlarged and edematous.

Histopathological examination of dermal nodules usually revealed superficial hyperkeratosis, hydropic degeneration of keratinocytes, microvesicles of various sizes filled with pink fluid, vasculitis and infiltration of macrophages and fibroblasts (Figure 8). Many eosinophilic cytoplasmic inclusions were seen in the stratum spinosum (Figure 9, empty triangle); the nuclei were vacuolated, "punched out-like" appearance (Figure 9, full triangle). Pulmonary nodules were characterized by proliferative bronchiolitis and alveolitis giving the appearance of gland-like structures

(Figure 10,a). There were also large eosinophilic cytoplasmic inclusions in the pulmonary macrophages (Figure 10, triangles). Nuclear degeneration was less pronounced than in the skin lesions. Ultrastructurally, "sheep pox" cells in the skin and lungs had nuclear vacuolation and virions with dumbbell-shaped cores, similar to those described previously by Murray *et al* (8).

Serum neutralization tests

The development of neutralizing antibodies is shown in Figure 11. All four sheep developed neutralizing antibody detectable as early as day 14 PI. However, lower SN titers were recorded for the two sheep (S5 and S7) that received the virus by the intradermal route only.

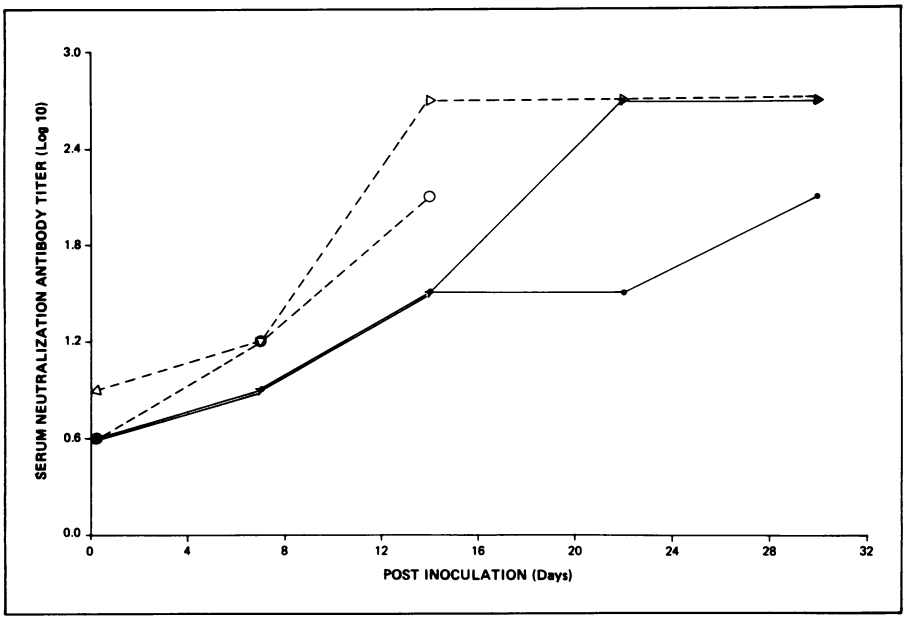

FIGURE 11. Serological response of sheep to experimental inoculation with a Nigerian strain of sheep pox virus.

Electron microscopy findings

The nodular lesions and the infected cell culture material from the sheep pox specimens both yielded characteristic large cuboidal virus particles with rough irregular outer membrane. The virions were approximately 300 x 350 nm (Figure 12). In comparison the contagious ecthyma scabs yielded virions measuring approximately 300 x 200 nm and displaying the regular criss-cross surface pattern characteristic of CEV (Figure 13). Both the SP and CE tissue specimens contained enough virions to be readily identified from direct preparations of clinical specimens using negative contrast electron microscopy.

DISCUSSION

The clinical manifestation in the sheep experimentally infected with a West African strain of SP virus is similar to those described in the natural cases of infection reported from Nigeria where this virus originated (6). In the acute phase of the disease, the skin lesions were predominantly nodular and except for occasional pus-filled eruptions which were probably due to secondary bacterial infection, no lesions resembling vesicles were observed. In their brief communication on the transmission of this isolate to Merino lambs, Asagba and Nawathe (6) report the occurrence of a succession of SP lesions from papules through pustules. However, the nature of the pustules is not described and no mention of vesiculation of lesions as reported in "classical" SP (3,8,11) is recorded (6). Apart from some nasal discharge, coughing, and some pox lesions in the lungs and rumen, other visceral organs were not involved in the experimental cases reported here. Generally the sheep regained their condition and appetite after the initial phase of the disease. It is possible that under field conditions other factors such as opportunistic bacteria, parasites and/or environmental stresses may result in severe systemic changes and/or death (3,6).

