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EXPERIMENTAL INFECTION OF GAME ANIMALS WITH LUMPY SKIN DISEASE VIRUS (PROTOTYPE STRAIN NEETHLING)

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Abstract

YOUNG, E., BASSON, P. A. & WEISS, K. E. Experimental infection of game animals with lumpy skin disease virus (prototype strain Neethling). *Onderstepoort J. vet. Res.* (1970), 37 (2), 79–88.

Calves of a giraffe, an impala, two buffaloes, and two adult black wildebeests were artificially infected with the Neethling type of lumpy skin disease virus. The giraffe and impala died with typical symptoms and lesions of the disease. Virological examinations confirmed the presence of lumpy skin disease virus in lesions of these animals. Histopathological studies revealed microscopic lesions typical of those reported in cattle suffering from lumpy skin disease. Both intracytoplasmic and intranuclear inclusions were noticed in various cell types and some additional histopathological changes are reported. Neither the wildebeests nor the buffaloes reacted clinically to artificial infection and they failed to show a rise in antibody titre subsequently.

Introduction

Following the isolation in tissue culture of several cytopathic agents associated with lumpy skin disease of cattle (Alexander, Plowright & Haig, 1957), it has been shown conclusively that virus strains, indistinguishable from the prototype Neethling virus, are responsible for true lumpy skin disease (Prydie & Coackley, 1959; Weiss, 1963). Evidence has also been presented that the lumpy skin disease virus is a member of the pox group of viruses (Weiss, 1968).

The pathogenicity of the virus for cattle, sheep, goats and rabbits by means of experimental inoculation has been investigated by Alexander *et al.* (1957) and Capstick (1959) and was reviewed by Weiss (1963, 1968). However, no information was available concerning the susceptibility of game animals to the virus of lumpy skin disease. The purpose of this paper is to record the results of the experimental infection of game in the Kruger National Park with this virus.

Materials and Methods

Experimental animals

For the purpose of these experiments a male giraffe [*Giraffa camelopardalis* (Linnaeus, 1758)] about 4 months old, a female impala [*Aepyceros melampus* (Lichtenstein, 1812)] a few weeks of age, a male and a female buffalo [*Syncerus caffer* (Sparrman, 1779)] less than 3 weeks old and two adult male black wildebeests [*Connochaetes gnou* (Zimmermann, 1780)] were obtained. The giraffe was captured in the veld as a newly born calf before it had suckled. The impala and buffaloes were captured in the southern district of the Kruger National Park presumably after they had received colostrum from their mothers. These animals were housed separately in isolation units in the Kruger National Park and fed on a diet of commercial milk substitute and lucerne hay. The two adult male black wildebeests were obtained from the Department of Nature Conservation, Provincial Administration, Transvaal and housed at the Veterinary Institute, Onderstepoort for experimental purposes. Serum samples from all these animals except the giraffe were collected before infection and tested for antibodies against lumpy skin disease virus.

Inoculation of virus

Virulent lumpy skin disease virus (Neethling) propagated serially in tissue cultures through four calf kidney and five lamb kidney passages was used. The virus suspension was preserved at 20°C in the freeze-dried form and had a titre of $10^{5.0}$ TCID 50 per ml. Prior to inoculation the freeze-dried material was reconstituted to its original volume. The giraffe received 1.0 ml of virus suspension subcutaneously on the left side of the neck and a further 1.0 ml of virus suspension intramuscularly into the left buttock. The impala and buffaloes each received 1.0 ml subcutaneously on the left side of the neck as well as 1.0 ml intravenously into the jugular veins. The wildebeests each received 1.0 ml subcutaneously on the left side of the neck.

Observation, collection and examination of specimens

Clinical examinations and temperature recordings of the infected animals were regularly carried out and serum for serological tests was collected before and at various intervals after experimental infection except in the giraffe. The animals that contracted the disease were given antibiotic treatment with penicillin, viz. 1 500 000 and 300 000 units per day for the giraffe and impala respectively until they died.

Post mortem examinations were carried out on both the giraffe and impala and specimens for histopathological studies collected in 10 per cent formalin. Appropriate blocks from these specimens were embedded in paraffin wax, cut at 3 μ thickness and stained with haematoxylin and eosin (HE) for light microscopy. Haematoxylin-phloxin (HP), Lillie's modification of Langeron's Alizarin Red S and oil red O (ORO) were employed as special staining techniques.

Specimens for virological examination were collected aseptically and kept in a deep freeze refrigerator before isolation of the virus on primary lamb kidney cultures and identification by neutralization tests, according to the methods described by Weiss (1963).

Results

Clinical Signs

Giraffe

After an incubation period of 6 days, swellings developed at the sites of injection. These swellings were very painful to the touch, hard and firmly attached to the skin. They gradually increased in volume and by the ninth day the larger of the two was $10 \times 6 \times 3$ cm in size [Plate 1 (1)]. The skin over the swellings became completely black. Thirteen days after their onset one lesion became necrotic and partially liquefied and exuded a

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serosanguineous fluid. Several small nodules could be palpated in the region of the inner thighs and a single ulceration was observed on the ventral aspect of the base of the tail.

