Pathogenesis of Ovine Pseudorabies (Aujeszky's Disease) Following Intratracheal Inoculation

Stephen P. Schmidt, Wayne A. Hagemoser, John P. Kluge and Howard T. Hill*

ABSTRACT

Pseudorabies virus was inoculated intratracheally into sheep to investigate the pathogenesis of pseudorabies virus infection.

Clinical signs of pyrexia, depression, frequent swallowing, facial fasciculations, chorea, excessive salivation, mild tympanites, labored breathing and focal pruritus were followed by death Macroscopic lesions were severe focal facial trauma, petechiae in cervicothoracic ganglia and dilated esophaguses. The medulla oblongata and the trigeminal, cranial cervical, cervicothoracic and parabronchial ganglia contained pseudorabies virus and pronounced nonsuppurative inflammatory changes.

The neural distribution of lesions and virus suggests that the virus travelled from the respiratory mucosa to the central and sympathetic nervous system by two routes: 1) in the vagus and glossopharyngeal nerves to the medulla oblongata and 2) in the postganglionic fibers to the sympathetic ganglia. The presence of virus in the nasal mucus indicated that horizontal transmission of pseudorabies virus may occur among sheep.

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

RÉSUMÉ

Cette expérience consistait à injecter le virus de la pseudo-rage dans la trachée de moutons, afin d'étudier la pathogénèse de l'infection par ce virus.

Ils manifestèrent les signes cliniques suivants: pyrexie, dépression, ingurgitation fréquente, fasciculations faciales, chorée, salivation excessive, léger tympanisme, respiration laborieuse et prurit focal, avant de mourir. Les lésions macroscopiques incluaient de sévères traumas faciaux, des pétéchies dans les ganglions cervico-thoraciques et une dilatation de l'oesophage. Les lésions microscopiques se traduisaient par une inflammation non suppurante du bulbe rachidien et des ganglions trigéminaux, cervicaux crâniaux, cervicothoraciques et parabronchiques; tous ces tissus contenaient aussi du virus.

La distribution neurale des lésions et du virus font penser qu'il voyagea de la muqueuse respiratoire jusqu'au système nerveux central et sympathique, de deux façons: 1) par les nerfs vague et glosso-pharyngien, jusqu'au bulbe rachidien, et 2) par les fibres postganglionnaires, jusqu'aux ganglions sympathiques. La virulence du mucus nasal indiqua la possibilité de la transmission horizontale du virus de la pseudo-rage, chez les moutons.

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

INTRODUCTION

In 1902, Aujeszky, described pseudorabies (Pr) in a cat, a dog and an ox (1). The first reports of Pr in sheep are from experimental work in 1910 (2) and 1911 (3). There are no reports of natural Pr in sheep prior to 1912, but three outbreaks occurred in sheep flocks from 1912 to 1933 (4-6). Kojnok's observations of outbreaks in cattle and sheep led to recognition that swine could be a source of pseudorabies virus (PrV) for sheep (7). Through the 1960s, several published accounts from eastern Europe recorded large losses of sheep from pseudorabies (8-12). The articles described clinical signs and macroscopic and microscopic lesions. In 1964, Dow and McFerran reported the characteristics and distribution of lesions and the distribution of virus in PrV infected sheep (13, 14). They also proposed the pathogenesis of ovine Pr from viral exposure by several different routes.

Researchers have suggested that PrV carrier rats (6) and virus contaminated fomites (15), urine (9,10), swine pen effluents (12), swine secretions (7), and direct contact with pigs (7,16) could infect sheep. Sheep have contracted Pr after experimental PrV inoculation by scarification, subcutaneous injection, intranasal and oral administration and intraconjunctival instillation (13,14). Air can be a vector

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for the transmission of pathogenic and nonpathogenic viruses (17), and the airborne spread of aerosolized PrV among swine on the same and different premises has been documented by Donaldson *et al* (18) and Gloster *et al* (19). Air could be a suitable vector for transmitting PrV to nonporcine hosts.

The purpose of this research was to determine the pathogenesis of PrV in sheep exposed intratracheally to aerosolized virus.

MATERIALS AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN

Thirty-two male or female Suffolk and Suffolk-cross domestic sheep approximately five to six months old were used in the project. The sheep were randomly assigned to eight groups of four animals each. There were six PrV inoculated groups and two control groups. For inoculation, a group of four animals was exposed at the same time intratracheally to a virulent field strain of pseudorabies virus. One animal was killed at 24, 48, 72 and, if it survived long enough, 96 hours postinoculation (HPI). Control animals received sterile Earle's balanced salt solution intratracheally and were killed 24, 48, 72 or 96 hours postinoculation. Before inoculation with PrV, the animals were tested for Pr antibodies using the microtitration serum neutralization test (20) and found to be negative.

VIRUS INOCULUM

A field strain of PrV isolated from lambs provided the seed virus for this study. The isolate was plaque purified and propagated in a monolayer of 48 hour old porcine kidney cells. The virus was dispensed in 1 mL aliquots containing 1.0 x 10^7 plaque forming units (PFU) and frozen at -70°C.

