A Necrotizing Pneumonia in Lambs Caused by Pseudorabies Virus (Aujesky's Disease Virus)

Stephen P. Schmidt, Eugene C. Pirtle, Wayne A. Hagemoser and Dennis P. Wages*

ABSTRACT

An outbreak of pseudorabies occurred in sheep housed with swine in the same building. Although the sheep and swine were not in physical contact, the lambs and ewes were exposed to air from the sows' section. Three dead lambs were submitted to the Iowa State University Veterinary Diagnostic Laboratory for necropsy.

Grossly there were pulmonary congestion and multifocal pulmonary hemorrhages. Microscopic lesions were severe acute multifocal necrotizing bronchopneumonia with necrotizing vasculitis and intranuclear inclusion bodies within the neurons of the parabronchial ganglia.

Bacterial cultures were negative for pathogenic agents; pseudorabies virus was isolated from ovine brain tissue. Viral antigen was demonstrated in the neurons of the parabronchial ganglia by immunoperoxidase staining. Electron microscopy revealed nucleocapsids in the parabronchial ganglionic neurons which contained basophilic intranuclear inclusion bodies. Viral DNA prepared from the ovine pseudorabies virus isolate was found by restriction endonuclease analysis to be related to the Indiana Funkhauser strain of pseudorabies virus.

Key words: Aujeszky's disease virus, pseudorabies, sheep, pneumonia.

RÉSUMÉ

La pseudo-rage a sévi chez des moutons qu'un éleveur gardait avec des porcs, dans une étable remodelée. Même si les premiers n'étaient pas en contact physique avec les seconds, les agneaux et les brebis respiraient l'air qui provenait de la section réservée aux truies. Le cultivateur soumit trois agneaux morts au laboratoire de diagnostic vétérinaire de l'université de l'Iowa, afin qu'on en effectue la nécropsie.

Les lésions macroscopiques affectaient les poumons et elles se traduisaient par de la congestion et de multiples foyers hémorragiques. L'histopathologie révéla plusieurs foyers de broncho-pneumonie nécrotique aiguë, qui s'accompagnaient de vasculite nécrotique et d'inclusions intranucléaires dans les neurones des ganglions parabronchiques.

L'examen bactériologique des poumons de ces agneaux donna des résultats négatifs, mais on isola de leur cerveau le virus de la pseudo-rage. La coloration à l'immunoperoxydase permit aussi de le démontrer, dans les neurones des ganglions parabronchiques. La microscopie électronique révéla la présence de nucléocapsides, dans les neurones des ganglions parabronchiques qui arboraient des inclusions intranucléaires basophiles. L'analyse de l'endonucléase de restriction révéla que l'acide désoxyribonucléique du virus en cause affichait une relation avec celui de la souche du virus de la pseudo-rage Funkhauser de l'Indiana.

Mots clés: virus de la maladie d'Aujeszky, pseudo-rage, moutons, pneumonie.

INTRODUCTION

Pseudorabies (Aujesky's disease), a disease of worldwide distribution affecting swine and other domestic animals, has been increasing in incidence and virulence in the midwestern United States (1,2).

Since the discovery of pseudorabies (Pr) by Aujeszky in 1902 (3), relatively few papers have described spontaneous outbreaks in sheep. The earliest reports were from Eastern Europe in 1912 (4), 1927 (5) and 1933 (6), followed by England in 1938 (7) and Brazil in 1939 (8). Kojnok observed in 1962 that swine were the source of pseudorabies virus (PrV) for ruminant infection (9). This finding was substantiated by other authors' reports of natural Pr outbreaks in sheep during the 1960s until the present (10-16).

A review of the literature disclosed no descriptions of necrotizing pneumonia or necrotizing pulmonary vasculitis in sheep (4-20) and only one report of necrosis in the parabronchial ganglia (17). The objective of this study was to characterize the pneumonic lesions in an outbreak of Pr in

*Department of Veterinary Pathology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803 (Schmidt), Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011 (Hagemoser), National Animal Disease Center, Ames, Iowa 50010 (Pirtle) and Department Food Animal and Equine Medicine, School of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606 (Wages).