Fever developed 7 days after commencement of the swellings. The normal temperature of 37.5°C (99.5°F) was sometimes exceeded by more than 2.2°C (4°F) and was not influenced by antibiotic treatment. It remained elevated until shortly before the animal's death.

Fourteen days after the appearance of the first swellings the giraffe manifested the first obvious clinical signs of systemic illness. He looked dull, had a rough coat, his ears drooped and his tongue protruded a few centimetres through the partially closed lips. General weakness, polydipsia, polypnoea and a slight mucopurulent nasal discharge were also evident. The mouth was obviously very sore and he refused to eat any solids. Closer examination of the oral cavity revealed numerous nodules [Plate 1 (2)], erosions and ulcerations, varying in size from a few millimetres to one centimetre in diameter. The ulcerations were white, had a granular base and occurred chiefly on the inside of the lips. The ventral surface of the tongue was virtually covered by small white necrotic foci [Plate 1 (3)]. Larger necrotic areas were also found on the dorsal surface of this organ [Plate 1 (4)].

During the terminal stages of the disease the giraffe became very emaciated. Haematological examinations at this stage revealed a severe leucopaenia. The animal eventually died 15 days after the appearance of the first lesions.

Impala

After an incubation period of 25 days a swelling developed on the outside of the lower lip [Plate 1 (5)]. This lesion was round and hard, became completely black and grew to a diameter of one centimetre. A large number of nodules developed over the body the following day. The lesions were firm, round and hard on palpation. Large lumps developed at the site of subcutaneous injection and behind the right elbow [Plate 2 (6)]. These large nodules became black and depilated. The necrotic centre of the large lump at the site of inoculation sloughed 6 days after its appearance [Plate 2 (7)]. Numerous smaller nodules could be palpated under the skin of the metacarpal and metatarsal regions [Plate 2 (8)]. They gradually grew and gave the legs a beaded appearance. Several small very superficial nodules, 2 to 5 mm in diameter, developed on the face. They became detached after a few days without ulcerating, the underlying surface being depilated but still covered by regenerating epidermis. Small cutaneous nodules developed on one vulvar lip and on the ventral aspect of the tail [Plate 2 (9)].

The animal became extremely emaciated, losing 25 per cent of its weight between 15 and 29 days after infection.

Mouth lesions appeared 30 days after infection [Plate 2 (10)]. The rapidly developing lesions on the lips and cheeks originally consisted of nodules varying in size from a few millimetres to a centimetre. White granular ulcerations developed at these sites after the necrotic plugs were shed. Flat, dull-white necrotic foci were observed on the dorsum of the tongue. The large number of necrotic mouth lesions evidently caused much pain and interfered with feeding.

During the last two days the animal looked dull, had a staring coat and developed posterior paresis. Death intervened 6 days after detection of the first lesions. Blood examinations on the last day revealed a severe leucopaenia. Contrary to the giraffe the body temperature of this animal fluctuated during the course of the disease, but it never exceeded the normal range.

Buffalo calves and wildebeests

These animals developed no clinical signs after administration of the virus.

Pathology

Macroscopic changes

Giraffe: Cachexia, localized areas of subcutaneous oedema and a general enlargement of all the lymph nodes were present. Prominent lesions, involving the skin, subcutis and underlying muscular tissue, were seen at the sites of inoculation. Upon incision these lesions were large, yellow, fibrotic masses. One lump was extensively necrotic and contained a serosanguineous exudate. Smaller nodules from which a straw-coloured fluid could be expressed were present in the subcutis of the inner thighs.

Most of the small disseminated lesions occurred in the oral cavity. More than 200 necrotic lesions, varying in size from less than 1 to 7 mm in diameter were counted on the tongue. These lesions varied in form; flat or raised, white, necrotic foci and shallow erosions being seen. A single ulceration with a diameter of a centimetre was found in the mucosa of the oesophagus.

Focal, dull, greyish-brown areas of suspected muscular necrosis and myositis were observed in different parts of the cervical musculature. These lesions varied in diameter from a few millimetres to a few centimetres. The spleen was moderately swollen and the splenic corpuscles were fairly distinct. Incision of the left prescapular lymph node revealed several small, dull, grey, necrotic or inflammatory foci. The lungs were congested and oedematous and the left diaphragmatic lobe revealed signs of purulent bronchopneumonia. The liver was swollen and degenerative and contained a few small disseminated foci of necrosis or hepatitis. Suggillations were detected in the mucosa of the abomasum.

Impala: Cachexia, a moderate enlargement of all the lymph nodes and pulmonary oedema were observed at autopsy. The skin and mouth lesions resembled those of the giraffe. The necrotic skin lesions at the site of subcutaneous inoculation were suppurative. Incision of the nodules in the metacarpal and metatarsal regions of the legs revealed a type of granulomatous reaction in the fascia, on the tendons and tendon sheaths. Fourteen lesions were observed in the mouth, 13 on the fore limbs, 35 on the hind limbs and four on the rest of the body.