VIRUS ISOLATION FROM TISSUE

Tissue samples for virus isolation (VI) were harvested at necropsy and stored at -70°C until processed (Table I). The tissues were emulsified, then processed for PrV isolation and identification on Madin-Darby bovine kidney cell monolayers (20).

TABLE I. Summary of Tissue Viral Isolations, Fluorescent Antibody Staining and Lesions for PrV Inoculated Sheep

Tissue	Hours Postinoculation											
	24			48			72			86-96		
	VI[a]	FA[b]	H[c]	VI	FA	H	VI	FA	H	VI	FA	H
Nasal turbinate	0/6[d]	0/4	0/6	0/6	0/4	0/6	0/6	0/4	0/6	0/6	0/3	0/6
Tonsil	0/6	0/4	0/6	0/6	0/4	0/6	0/6	0/4	0/6	0/6	0/3	0/6
Retropharyngeal lymph node	0/6	N[e]	0/6	0/6	N	0/6	0/6	N	1/6	1/6	N	3/6
Lung	2/6	0/5	0/6	2/6	0/4	0/6	0/6	0/4	0/6	1/6	1/3	1/6
Bronchial lymph node	0/6	0/5	0/6	1/6	0/6	0/6	2/6	0/5	0/6	3/6	0/6	3/6
Adrenal gland	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
Trigeminal ganglia	1/6	0/5	0/6	0/6	0/6	1/6	1/6	2/6	2/6	2/6	3/6	2/6
Cranial cervical ganglia	0/6	0/6	0/6	0/6	0/6	2/6	1/6	4/6	4/6	4/6	6/6	4/6
Cervicothoracic ganglia	0/6	0/6	0/6	0/6	0/5	1/6	1/6	3/6	4/6	3/6	4/5	3/6
Olfactory bulbs	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Cerebral cortex	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Basal ganglia	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Mesencephalon	0/6	0/6	0/6	0/6	0/6	0/6	2/6	1/6	1/6	2/6	3/6	3/6
Metencephalon	0/6	N	0/5	0/6	N	0/5	2/6	N	1/4	2/6	N	4/5
Medulla oblongata	0/6	0/6	0/6	0/6	0/6	0/6	2/6	4/6	4/6	5/6	6/6	6/6
Cervical spinal cord	0/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6	2/6	1/6	0/6
Thoracic spinal cord	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	3/6	1/6	0/6
Lumbar spinal cord	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/6

[a]Virus isolation

[b]Direct fluorescent antibody

[c]Histological lesions

[d]Number positive/Number examined

[e]Not done

VIRUS ISOLATION FROM NASAL SECRETIONS, CEREBROSPINAL FLUID AND URINE

Nasal mucus was collected one day prior to PrV inoculation and at 24 hour intervals beginning at 24 HPI and continuing until the animals were euthanized. Cerebrospinal fluid (CSF) and urine were harvested only from animals sacrificed between 86-96 hours postinoculation. The nasal swabs, urine, and CSF were stored at -70°C until processed.

VIRUS INOCULATION

Sheep were injected with an intravenous dose of Rompun (Bayvet, Cutter Laboratories, Inc., Shawnee, Kansas; 0.05 mg per kg body weight) followed one to two minutes later by an intravenous dose of Ketaset (Bristol Laboratories, Syracuse, New York; 2-5 mg per kg body weight). When recumbent, the animals were intubated with a 9 mm inside diameter Lo-Pro cuffed tracheal tube (National Center Company, Argyle, New York). A Stephen Slater Valve (Anesthesia Associates, Inc., San Marcos, California) was attached to the tracheal tube; affixed to the input port of the valve was a Pelco all-glass nebulizer (Ted Pella, Inc., Tustin, California). Two mL of either Earle's balanced salt solution or tissue culture fluid containing PrV at 1.0 x 10^7 PFU per mL was nebulized five to seven minutes by an 8 L per minute airflow at 1 atmosphere pressure generated by a Gast portable pressure/vacuum pump (Fisher Scientific, Itasca, Illinois).

CLINICAL SIGNS

Following inoculation, animals were observed for clinical signs twice daily. Rectal temperatures were recorded from all animals at 12 hour intervals and analyzed using a repeated measures split-plot analysis of variance (21). When significant effects were observed, individual differences between means were tested for statistical significance using Tukey's Honestly Significant Difference Test (22). The harmonic mean was used for all mean comparisons involving an unequal number of observations. In all analyses, statistical significance at the ($q<0.05$) level was accepted.

NECROPSY

The animals were painlessly killed at 24 hour intervals; however, three animals were killed when death was imminent at 86-96 hours postinoculation. Representative tissues were harvested, and macroscopic lesions were recorded. The brain was bisected along the longitudinal fissure. One half was fixed in 10% neutral buffered formalin. The other half was subdivided for VI or direct fluorescent antibody (DFA) examination. The spinal cord was divided into the cervical, thoracic and lumbar regions. Each segment was further subdivided into four equal pieces of tissue. The first and third pieces were formalin fixed for histological study while the second and fourth were saved for VI and DFA examination. Other tissues for histopathology were fixed in 10% neutral-buffered formalin except for the eyes, which were fixed in Bouin's solution for 24 hours. Tissues saved for VI and DFA examination were frozen in liquid nitrogen and stored at -70°C until processed.