Reprint requests to Dr. W.A. Hagemoser.

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young lambs which were presumed to have been infected by aerosolized PrV from farrowing sows.

MATERIALS AND METHODS

ORIGIN OF OVINE TISSUES

Three dead two to three week old lambs were submitted for necropsy to the Iowa State University Veterinary Diagnostic Laboratory. The lambs were from a flock of 35 ewes and 48 lambs.

A remodeled barn housed the animals (Fig. 1). The larger end of the building was subdivided into two units, one for farrowing sows (vaccinated against Pr with a modified live vaccine [Pr-Vac, Norden Laboratories, Inc., Lincoln, Nebraska]) and the other for lambing ewes. A solid plank wall, built from the floor to the bottom edge of the ceiling joists, separated the two areas. This construction permitted a 20 cm open space between the top of the wall and the haymow floor boards. A ventilation fan, constructed high in the wall, blew air from the farrowing to the lambing side. In the room adjacent to the lambing unit were penned ewes with older lambs. The next room contained market weight swine. The walls between these areas were as previously described, and the rooms were open at one end.

Seven ewes, 32 lambs (2-3 weeks old), and one cat died during the outbreak. The first Pr cases occurred among the ewes and older lambs while the remaining cases were in the neonatal lambs and lambing ewes. The initial losses were in the lambs followed by deaths of ewes later in the outbreak. The clinical signs beginning with muscle spasms and ending with pruritus, had a sudden onset. The lambs died within 12 hours after clinical signs began. Pruritus was common in the lambs but was evident in only one ewe.

HISTOTECHNIQUE

Representative tissues were harvested at necropsy for histological examination. Portions of cerebrum, cerebellum, brainstem (diencephalon, mesencephalon, metencephalon and medulla oblongata), lung, heart, liver and kidney were fixed in 10% neutral buffered formalin. Tissues were processed by routine paraffin techniques, sectioned at 6 μ and stained with hematoxylin and eosin (H & E) by the Harris method (21).

VIRUS ISOLATION

Ovine brain tissue was processed for the isolation and identification of pseudorabies virus (22).

RESTRICTION ENDONUCLEASE ANALYSIS

Virus isolated from the outbreak (VDL 82P2294) along with Norden vaccine virus (PR-Vac, Norden Laboratories, Inc., Lincoln, Nebraska) and Indiana Funkhauser (Ind[FH] strain of PrV were subjected to restriction endonuclease (RE) analysis utilizing RE enzymes (BAM HI, Sal I, and Hinf I, Bethesda Research Laboratories, Gaithersburg, Maryland) as described in the literature (23,24).

IMMUNOPEROXIDASE STAINING

In an attempt to localize and prove that viral antigen was present in the lungs and brainstem of the submitted lambs, an immunoperoxidase staining procedure (Vectastain™ ABC Kit, Vector Laboratories, Inc., Burlingame, California) was performed on formalin fixed paraffin embedded tissues. Tissue sections, cut at 6 μ, were mounted on glass slides and incubated for 12 hours at 60° C in dry heat. The sections were deparaffinized and immunoperoxidase stained as outlined by the Vectastain™