Microscopic lesions

Giraffe: Skin: The lesions were mainly confined to the subcutis, subcutaneous muscles, stratum papillare of the dermis and epidermis. The stratum reticulare appeared to be less severely affected. Foci of colliquation, vacuolation and microcavitation, sometimes in association with a small number of polymorphonuclear cells, were noticeable in the epidermis. Necrosis and sloughing of the outer layers were apparent in some areas [Plate 3 (11)]. A few suspected intracytoplasmic (IC) and intranuclear (IN) inclusions were present in the epidermis within or adjacent to the necrotic areas.

The lesions in the stratum papillare and subcutis were very similar, the only difference being the more advanced changes in the latter. Marked necrosis and oedema of the surrounding tissues made the determination of the exact nature of the lesions and cell reactions very difficult. It seemed to consist mainly of a mixed cell reaction, with round cells predominating in most areas, mobilization of histiocytes and an abortive attempt of

PLATE 1 1. Giraffe: Large swelling on the side of the neck at the injection site. 2. Giraffe: Nodules (arrow) on the inner aspect of the upper lip. 3. Giraffe: The ventral surface of the tongue showing numerous small necrotic foci. 4. Giraffe: Dorsal surface of the tongue with a few larger necrotic areas. 5. Impala: Raised, swollen lesion on outer lower lip.

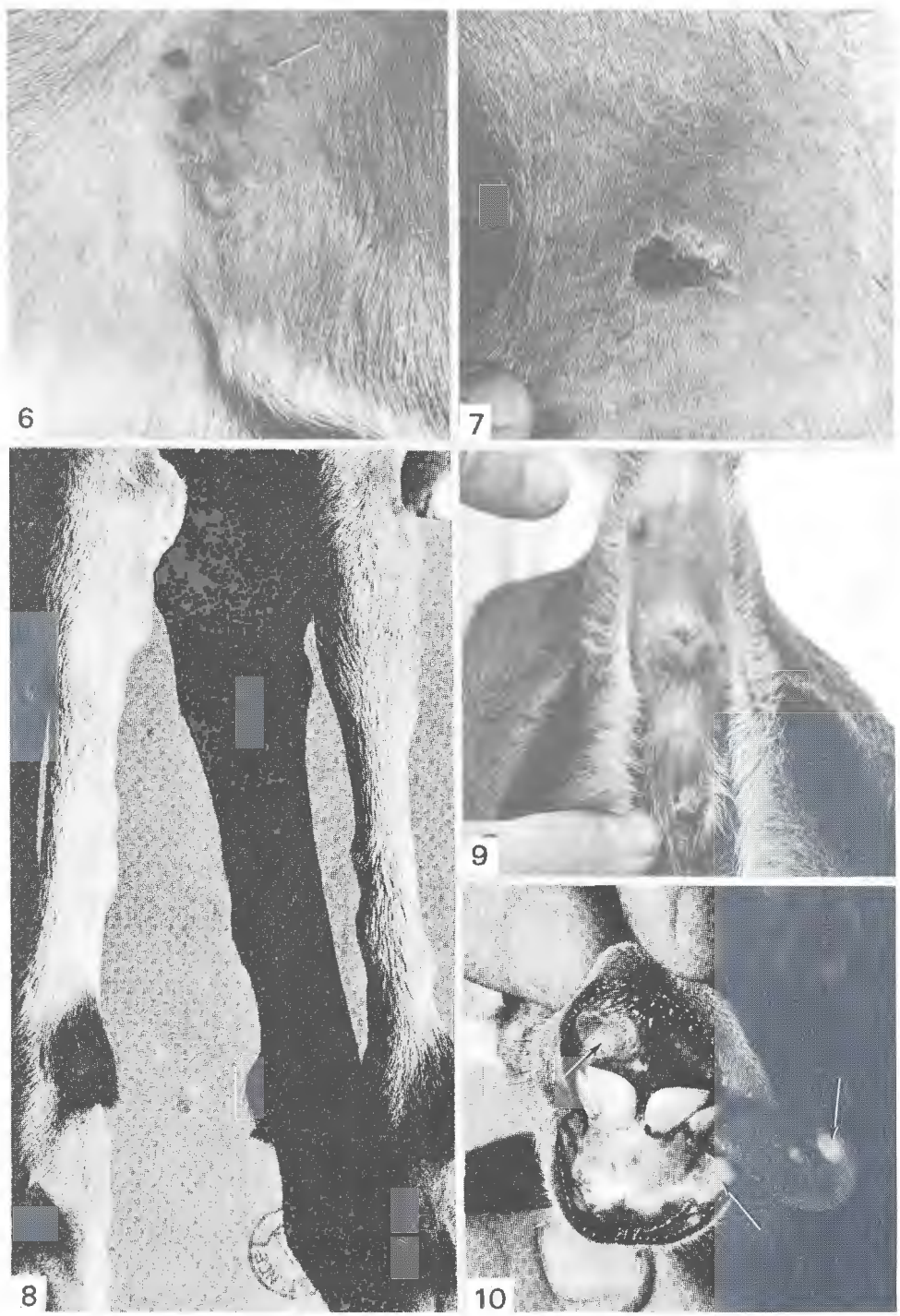

PLATE 2 6. Impala: Focal area of dermatitis and necrosis behind the elbow. 7. Impala: Sloughing of the skin at the site of injection on the side of the neck. 8. Impala: Subcutaneous nodules in the metatarsal and hock regions. 9. Impala: Nodules on one vulvar lip and on the ventral aspect of the tail. 10. Impala: Mouth lesions which appeared 30 days after infection.