HISTOTECHNIQUE

Tissues harvested for histological examination are listed in Table I. The other tissues saved were eyes, spleen, thymus, salivary glands, heart, adrenal glands, kidneys, liver, pancreas, duodenum, jejunum, ileum and spiral colon. The nasal turbinates, following fixation, were decalcified in a saturated aqueous solution of ethylenediamine tetraacetate for 48-72 hours. The tissues were processed by routine paraffin techniques, sectioned at 6 μ, and stained with hematoxylin and eosin (H & E) by the Harris method.

IMMUNOPEROXIDASE STAINING

All six PrV inoculated animals killed between 86-96 HPI and two of six animals killed at 72 HPI with moderate to severe lesions in the medulla oblongata were tested for PrV antigen. Tissue sections, from the medulla at the level of the obex, were cut at 6 μ, mounted on glass slides, and incubated for 12 hours at 60°C in dry heat. The sections were then stained as outlined by the Vectastain™ protocol (Vectastain™ ABC kit, Vector Laboratories, Inc., Burlingame, California). Rabbit antipseudorabies serum was the primary antiserum and Harris hematoxylin was the counter stain.

DIRECT FLUORESCENT ANTIBODY TEST

The tissues saved for DFA examination, listed in Table I, were placed in 5 mL sample vials (Nagle Co., Rochester, New York), quick frozen in liquid nitrogen, and maintained at -70°C until processed. The procedure for DFA staining and blocking test has been described (20). The preparations were examined with a fluorescent microscope and classified positive or negative depending upon the presence or absence of bright yellow-green fluorescence.

ELECTRON MICROSCOPY

Tissues sampled for electron microscopy were from the spinal tract nucleus of the trigeminal nerve in the medulla oblongata at the level of the obex, cranial cervical ganglia and cervicothoracic ganglia. These fixed tissues were cut into 1 mm^3 pieces and postfixed in 1% osmium tetroxide. Thick sections were stained with 1% toluidine blue in 1% aqueous sodium borate. Specific areas were selected, thin sections stained with uranyl acetate and lead citrate, and examined in a Hitachi HS-9 electron microscope (Hitachi, Ltd., Tokyo, Japan).

RESULTS

CLINICAL SIGNS

The mean temperature differences between the control and inoculated sheep approached statistical significance at 60 HPI ($q<0.05$) and was significant at 72, 84 and 96 hours postinoculation (Fig. 1).

The first visible clinical signs occurred in two animals at 72 HPI and were mild depression, abnormal facial movements, salivation and frequent swallowing. By 84-96 HPI, the remaining five animals were either ill or dead. The severity of the signs increased with time to depression, facial fasciculations, chorea, excessive salivation,

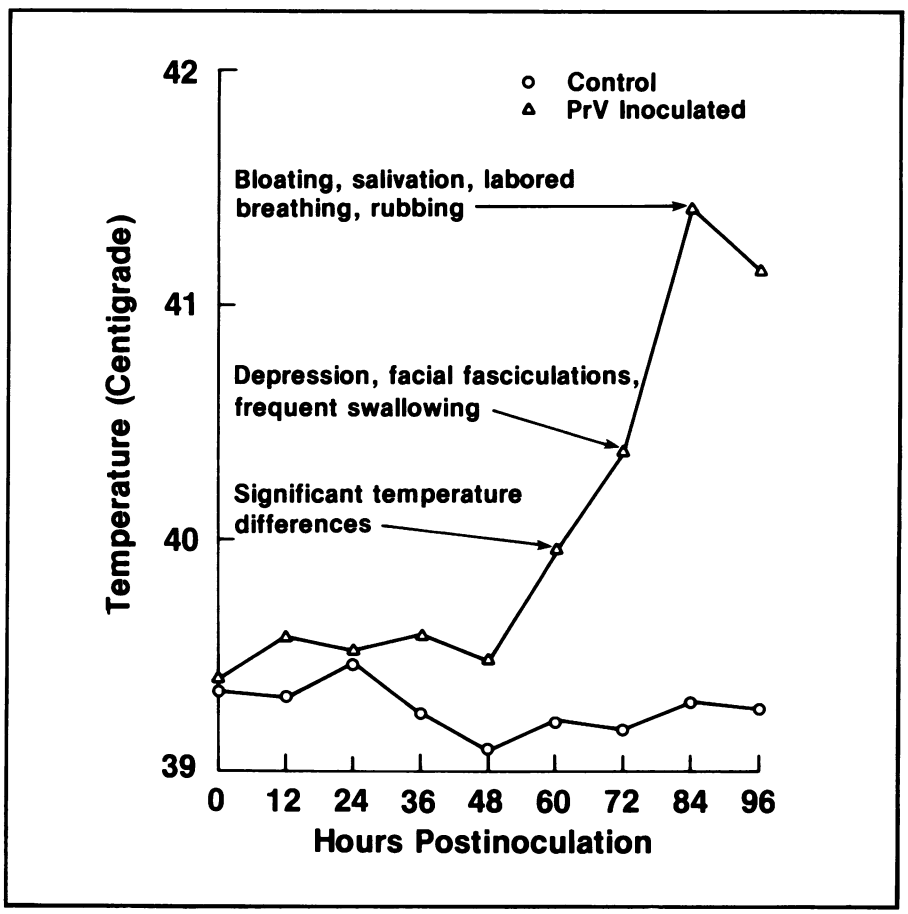

Fig. 1. Average rectal temperatures at 12 hour intervals from the control and PrV inoculated sheep. Arrows indicate the times clinical signs occurred.

labored breathing, moderate tympany, self-mutilation and finally death. The control animals had no clinical signs of pseudorabies.