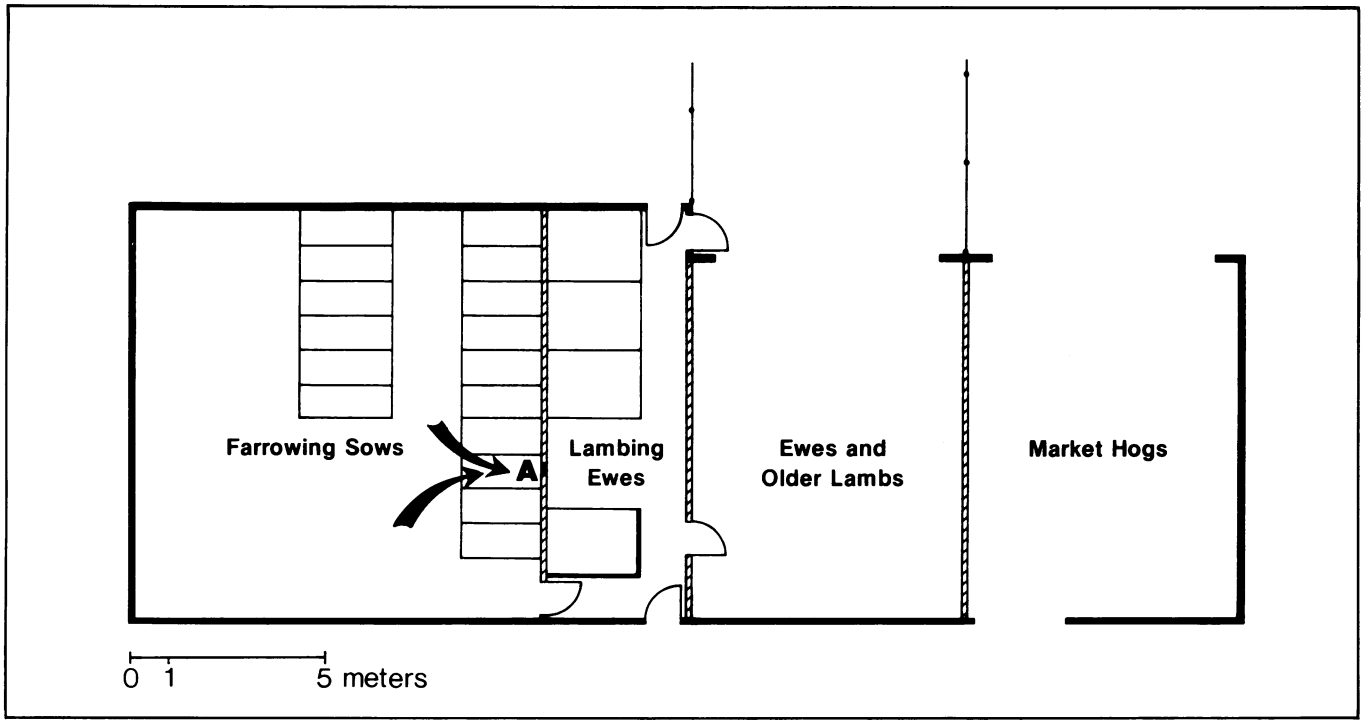

Fig. 1. Portion of the converted barn subdivided into areas for farrowing sows, lambing ewes, ewes and older lambs, and market hogs. The black walls represent outer walls while inner walls, marked with oblique lines, partitioned the barn. A ventilation fan (A) was located between the compartments for farrowing sows and lambing ewes. The arrows indicate the air flow from the farrowing to the lambing unit.

protocol. Rabbit antipseudorabies serum was the primary antiserum and Harris hematoxylin was the counterstain.

ELECTRON MICROSCOPY

Selected sections from the H & E slides were processed for the electron microscopy to demonstrate viral particles in the neurons of the parabronchial ganglia (25).

RESULTS

GROSS AND MICROSCOPIC FINDINGS

Necropsy disclosed diffuse pulmonary congestion and multifocal pulmonary hemorrhages.

Histological examination of the pulmonary parenchyma revealed severe generalized edema, hemorrhage and congestion. A few of the pulmonary vessels, bronchioles and adjacent parenchyma contained multifocal areas of necrosis (Fig. 2). These necrotic foci were characterized by the loss of tissue cytoarchitecture and contained mixed leukocyte infiltrates of neutrophils, macrophages and lymphocytes (Fig. 3). In some foci, a few degenerate and unidentifiable cells contained eosinophilic intranuclear inclusion bodies. Within the parabronchial ganglia were degenerate neurons with eosinophilic inclusion bodies (Fig. 4). In other ganglionic neurons, the nuclei were filled with a basophilic inclusion body encircled by fragmented and particulate chromatin at the nuclear membrane.