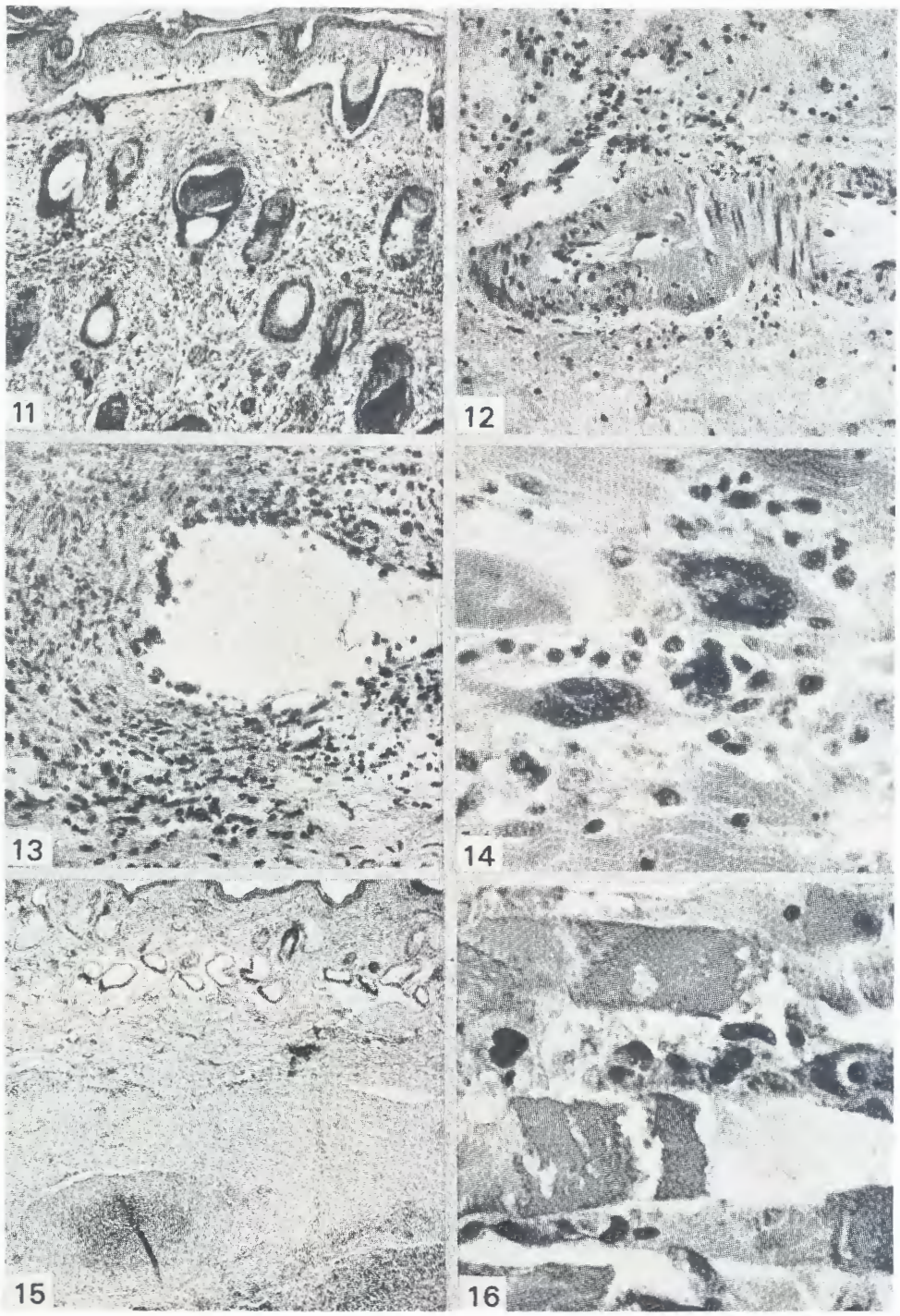

PLATE 3 Photomicrographs from microscopical sections stained HE. 11. Giraffe: Skin showing inflammatory changes, vacuolation and sloughing of the outer epidermal layers. × 75. 12. Giraffe: Acidophilic, coagulative necrosis of an artery in the subcutis. × 192. 13. Giraffe: Advanced necrosis of a vessel in the stratum reticulare. × 192. 14. Giraffe: Two giant cells in the subcutis. × 500. 15. Impala: Skin showing thrombosis in a lymphatic surrounded by a zone of fibroplasia. × 30. 16. Giraffe: Subcutaneous musculature showing typical Zenker's necrosis, fragmentation and myolysis. × 480.