MACROSCOPIC LESIONS

There were no significant lesions in the control sheep. The lesions in the principals were epicardial hemorrhage, enlarged bronchial lymph nodes, dilated esophaguses, petechiae in the cervicothoracic ganglia and facial trauma (Table II).

MICROSCOPIC LESIONS

In the principal sheep, atrial lesions occurred in two of six animals from 86-96 hours postinoculation. One animal had a very mild myocardial infiltrate of neutrophils while the other had a focus of histiocytes adjacent to a nerve. The septal myocardium in one of six animals at 48 HPI had a microfocus of histiocytes infiltrating several Purkinje fibers.

The retropharyngeal and bronchial lymph nodes contained several microfoci of necrosis in the subcapsular and paracortical sinuses. The spleen contained necrotic foci in the germinal centers in two of six principal sheep from 86-96 hours postinoculation.

The neural tissues contained microscopic lesions typical for pseudorabies (TableI). In two parabronchial ganglia in one of six sheep from 86-96 HPI, there were eosinophilic and basophilic intranuclear inclusion bodies (Fig. 2). The lesions in the trigeminal ganglia ranged from randomly scattered neuronal intranuclear inclusion bodies to foci of mononuclear cells encompassing degenerate neurons (Fig.3).

Cranial cervical ganglia and cervicothoracic ganglia contained lesions characterized by one or two microfoci of mononuclear cells to extensive hemorrhage, large multifocal mononuclear infiltrates, and degenerate to necrotic neurons containing numerous intranuclear inclusion bodies (Figs. 4 and 5).

The mesencephalon and metencephalon contained a few widely scattered neurons with basophilic intranuclear inclusion bodies. The medulla oblongata contained consistent lesions

TABLE II. Gross Lesions in PrV Inoculated Sheep

Tissue	Lesion	Hours Postinoculation			
		24	48	72	86-96
Bronchial lymph node	Enlargement	1/6[a]	0/6	0/6	1/6
Esophagus	Dilatation	0/6	0/6	1/6	1/6
Cervicothoracic ganglia	Petechiae	0/6	1/6	1/6	2/6
Head	Trauma	0/6	0/6	0/6	3/6

[a]Number with lesions/Number examined

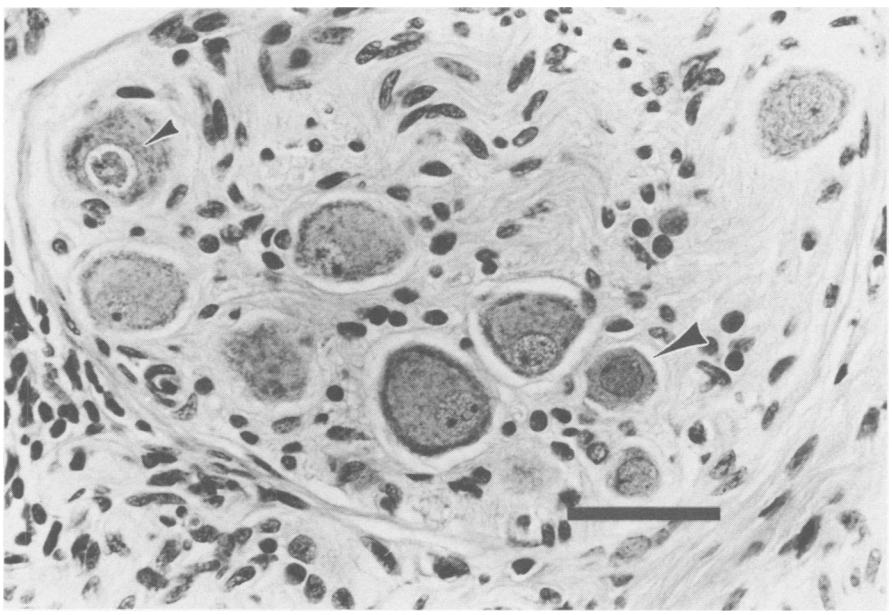

Fig. 2. Parabronchial ganglion from a PrV inoculated sheep between 86-96 HPI. Note the basophilic intranuclear inclusion body encircled by particulate chromatin at the nuclear membrane (large arrow), and the eosinophilic inclusion occupying the central portion of the nucleus (small arrow). H & E. Bar=47 μ.