The brainstem, slightly caudal to the cerebellar peduncles, contained degenerate neurons with basophilic intranuclear inclusion bodies. These neurons were medial and dorsal to the facial nucleus. A mild lymphocytic perivascular infiltrate involved some of the neuroparenchymal vessels. No microscopic lesions were observed in the cerebral cortex or in the olfactory bulbs.

VIRUS ISOLATION

Pseudorabies was confirmed by PrV isolation from emulsified ovine brain tissue.

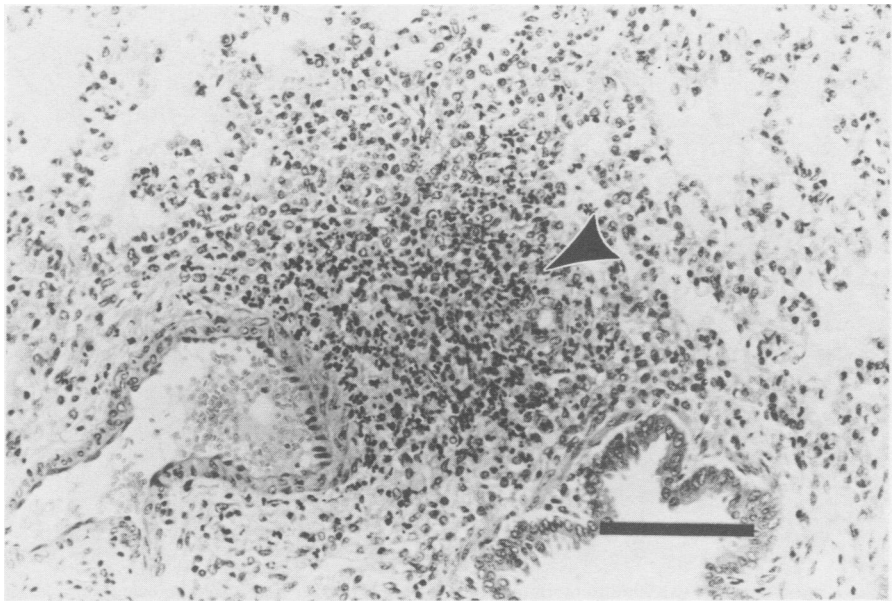

Fig. 2. Lung from lamb. Note the area of pneumonia peripheral to a bronchiole (arrow). H & E. Bar=125 μ.

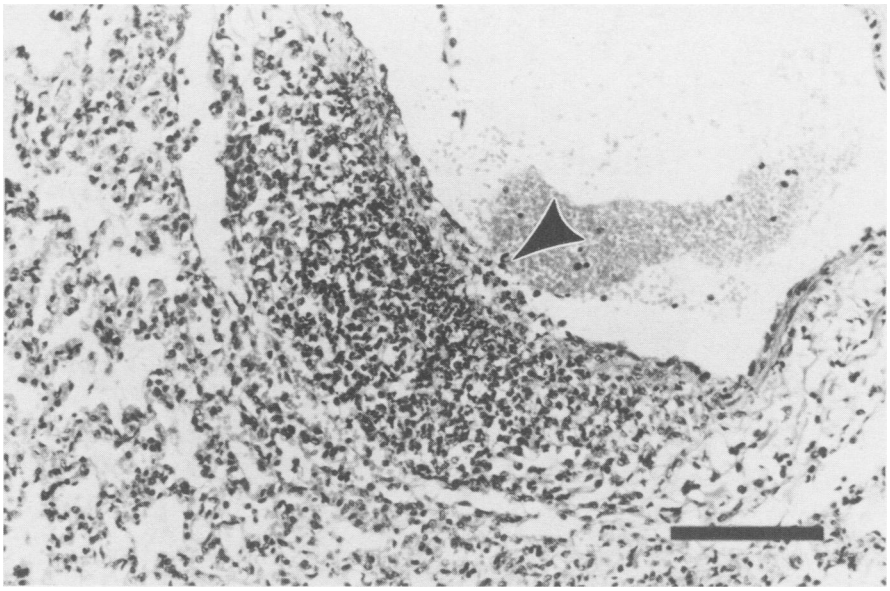

Fig. 3. Vessel wall in pulmonary parenchyma. Note the focal vasculitis obliterating the cytoarchitecture of the vessel wall (arrow). H & E. Bar=86 μ.