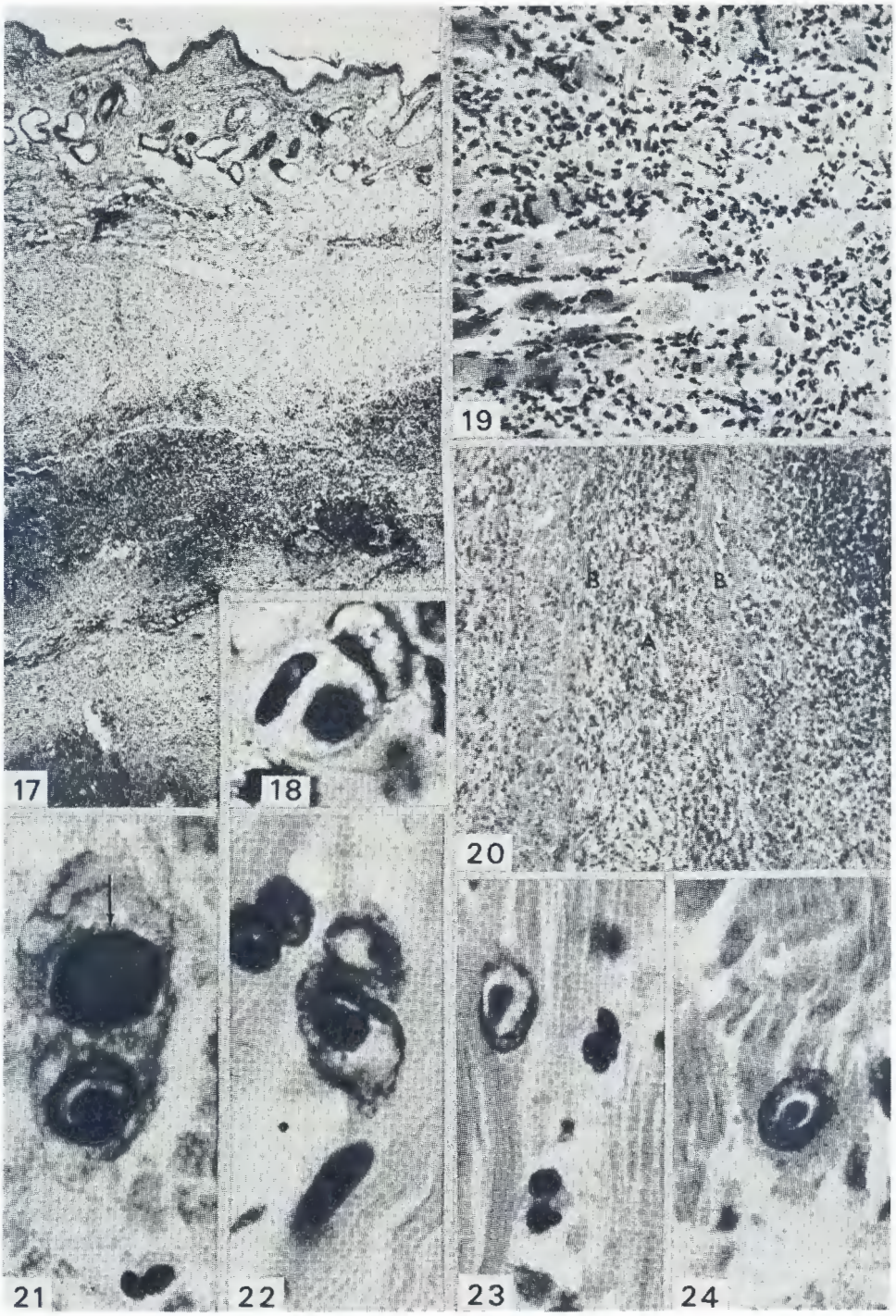

PLATE 4 Photomicrographs from microscopical sections stained HE. 17. Impala: Skin, subcutis and subcutaneous musculature showing marked and advanced reactions and even some calcification. ×30. 18. Impala: One IC inclusion in an epidermal cell. × 1200. 19. Giraffe: Inflammatory changes in the subcutaneous muscles. × 192. 20. Giraffe: Lymph node with inflammatory and necrotic changes in a trabecula (A) and adjacent sinuses (B). × 90. 21. Giraffe: A foamy macrophage with both an IC (arrow) and an IN inclusion. × 1200. 22–24 Giraffe: IN sarcolemmal inclusions in the subcutaneous muscles. × 1200.

fibroplasia. These changes were frequently associated with the distribution of veins and arteries, some of which showed perivascular round cell infiltration, vasculitis and thrombosis. Degenerative or necrotic lesions were found in several veins and lymphatics and a few arteries either in the presence or absence of vasculitis or thrombosis [Plate 3 (12 and 13)]. In the affected parts one or more layers of the vessel wall were involved. The lesions had either progressed from the adventitia to the intima or vice versa and were characterized by acidophilic fibrinoid changes and karyorrhexis.