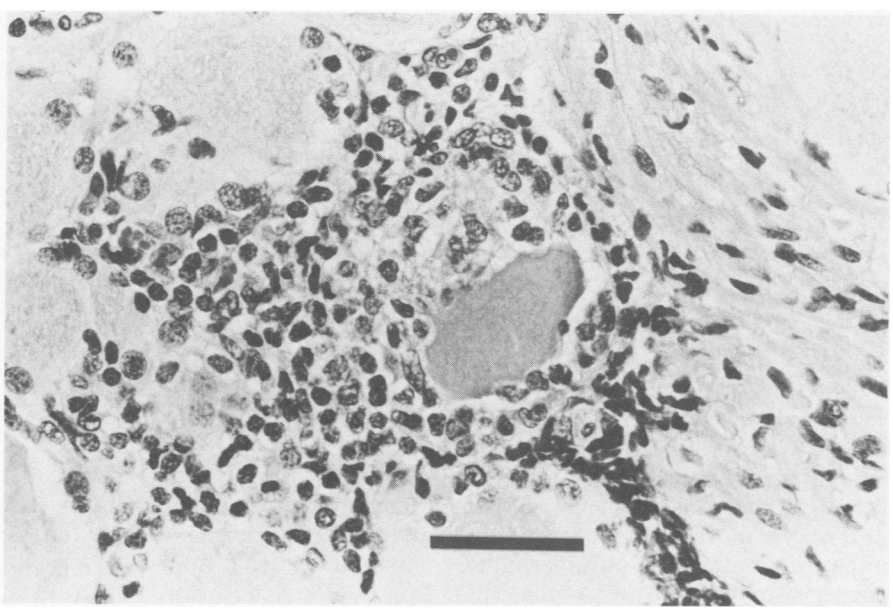

Fig. 3. Trigeminal ganglion from a PrV inoculated sheep from 86-96 HPI. Note focus of histiocytes encircling a degenerate neuron. H & E. Bar=40 μ.

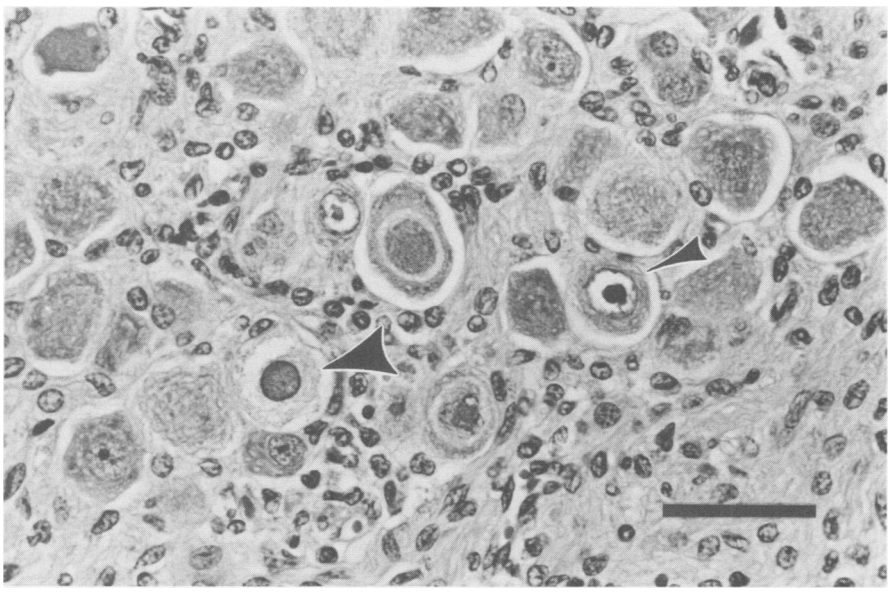

Fig. 4. Cranial cervical ganglion from a PrV inoculated sheep from 86-96 HPI. Note the neurons containing a basophilic intranuclear inclusion body (large arrow), and the eosinophilic intranuclear inclusion body (small arrow). H & E. Bar=38 μ.

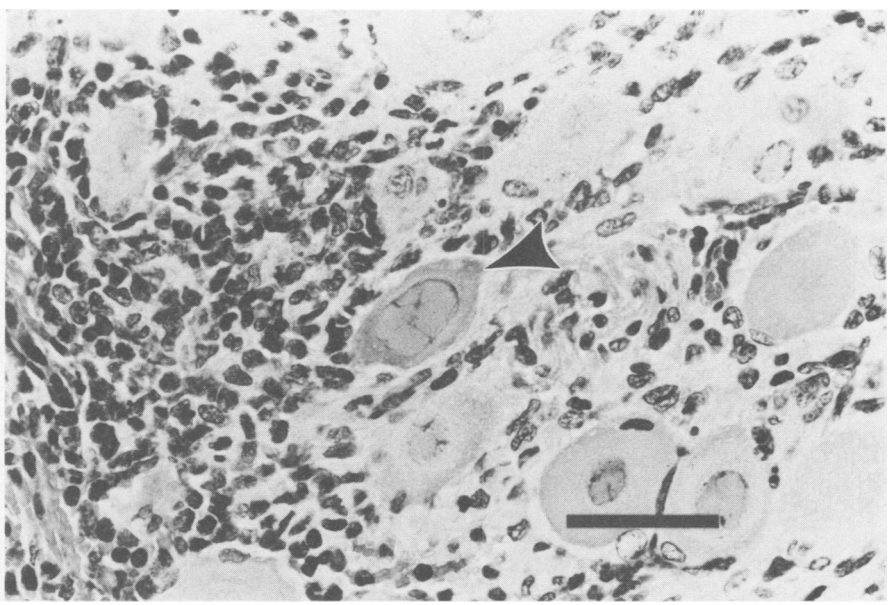

Fig. 5. Cervicothoracic ganglion from a PrV inoculated sheep from 86-96 HPI. Note glial focus and a basophilic intranuclear inclusion body encircled by a particulate margin of chromatin (arrow). H & E. Bar=49 μ.