In contrast to the report by Asagba and Nawathe (6), by histological examination, we observed numerous eosinophilic intracytoplasmic inclusion bodies both in the affected dermal and lung cells. Electron microscopy examination of affected cells revealed that the inclusions contained virions. In addition, by histological examination, we observed an abundance of "sheep pox" cells with typical "punched-out" nuclei both in the skin and lung lesions. These pathognomonic lesions were similar in character to those reported by Plowright *et al* (7) and Murray *et al* (8) for the "classical" SP.

The clinical and histopathological findings of the present study with a

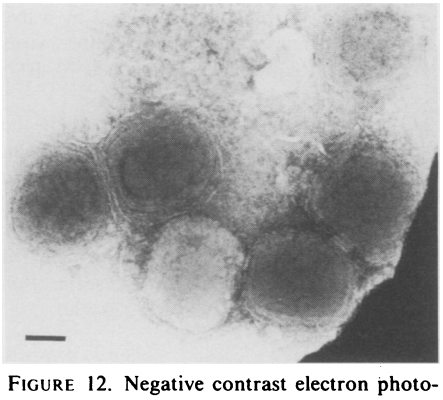

FIGURE 12. Negative contrast electron photomicrograph of sheep pox virions from nodule ground in water. Bar = 100 nm.

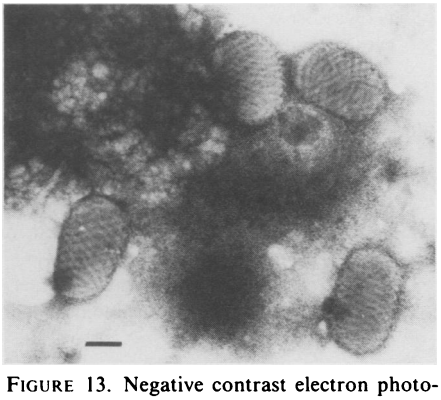

FIGURE 13. Negative contrast electron photomicrograph of contagious ecthyma virions from mouth scales ground in water. Bar = 100 nm.

West African isolate and those reported by Asagba and Nawathe (6) for the natural cases are comparble to the nodular form of SP or "stone pox" (3) Nodular lesions of SP are similar to those of LSD of cattle, which is caused by Neethling virus, another member of the Capripoxviruses (12). Since LSD is widespread in Nigeria (13) and its experimental transmission to sheep has been documented (14), it is tempting to speculate that the Nigerian isolate of sheep pox could have originated from LSD virus. The pathogenicity of the Nigerian isolate used in this study for cattle remains also to be tested. This may help to clarify the role of sheep as possible carriers of LSD virus in West Africa as speculated previously by Capstick (14) and Weiss (15).

As we have demonstrated with clinical material in this report, direct negative contrast electron microscopy is a simple and rapid method of diagnosis of SP. The virus particles resembled other members of the Capripoxviruses (16), and appeared similar to the C-form of vaccinia virus described by Westwood *et al* (17). Based on the virus structure alone (Figure 12 and 13), differential diagnosis between SP and CE, a parapoxvirus infection which occurs sporadically in Canada (18) can easily be made. Furthermore, electron microscopy examination of lesions is crucial for the differential diagnosis of cases of SP if the lesions are restricted to the mouth and head region as is the case in CE infection.

The results of the present study indicate that following infection, a significant rise in serum neutralizing antibody titers was demonstrable by a microneutralization test. Similar to the results reported by Davies and Otema (19) for a Kenyan strain of SP, this test was suitable for the detection of neutralizing antibodies in sera one week after the development of nodular SP when fever was subsiding (Figure 1). In contrast to Martin *et al* (20) we found that a constant virus-variable serum neutralization test, without complement enhancement, was adequate for demonstrating neutralizing antibodies by the microneutralization test. Davies and Otema (19) in a comparative study and a similar constant virus-variable serum system in a microneutralization assay, using bovine fetal muscle cells, finds the test to be as valuable as the indirect fluorescent antibody test in evaluation of antibody responses in experimentally infected sheep. These investigators show that neutralizing antibodies peak one month after infection, then gradually decline for up to six months, and remain relatively stable for 18 months, the period of their study. Immunization of sheep with vaccine strain of SP also results in development of neutralizing antibodies which last at least for one year (21-23).