The subcutaneous muscles were markedly affected by Zenker's necrosis; myolysis and fragmentation [Plate 3 (16)] and frequently surrounded by a marked mononuclear reaction [Plate 4 (19)]. Some of the nerves in the affected areas were also involved in the necrotic process. Foamy macrophages were present regularly. A few multinucleated giant cells were seen in the subcutis [Plate 3 (14)].

The sarcolemmal nuclei in the affected areas were usually hypertrophic and contained one or more small, well circumscribed, homogeneous and mildly eosinophilic or purplish-red inclusions, which eventually appeared to coalesce. These structures were either round or oval, surrounded by a clear halo and stained a deeper purplish-red with HP stain [Plate 4 (22 to 24)]. Similar IN inclusions were also noticeable in some of the interstitial cells, endothelial cells and macrophages in the dermis and subcutis [Plate 4 (21)]. Large purplish-red and brick-red IC inclusions were observed in the same areas in the walls of some of the affected blood vessels, in the endothelium and in the interstitial cells, which apparently included histiocytes and probably also fibroblasts [Plate 4 (21)]. Mature fibrocytes were not involved. The IC inclusions were of various shapes and sizes, but usually more or less rounded and several even exceeded the size of the nucleus. Some of them had a somewhat foamy appearance, or contained a rather inconspicuous darker central mass, and were frequently encircled by a thin layer of more basophilic material. Halos around the inclusions were absent and they gave a deep purplish-red staining reaction with HP. Both IN and IC inclusions were noticed in a few nerves, presumably within walls of the vessels supplying these nerves. Proliferative intra- and extravascular *Besnoitia* organisms and a few young developing cysts were also encountered.

Striated muscle: In the affected areas, the muscles below those of the subcutis were also involved in the reaction. The changes consisted of Zenker's necrosis, fragmentation and myolysis with surrounding oedema, necrosis and mixed cell reaction. IN inclusions were present within some of the sarcolemmal nuclei. Some of the necrotic muscle fibres were mineralized and gave a positive Alizarin reaction for calcium. Both IN and IC inclusions were present in the histiocytes and walls of some of the veins.

Lymph nodes: Advanced necrotic changes in the prescapular lymph node chiefly involved the sinuses, vessels, capsule and trabeculae [Plate 4 (20)]. Most of the sinuses contained fibrinous thrombi or a somewhat amorphous proteinaceous substance admixed with necrotic leucocytes. Some of the veins were also thrombosed. The reaction centres of the lymph nodules, however, were unaffected. A few suspected small IN inclusions were noticed in the trabeculae, but they were not unequivocal. Two other lymph nodes contained many erythrocytes and macrophages in the sinuses, but no necrotic changes were present. One small developing *Besnoitia* cyst was also encountered.

Liver: A few small foci of necrosis and mixed cell infiltrates were seen just beneath Glisson's capsule and some small equivocal IN inclusions were detectable in these areas. The rest of the liver showed mild cloudy swelling and fatty changes as indicated by a positive ORO reaction.

Lung: A purulent bronchopneumonia, associated with interstitial oedema and lymphatic thrombi, and a few possible IN inclusions were noticed. One young developing *Besnoitia* cyst was encountered.

Spleen: The spleen was congested, but the splenic corpuscles were about normal in size. Many plasma cells were noticed in the red pulp.

Oesophagus: Focal necrosis of the epithelium and inflammatory reactions in the submucosa were present. A few suspected small IN inclusions were found in the epithelial cells, but they were not unequivocal.

Kidneys: The changes were very mild and consisted only of a rarefaction of the tubular epithelium and the presence of a proteinaceous substance in the tubules.

Brain: Congestion and mild focal vasculitis were the only noticeable changes.

No lesions were encountered in the myocardium and intestines.

Impala: Skin: Except for being more advanced the lesions resembled those of the giraffe very closely. Partial mural and obliterating hyalinoid thrombi were present in several small, medium and large veins and lymphatics in the subcutis [Plate 3 (15)]. They were accompanied by severe congestion, haemorrhages and marked focal necrosis and calcification in all layers of the skin and subcutis [Plate 4 (17)]. The leucocytic infiltration consisted mainly of mononuclear cells and some neutrophils; the former usually predominated, especially in the perivascular zones. Neutrophils were abundant in one subcutaneous focus. Fibroplasia in the subcutis was also more advanced than in the giraffe. Marked necrosis and calcification were present in the subcutaneous musculature underlying the skin lesions. A few multinucleated giant cells were seen in the subcutis.

A small number of the affected epithelial cells contained fairly large IC or small, rather inconspicuous and questionable IN inclusions [Plate 4 (18)]. These inclusions were more prominent and more frequently present in the histiocytes and probably even in some fibroblasts within the reactive zones. The IC inclusions were more conspicuous than the small number of IN inclusions, which were less frequently found than in the giraffe and absent in the sarcolemmal nuclei. Their general appearance and staining reactions in the two animals were similar.

Prescapular node: A small purulent and necrotic focus surrounded by epithelioid and giant cells was present in the hilus. A few histiocytes contained IC inclusions.

Virological and serological examinations

Re-isolation of virus

The following specimens from animals showing macroscopic lesions were processed for virus isolation in primary lamb kidney monolayers.