limited to the solitary tract, dorsomedial solitary nucleus, and spinal tract nucleus of the trigeminal nerve. The lesions were most common at the level of the obex and were less dramatic and more localized than in the ganglia. The early lesions were limited to neurons and were reflected by alterations in the nucleoplasm while later lesions had wider lymphocytic perivascular cuffs, larger and more frequent microglial nodules (Fig. 6), and more extensive gliosis and edema. Intranuclear inclusion bodies were focally numerous in the neurons and neuroglia.

IMMUNOPEROXIDASE STAINING

Sections of medulla oblongata from two of six sheep killed at 72 HPI and six of six at 86-96 HPI found to have lesions by light microscopy were examined by the immunoperoxidase method. Traces of Pr viral antigen were demonstrated in the solitary tract, solitary nucleus, and spinal tract nucleus of the trigeminal nerve. Of the two sympathetic ganglia tested from two of six animals from 86-96 HPI, a positive reaction for viral antigen was observed in the cranial cervical ganglion and was limited exclusively to the neurons.

DIRECT FLUORESCENT ANTIBODY TEST

Numerous positive fluorescent tissues were found in the animals from the last two time periods (Table I). The cranial cervical and cervicothoracic sympathetic ganglia and the medulla oblongata at the level of the obex were consistently positive (Fig. 7).

VIRUS ISOLATION FROM TISSUE

The positive PrV isolations were dispersed over all of the time periods (Table I). The pulmonary tissues were positive for virus early in the experiment, and bronchial lymph node isolations increased with time. Pseudorabies virus isolations occurred most frequently in the ganglia, brainstem, and spinal cord from later groups. More isolations were made from the medulla oblongata than from the mesencephalon and metencephalon.

VIRUS ISOLATION FROM NASAL SECRETIONS, CEREBROSPINAL FLUID AND URINE

Pseudorabies virus was isolated from the nasal mucus of two animals killed in agonal stages between 86-96 hours postinoculation. No virus was isolated from urine of two of two animals or from the CSF of five of five animals from 86-96 hours postinoculation.

ELECTRON MICROSCOPY

In one sheep killed from 86-96 HPI, there were numerous herpesvirus nucleocapsids located in neurons of the cranial cervical ganglia, cervicothoracic ganglia and spinal tract nucleus of the trigeminal nerve (Fig. 8).

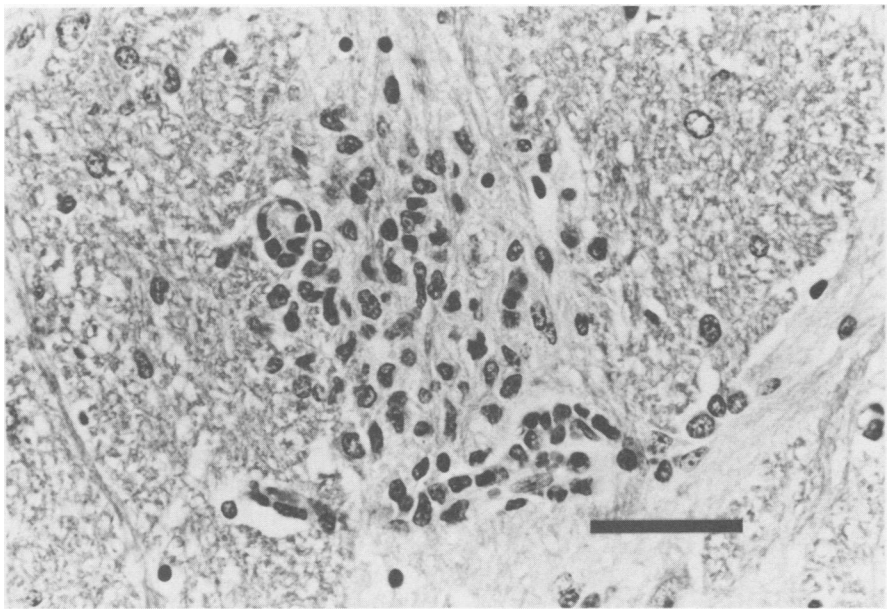

Fig. 6. Spinal tract nucleus of the trigeminal nerve (transection 1 mm caudal to the obex) in a PrV inoculated sheep from 86-96 HPI. Note microglial nodule within the neuropil. H & E. Bar=20 μ.