RESTRICTION ENDONUCLEASE BANDING PATTERNS

The VDL 82P2294 ovine isolate and Ind(Fh) strain had relatively similar banding patterns with all three restriction enzymes (Fig. 5). The banding patterns of the Norden vaccine strain were different from both VDL 82P2294 and Ind(Fh) strains. In the Bam HI pattern (Lane B), there was an area of heterogeneity between 5.0 and 6.0 megadaltons. In the Sal I pattern (Lane B), there were numerous differences in numbers and migration of bands. In the Hinf I pattern, the Norden PrV DNA had no 4.2 megadalton band, differentiating Norden from the VDL 82P2294 and Ind(Fh) strains.

IMMUNOPEROXIDASE STAINING

Viral antigen positive neurons were in the transverse tissue sections from the medulla oblongata slightly caudal

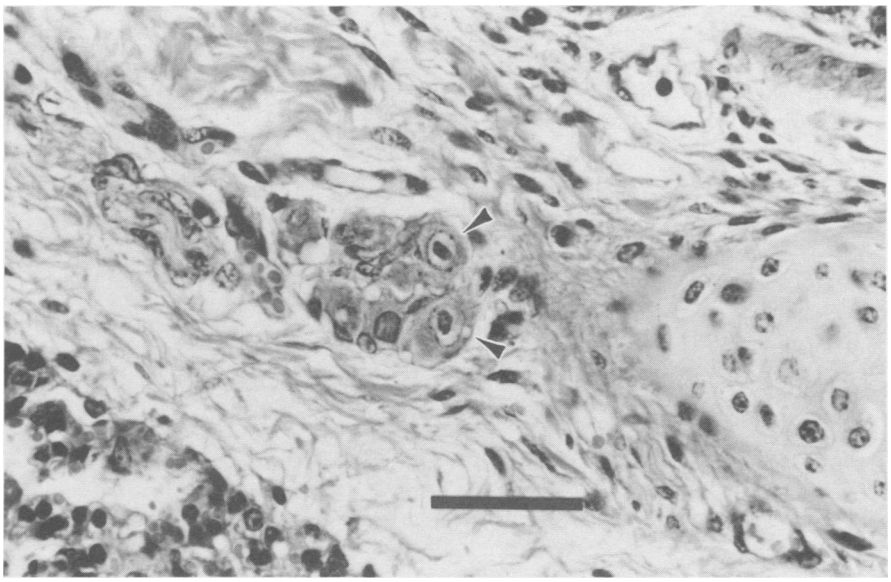

Fig. 4. Parabronchial ganglion in lamb lung. Note the degenerate neurons and eosinophilic intranuclear inclusion bodies (arrows). H & E. Bar=50 μ.