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REFERENCES

1. SINGH IP, PANDEY R, SRIVASTAVA RN. Sheep pox: a review. Vet Bull 1979; 49: 145-154.
2. DAVIS FG. Characteristics of a virus causing a pox disease of sheep and goats in Kenya, with observations on the epidemiology and control. J Hyg (Camb) 1976: 76: 163-171.
3. DAVIES FG. Sheep and goat pox. In: Gibbs EPJ, ed. Virus diseases of food animals. New York: Academic Press, 1981: 733-749.
4. DAVIES FG. Lumpy skin disease. In: Gibbs EPJ, ed. Virus diseases of food animals. New York: Academic Press, 1981: 751-766.
5. PLOWRIGHT W, FERRIS RD. The growth and cytopathogenicity of sheep pox virus in tissue cultures. Br J Exp Pathol 1958; 39: 424-435.
6. ASAGBA MO, NAWATHE DR. Evidence of sheep pox in Nigeria. Trop Anim Health Prod 1980; 13: 16.
7. PLOWRIGHT W, MacLEOD WG, FERRIS RD. The pathogenesis of sheep pox in the skin of sheep. J Comp Pathol 1959; 69: 400-413.
8. MURRAY M, MARTIN WB, KOYLU A. Experimental sheep pox. A histological and ultrastructural study. Res Vet Sci 1973; 15: 201-208.
9. MUSHI EZ, PLOWRIGHT W. A microtiter technique for the assay of malignant catarrhal fever virus and neutralizing antibody. Res Vet Sci 1979; 27: 230-232.
10. LENNETTE EH. General principles underlying laboratory diagnosis of viral and rickettsial infections. In: Lennette EH, Schmidt NJ, eds. Diagnostic procedures for viral and rickettsial infections. New York: American Public Health Association Inc., 1969: 1-65.
11. DARDIRI AH. Sheep and goat pox. In: Foreign animal diseases. Committee on Foreign Animal Diseases of the United States Animal Health Association Publication, 1984: 309-316.
12. MONLOX WS. Lumpy skin disease. In: Foreign animal disease. Committee on Foreign Animal Diseases of the United States Animal Health Association Publication, 1984: 232-243.
13. BIDA SA. Confirmation by histopathology of the probable widespread of lumpy skin disease (LSD) in Nigeria. Bull Anim Health Prod Afr 1977; 25: 371-324.
14. CAPSTICK PB. Lumpy skin disease — experimental infection. Epiz Dis Afr 1959; 7: 5-62.
15. WEISS KE. Lumpy skin disease virus. In: Virology monographs. New York: Springer-Verlag, 1968; 3: 111-131.
16. FENNER F. The classification and nomenclature of the viruses. Intervirology 1976; 6: 1-12.
17. WESTWOOD JCN, HARRIS WJ, ZWARTOUW HT, TITMUS DHJ, APPLEYARD G. Studies on the structure of vaccinia virus. J Gen Microbiol 1964; 34: 67-78.
18. GREIG AS. Contagious ecthyma of sheep. Can J Comp Med 1956; 20: 448-452.
19. DAVIES FG, OTEMA C. The antibody response in sheep infected with a Kenyan sheep and goat pox virus. J Comp Pathol 1978; 88: 205-210.
20. MARTIN WB, ERHAN M, ONAR B. Studies on sheep pox vaccine-serum-virus neutralization tests. Pendik Vet Kontrol Ara Enst Derg 1975; 8: 26-47.
21. RAMYAR H, HESSAMI M. Studies on the duration of immunity conferred by a live modified sheep pox tissue culture virus vaccine. Zentralbl Veterinaermed 70; B17: 869-874.
22. UPPAL PK, NILAKANTAN PR. Studies on the serological relationships between avian pox, sheep pox, goat pox and vaccinia virus. J Hyg (Camb) 1970; 68: 349-358.
23. PENKOVA VM, JASSIM FA, THOMPSON JR, ALDOORI TM. The preparation of an attenuated sheep pox virus and its use as a vaccine. Bull Off Int Epizootiol 1974; 81: 329-339.