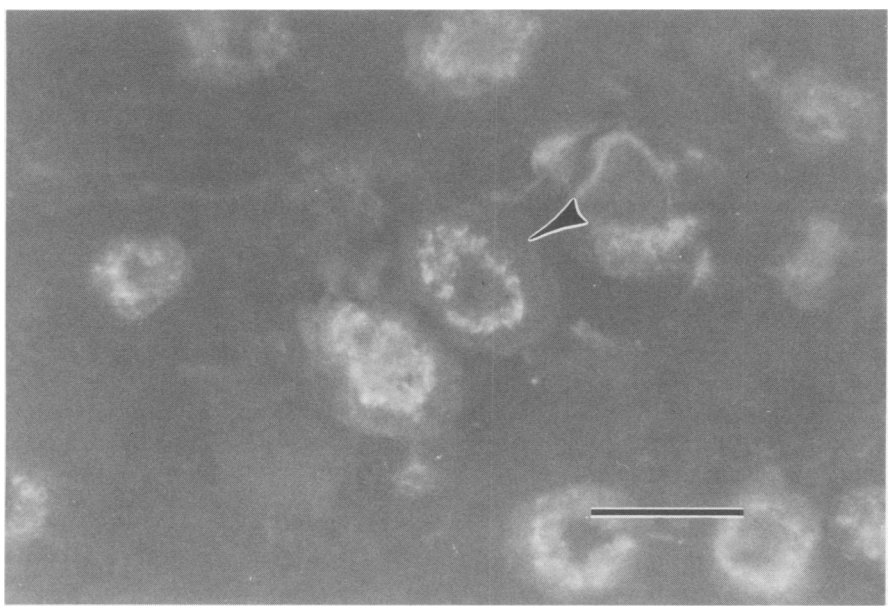

Fig. 7. Cervicothoracic ganglion from a sheep inoculated with PrV from 86-96 HPI. Note the neurons with the prominent intranuclear, nuclear membrane, and perinuclear cytoplasmic fluorescence (arrow). DFA. Bar=40 μ.

DISCUSSION

The susceptibility of sheep to PrV by the intratracheal route correlated closely with their susceptibility to oral, nasal and intraconjunctival viral exposure (13).

A temperature range of 40° - 41.4°C occurred from 60-96 HPI in the principals. Elevated temperatures from 40.5 - 42.2°C were reported from natural outbreaks (6,10,12) and other experimental work (13,14,23).

The clinical signs were depression, abnormal facial movements and twitches, occasional chorea, frequent swallowing, tympanites, excessive salivation, labored breathing and rubbing of the head. These and similar signs have been previously reported in natural outbreaks (5,6,8-12) and in experimental infections (13,23). Clinical pneumonia did not occur in the intratracheally inoculated sheep.

The gross changes in the inoculated sheep were facial trauma, dilated esophaguses, and multifocal ganglionic petechiae. Previously reported findings of pulmonary congestion (5,11), pulmonary edema (10,11), meningeal congestion (5), hepatic congestion (5), abomasal impaction (6), subepicardial petechiae (11,13,23), and increased cerebrospinal fluid (23) were not observed in these experimental animals.

Previous reports of natural PrV infections characterized neural lesions as either a nonsuppurative encephalitis involving all major anatomical divisions of the brain (11) or a multifocal necrotizing encephalitis in the brainstem and cerebellum (24). In other reported Pr cases, the presence of lesions in the parabronchial (25,26) and cervicothoracic ganglia (25) were evidence that PrV can be effectively distributed in the ovine autonomic nervous system. Dow and McFerran reported a nonsuppurative encephalomyelitis in the PrV infected experimental animals (23). In animals inoculated intranasally and orally, lesions occurred in the solitary tract, solitary nucleus, spinal tract nucleus of the trigeminal nerve and other nuclei of the medulla (13).

The most significant findings were the neuroparenchymal lesions in the medulla oblongata and sympathetic ganglia of the principals killed at 72 and 86-96 hours postinoculation. The lesions in the medulla oblongata were limited to the solitary tract, dorsomedial solitary nucleus and spinal tract nucleus of the trigeminal nerve. The lesions were classifiable as a nonsuppurative encephalitis and could be correlated with clinical signs.

The precise mechanism and the exact location whereby the virus entered the nervous system could not be determined in this study. There were no sequential lesions attributable to or compatible with PrV entry in the nonneural epithelial tissues of the oropharynx or respiratory tract. The only nonneural tissues containing lesions were the retropharyngeal and bronchial lymph nodes and spleens from animals killed from 86-96 hours postinoculation. The micronecroses in the retropharyngeal and bronchial lymph nodes probably resulted from