Giraffe: Virus isolates were obtained from an excised skin nodule, subcutaneous tissue at the site of inoculation and a mouth lesion. The former two specimens gave rise to cytopathic changes, typical for lumpy skin disease virus, on the 6th day after infection and progressed so that they involved 90 per cent of the cells in the monolayers by the 10th to 13th day. Cytopathic effects with the mouth lesion material were first observed on the 10th day after infection and involved most of the cells by the 17th day. In subsequent passages cytopathic

effects became visible on the 2nd and 3rd days and were complete by the 15th to 18th day.

Stained preparations of infected monolayers of the second passage of all three virus isolates, prepared according to the method described by De Lange (1959), revealed intracytoplasmic inclusion bodies and cellular changes indistinguisable from those produced by lumpy skin disease virus (De Lange, 1959; Prydie & Coackley, 1959).

In a neutralization test using the fourth tissue culture passage material from each of the three virus isolates as antigen, known specific lumpy skin disease antiserum neutralized 1 000 to 10 000 TCID 50 of virus in each case.

A specimen of prescapular lymph node failed to yield virus.

Impala: Lumpy skin disease virus was re-isolated from a skin nodule in the metatarsal region of the leg and a prescapular lymph node. Cytopathic changes appeared on the 3rd day with the skin nodule material and was complete on the 5th day. With the isolation of virus from the prescapular lymph node cytopathic changes were first observed on the 6th day and had involved most of the cells by the 10th day. In subsequent passages cytopathic changes appeared on the 2nd and 3rd days and were complete by the 5th to 9th day.

Stained preparations of infected monolayers of the second passage in both cases revealed changes indistinguishable from those produced by lumpy skin disease virus. The two virus isolates were finally identified in a neutralization test in which a specific antiserum neutralized 1 000 to 10 000 TCID 50 of virus in each case.

Specimens of brain and subcutaneous tissue from the site of inoculation failed to yield virus after repeated attempts at isolation.

Serological tests

Serum virus neutralization tests were performed on serum samples collected from the buffalo and wildebeests prior to experimental infection and again 4 to 6 weeks later. The laboratory prototype Neethling strain of lumpy skin disease virus with a $10^{6.5}$ TCID 50 per ml was used for these tests. The results revealed that none of these animals had significant levels of antibody in their sera either before or after experimental infection.

Serum samples collected from the impala on the 5th and 13th day after infection showed no antibodies to the virus. The animal was subsequently bled on the 26th day, when the first clinical signs of disease were noted, and again on the 33rd day, at the time of death. A significant rise in antibodies was detected in these serum samples in that they both neutralized more then 10 TCID 50 of virus.

Discussion

The present studies proved that both the giraffe and impala are susceptible to experimental infection with the Neethling type virus of lumpy skin disease and that the disease can be fatal in these animals. Virus was re-isolated from lesions in both cases. Although minor variations were encountered, the pathogenesis of the disease in the giraffe and impala closely resembled that in the bovine. The incubation period in the giraffe was within the normal range seen in the bovine but was comparatively long in the impala.

The duration of the disease in cattle is about 14 days (Von Backström, 1945), whereas the impala and giraffe were affected for 6 and 15 days respectively. The first phase of the disease in both species was characterized by the presence of isolated lesions only. At this stage the animals were not obviously sick and such animals could have been passed unnoticed in the veld. Signs of systemic illness only became evident a few days before death, when generalization of the lesions occurred. Death, however, was probably postponed by forced feeding and treatment with antibiotics.

The fact that both the wildebeests and buffaloes failed to react and develop antibodies suggests that they are resistant to infection with this virus. However, the possibility that the buffalo calves had acquired passive colostral immunity before they were artificially infected should be borne in mind. The serological response of the impala is interesting. This animal first developed symptoms after an extended incubation period of 26 days and died on the 33rd day despite the fact that a significant rise in antibodies had already developed at the time.

Most of the lesions in the impala were confined to the metacarpal and metatarsal regions. In the giraffe, on the contrary, the skin lesions developed primarily at the sites of inoculation and most of the macroscopic lesions were present in the oral cavity. In both species severe stomatitis did not cause any salivation. This fact could be a limiting factor to the spreading of such a disease among game, as saliva of cattle has been shown to be infectious in the acute stage of lumpy skin disease (Weiss, 1963).