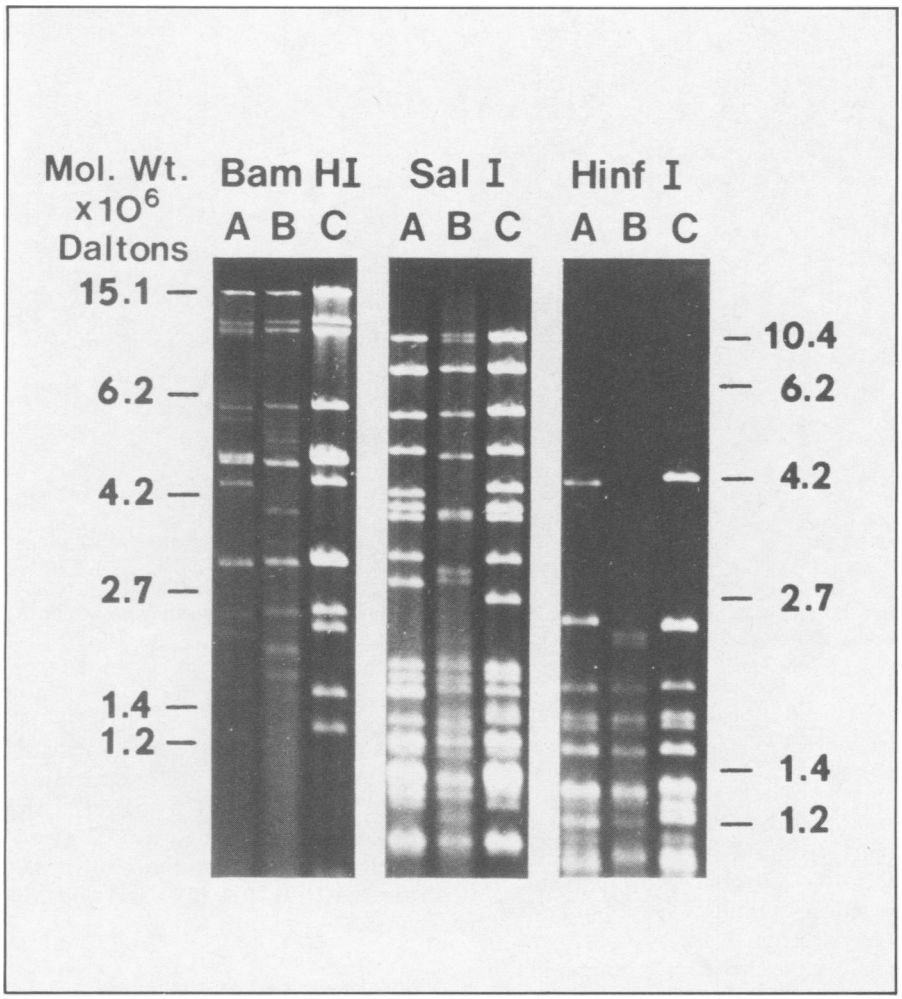

Fig. 5. Electrophoretic banding patterns of Pr viral DNA, VDL virus (Lane A). Norden vaccine virus (Lane B), and Ind(FH) (Lane C). The molecular weights on the left apply to the BAM HI restriction enzyme banding patterns, and the molecular weights on the right apply to Sal I and Hinf I banding patterns.

to the cerebellar peduncles. The affected neurons tended to be located medial and dorsal to the facial nucleus in the nucleus reticularis gigantocellularis.

ELECTRON MICROSCOPY

Nonenveloped nucleocapsids were demonstrated within the nuclei of the neurons in the parabronchial ganglia (Fig. 6). The virus particles were in the nuclei which contained granular pale basophilic intranuclear inclusion bodies in the H & E stained sections.

DISCUSSION

The history, clinical signs, macroscopic and histological lesions, virus distribution, and RE analysis of viral DNA have been described for an outbreak of Pr in ewes and two to three week old lambs. Although PrV isolations and serology were not attempted from the swine, the close association between the sheep and the pigs suggested the possible shedding of virulent aerosolized PrV (26) from vaccinated sows with latent or subclinical PrV infections (27-29). The results of RE analysis clearly indicate that the ovine PrV isolate was related not to a vaccine strain of PrV, but to the virulent Ind(Fh) strain.

The necrotizing pneumonia and the presence of viral antigen and nucleocapsids in the parabronchial ganglionic neurons are evidence for the aerogenous spread of PrV in this outbreak. With this type of viral transmission, ventilation and airflow patterns should be considered when Pr is suspected in swine or other farm animals. The specific involvement of the lungs warrants submission of this tissue for histological and virological examination when aerogenous spread of Pr is suspected.