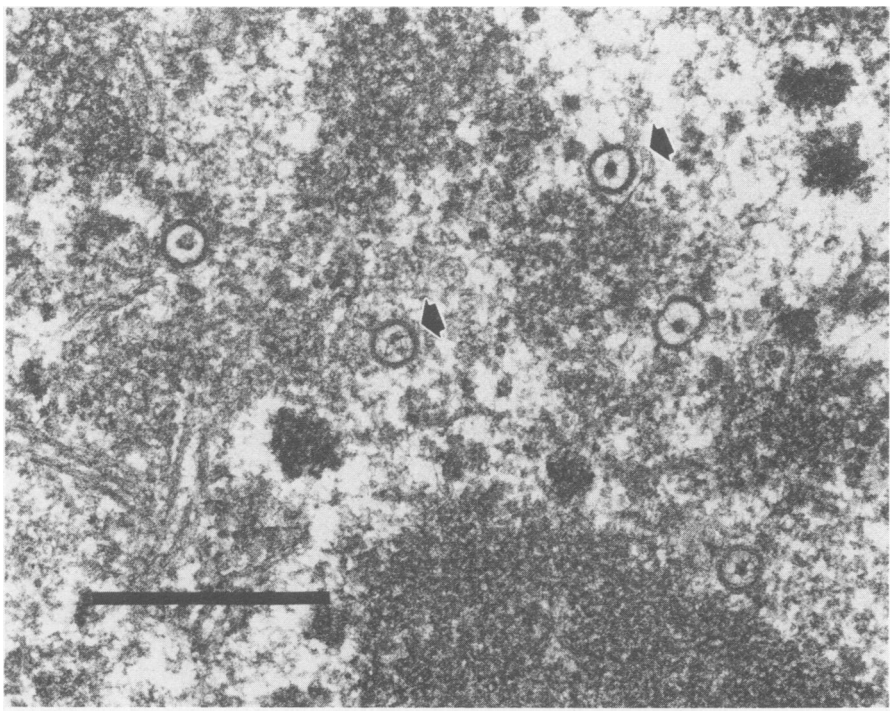

Fig. 8. Cranial cervical ganglion from a PrV inoculated sheep from 86-96 HPI. Note the nucleocapsids near the nucleolus of a neuron (arrow). Electron microscopy. Bar=0.5 μ.

virus entering the lymphatics in the oropharyngeal and deep pulmonary tissues.

The early virus isolations from the lung were probably due to residual inoculum since the number of isolations declined with time. In contrast, virus isolations increased during the study from the lymph nodes draining the respiratory and pharyngeal areas. This suggests that virus entered the submucosa. The isolation of virus from the lung of the animal with intranuclear inclusion bodies in the parabronchial ganglia is highly suggestive of viral replication in local pulmonary neural tissue.

The virus distribution within the central and sympathetic nervous systems was similar in all principals. The extent of viral distribution within the neural tissues was dependent upon the length of the incubation period and the duration of clinical illness. The mild and infrequent tissue changes and fewer virus isolations in the trigeminal ganglia suggest that intratracheal inoculation of aerosolized PrV allowed most of the virus to bypass the dendritic fields of the afferent fibers in the nasal and buccal mucosa which project centrally via the trigeminal nerve (27). The neural distribution of lesions, virus, and viral antigen in the solitary tract, solitary nucleus, and spinal tract nucleus of the trigeminal nerve was consistent with viral dissemination along afferent fibers of the glossopharyngeal nerve from the pharynx (28) and the afferent fibers in the vagus nerve from the lung and airways (29, 30). This distribution is compatible with the ability of the virus to be transported axoplasmally in the neural tissues (27,31).

In the cranial cervical and cervicothoracic ganglia, the random distribution of PrV antigen positive neurons and the absence of viral antigen in satellite cells are compatible with the axoplasmic transport of virus (27,31) in the sympathetic postganglionic fibers distributed in the pharyngeal (28) and respiratory mucosa (29,30). Virus in the cervical spinal cord segments could correspond to the caudal projections of the spinal tract nucleus of the trigeminal nerve (32). Virus in the cranial thoracic spinal cord would correspond to the distribution of the preganglionic neurons which synapse with the neurons in the cranial cervical ganglia and cervicothoracic ganglia (27).

The presence of PrV cytopathic effects, viral antigen, and nucleocapsids in the neurons indicated viral replication. These necrotic neurons along with ganglionic inflammation reduce the functional capacity of the neuroparenchyma. The spontaneous intermittent discharges from PrV infected neurons, which extend beyond normal bounds (33,34), may be the physiological cause of the clinical signs and death.

The lesions in the sympathetic ganglia were strikingly different from those in the medulla oblongata. These differences probably resulted from the lengths of time the tissues were infected with virus. The postganglionic fibers from the cranial cervical and cervicothoracic ganglia to the respiratory mucosa are shorter than the fibers from the pulmonary and tracheal mucosal irritant receptors to the respective nuclei in the medulla oblongata. In addition, before entering the medullary nuclei and tracts, the virus would have to replicate first in the neurons of the distal ganglia of the vagus and glossopharyngeal nerves. The effect is that the lesions in the sympathetic ganglia would be older and consequently the inflammatory changes more developed. At the same time, the cytopathic effects in the neurons of the medulla oblongata would be fatal before inflammatory foci as extensive as those in the sympathetic ganglia could occur.

This study indicates the potential for horizontal transmission of PrV in a flock of sheep. In contrast to McFerran and Dow (14) and in agreement with Lee *et al* (35),sheep were found to shed virus in the nasal mucus during the agonal phase of the disease. Contrary to the proposals of Bogdan (9) and Berecz (10) and in agreement with the data of McFerran and Dow (14), no virus was isolated from urine samples from 86-96 hours postinoculation.

Although, the absence of PrV in the CSF tends to agree with McFerran and Dow's findings of no viremia (14), the possibility of terminal or concurrent hematogenous viral dissemination cannot be ignored because of the occurrence of virus in retropharyngeal and bronchial lymph nodes, and adrenal glands.

Under the conditions of this study, sheep were found to be very susceptible to a typical Iowa field isolate of pseudorabies virus. Based upon the lesion distribution, the ease of location, and the striking nature of the

sympathetic ganglionic inflammatory lesions, the harvesting of these tissues for histological and virological examination is warranted.

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