Thomas & Maré (1945) described the presence of IC inclusions in lumpy skin disease and dealt in some detail with the lesions in the skin and mucous membranes. Proliferation of histiocytic and fibroblastic elements and their primary association with smaller blood vessels were emphasized. Thrombosis of these smaller vessels or a toxic agent, or both, were thought to be responsible for the necrosis. Mention was made of a very marked increase in histiocytic elements in the lymph nodes. Burdin (1959) confirmed most of these lesions in the skin and reported some degenerative and necrotic nuclear changes in inclusion-bearing cells and vacuolation of epithelial cells. De Lange (1959) described in detail the development of IC inclusions in lamb kidney cells inoculated with Neethling virus and IN inclusions in cells inoculated with Allerton virus. The histopathological lesions in the giraffe and impala resembled those described by these authors in cattle very closely. Some additional changes and variations, however, were evident. The involvement of the circulatory system — particularly, but not exclusively, the smaller vessels, and mainly the veins and lymphatics — appeared to be of primary significance. Some arteries, however, were also affected. The walls of some vessels showed either partial or complete necrosis; others were inflamed and thrombosed. In some of the regional lymph nodes the necrotic lesions were clearly confined to the trabeculae and adjoining sinuses, indicating the involvement of both blood vessels and lymph beds. It is evident that such lesions would offer an explanation for the marked swelling of certain limbs which is occasionally encountered in cattle. These vascular changes are expected to be of paramount significance in the development of the typical disseminated, necrotic lesions in the skin, mucous membranes and elsewhere. Intranuclear inclusions, particularly in the sarcolemmal and histiocytic nuclei, as well as both IN and IC inclusions in some endothelial cells, have hitherto not been described in cattle. The IN inclusions appeared to develop from the nucleoli. They were absent in the sarcolemmal cells of the impala. The IC inclusions were more frequent in the histiocytes

than in the epidermal cells. The staining reaction of the inclusions with HP was more intense than with HE and was in accordance with the findings of De Lange (1959). Necrosis of the subcutaneous muscles beneath the skin lesions was marked in both animals. The disease in the giraffe was unfortunately complicated with mild besnoitiosis (Van Niekerk, McCully & Basson, 1969, unpublished observations). *Besnoitia* has also been shown to cause both vascular lesions and mononuclear reactions (Basson, McCully & Bigalke, 1970). These lesions evidently augmented those of lumpy skin disease in the giraffe.

Lumpy skin disease is known to occur in cattle on the farms adjoining the Kruger National Park. Weiss (1963) has stated that this disease is contagious and possibly transmissible by insects and therefore it can be assumed that the disease is also present in the susceptible game species of the Park. However, as diseased animals are frequently killed by predators, the detection of such clinical cases in nature is merely fortuitous. The proper evaluation of the effect of lumpy skin disease on the game population of the Kruger National Park is therefore impossible at this stage and must await further observations. Furthermore, as other diseases — particularly those affecting the skin and mucous membranes such as foot-and-mouth disease, filariasis and demodicidosis — could very easily be confused with lumpy skin disease, a diagnosis should always be confirmed by histopathological and virological examinations.

Summary

Calves of a giraffe, an impala and two buffaloes and two adult black wildebeests were artificially infected with the Neethling type of lumpy skin disease virus. The giraffe and impala died after manifesting typical symptoms and lesions of the disease. Virological examinations confirmed the presence of lumpy skin disease virus in lesions of these animals. Histopathological studies revealed microscopic lesions typical of those reported in cattle suffering from lumpy skin disease. Both IC and IN inclusions were noticed in various cell types and some additional histopathological changes are reported. Neither the wildebeests nor the buffaloes reacted clinically to artificial infection, and serum samples from these animals did not reveal a subsequent rise in antibody titre.

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References

Alexander, R. A., Plowright, W. & Haig, D. A., 1957. Cytopathogenic agents associated with Lumpy skin disease of cattle. *Bull. epizoot. Dis. Afr.,* 5, 489–492.

Basson, P. A., McCully, R. M. & Bigalke, R. D., 1970. Observations on the pathogenesis of bovine and antelope strains of *Besnoitia besnoiti* (Marotel, 1912) infection in cattle and rabbits. *Onderstepoort J. vet. Res.*, 37 (2), 105-106.

Burdin, M. L., 1959. The use of histopathological examinations of skin material for the diagnosis of lumpy skin disease in Kenya. *Bull. epizoot. Dis. Afr.,* 7, 27–36.

Capstick, P. B., 1959. Lumpy skin disease — experimental infection. *Bull. epizoot. Dis. Afr.,* 7, 51–62.

De Lange, M., 1959. The histology of the cytopathogenic changes produced in monolayer epithelial cultures by viruses associated with lumpy skin disease. *Onderstepoort J. vet. Res.,* 28, 245–255.

Lillie, R. D., 1954. Histopathologic technic and practical histochemistry. 2nd ed. New York and Toronto: The Blakiston Company, Inc.

Prydie, J. & Coackley, W., 1959. Lumpy skin disease — tissue culture studies. *Bull. epizoot. Dis. Afr.,* 7, 37–50.

Thomas, A. D. & Mare, C. Van E., 1945. Knopvelsiekte. *Jl S. Afr. vet. med. Ass.,* 16, 36–43.

Von Backström, U., 1945. Ngamiland cattle disease: preliminary report on a new disease, the aetiological agent being probably of an infectious nature. *Jl S. Afr. vet. med. Ass.,* 16, 29–35.

Weiss, K. E., 1963. Lumpy skin disease. Emerging diseases of animals. FAO Agricultural Studies, No 61, 179–201. F.A.O. Rome, 1963.

Weiss, K. E., 1968. Lumpy skin disease virus. Monographs in Virology 3, 111–131. Springer-Verlag, Wien-New York, 1968

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