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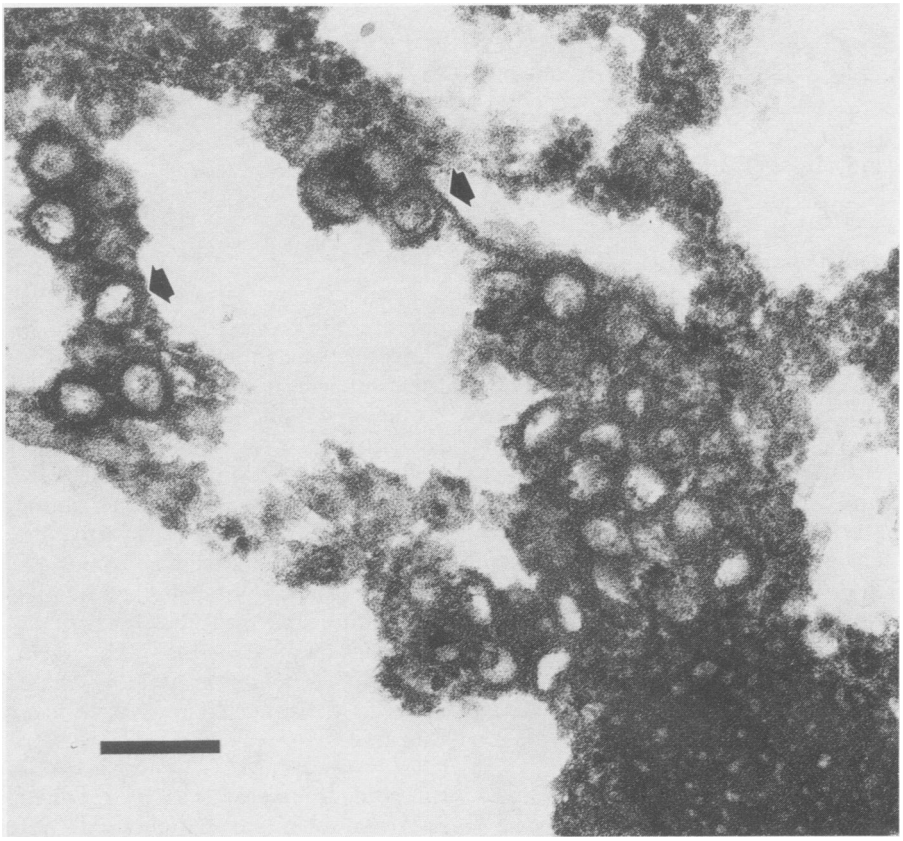

Fig. 6. Parabronchial ganglia from lamb. Electron micrograph from an H & E stained section. Note the nucleocapsids near the nuclear membrane (arrows). Bar=0.2 μm.

REFERENCES

1. **GLOCK RD, HILL HT.** Aujesky's disease: Clinical significance and control. US Anim Health Assoc Annu Meet 1976; 80:414-417.
2. **HAGEMOSER WA, KLUGE JP, HILL HT.** Studies on the pathogenesis of pseudorabies in domestic cats following oral inoculation. Can J Comp Med 1980; 44:192-202.
3. **AUJESZKY A.** Über eine neue Infektionskrankheit bei Haustieren. Zentralbl Bakteriol Parasitenkd Abt I 1902; 32:353-357.
4. **KOLONITS B.** Paralysis bulbaris infectiosa acuta kutyan juhon es szarvasmarhakon. Allator Lapok 1912; 35:615-617.
5. **SZILÁRD J.** Fertözö nyúltvelöbên ulás. Allator Lapok 1927; 50:123-124.
6. **MARCIS A.** Juhok tömeges megbetegedése fertözö nyúltvelöbénuláshan. Allator Lapok 1933; 61:193-195.
7. **STEWART SG.** A suspected case of Aujeszky's disease. Vet Rec 1938; 50:829-830.
8. **CARNEIRO V, LEME O.** Sôbre a doenca de Aujeszky nos Ovinos e Caprinos. Revista da Sociedade Paulista de Medicina Veterinaria 1939; 5:160-164.
9. **KOJNOK J.** The role of pigs in the spreading of Aujeszky's disease among cattle and sheep. Acta Vet Sci Hung 1962; 12:53-58.
10. **BECKER CH.** Die Aujeszkysche Krankheit in deutschen Schweinebeständen. Monatshefte Veterinaermed 1961; 16:88-96.
11. **BOGDAN E.** Adatok a nagyüzemben tartott juhok Aujeskyféle járványanak leküzdéséröl. Magy Allatorv Lap 1961; 16:72.
12. **BERECZ L.** Aujeszky-féle betegség juhok között, és a járvány megállitása. Magy Allatorv Lap 1961; 16:73.
13. **SENF W, SEFFNER W.** Erfahrungen bei der Aujeszkyschen Krankheit unter besonderer Berücksichtigung einiger Fälle bei Schafen und Rindern. Monatshefte Veterinaermed 1966; 21:58-64.
14. **SCHÄFER M, GUDAT E.** Beitrag zum Auftreten von Aujeszkyscher Krankheit beim Schaf. Monatshefte Veterinaermed 1966; 21:201-203.
15. **THAWLEY DG, WRIGHT JC, SOLORZANO RF.** Epidemiologic monitoring following an episode of pseudorabies involving swine, sheep, and cattle. J Am Vet Med Assoc 1980; 176:1001-1004.
16. **JANOVITZ EB.** Pseudorabies in sheep. Proc North Central Confr Vet Lab Diagnost (Sioux Falls, South Dakota) 1984:21.
17. **BECKER CH.** Zur primären Schädigung vegetativer Ganglien nach Infektion mit dem *Herpes suis* Virus bei verschiedenen Tierarten. Experientia 1967; 23:209-210.
18. **DOW C, McFERRAN JB.** Experimental Aujeszky's disease in the sheep. Am J Vet Res 1964; 25:461-468.
19. **DOW C, McFERRAN JB.** Experimental studies on Aujeszky's disease in sheep. Br Vet J 1966; 122:464-470.
20. **McFERRAN JB, DOW C.** The distribution of the virus of Aujeszky's disease in experimentally infected sheep. Res Vet Sci 1964; 5:143-148.
21. **LEE LG.** Manual of the histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. New York; McGraw-Hill, 1968.
22. **HILL HT, CRANDELL RA, KANITZ CHL, McADARAGH JP, SEAWRIGHT GL, SOLORZANO RF, STEWART WC.** Recommended minimum standards for diagnosis of pseudorabies (Aujeszky's disease). Proc Am Assoc Vet Lab Diagnost 1977; 20:375-390.
23. **PIRTLE EC, WATHEN MW, PAUL PS, MENGELING WL, SACKS JM.** Evaluation of field isolates of pseudorabies (Aujeszky's disease) virus as determined by restriction endonuclease analysis and hybridization. Am J Vet Res 1984; 45:1906-1912.
24. **PAUL PS, MENGELING WL, PIRTLE EC.** Differentiation of pseudorabies (Aujeszky's disease) virus strains by restriction endonuclease analysis. Arch Virol 1982; 73:193-198.
25. **HALVORSEN JA.** Improvements in two techniques for rapid diagnostic electron microscopy. J Histotechnol 1978; 5:152-156.
26. **DONALDSON AI, WARDLEY RC, MARTIN S, FERRIS NP.** Experimental Aujeszky's disease in pigs: Excretion, survival and transmission of the virus. Vet Rec 1983; 113:490-494.
27. **DAVIES EB, BERAN GW.** Spontaneous shedding of pseudorabies virus from a clinically recovered postparturient sow. J Am Vet Med Assoc 1980; 176:1345-1347.
28. **GUTEKUNST DE, PIRTLE EC, MILLER LD, STEWART WC.** Isolation of pseudorabies virus from trigeminal ganglia of a latently infected sow. Am J Vet Res 1980; 41:1315-1316.
29. **MOCK RE, CRANDELL RA, MESFIN GM.** Induced latency in pseudorabies vaccinated pigs. Can J Comp Med 1981; 45:56-59.