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Authors: KIRKPATRICK, CHARLES M., KANITZ, CHARLES L., and McCROCKLIN, SARA M.

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POSSIBLE ROLE OF WILD MAMMALS IN TRANSMISSION OF PSEUDORABIES TO SWINE[1]

CHARLES M. KIRKPATRICK,[2] CHARLES L. KANITZ[3] AND SARA M. McCROCKLIN[3]

Abstract: Of 73 wild and domestic mammals tested from an area endemic for pseudorabies in swine, 16 showed natural pseudorabies virus infection, 8 from farms with no pseudorabies history. In transmission experiments with swine and raccoons *(Procyon lotor),* pseudorabies was not transmitted between raccoons but was transmitted reciprocally between raccoons and swine by contact and when either consumed infected carrion of the other. The fluorescent antibody tissue section test proved valuable in diagnosis of pseudorabies, especially when employed with the virus isolation test.

INTRODUCTION

The epizootiology of pseudorabies (Pr) is not completely understood. Although swine are considered natural hosts and the principal reservoir of the virus, the mechanism of transmission is not always clear. A number of investigators have suggested the carrier pig as the major source of infection for swine.[1,7,9,20] The introduction of carriers is probably the most frequent source of infection of swine herds. Stresses associated with transportation or farrowing may cause reactivation and shedding of the virus. How infection is initiated in closed breeder herds, with no history of recent introduction of new stock, is unknown.

One hypothesis for herd-to-herd transmission is that infected wild mammals may carry the disease from one farm to another. Only limited information exists concerning the pathogenesis of Pr in wild mammals and their role in the spread of the virus. The Norway rat *(Rattus norvegicus)* has been suggested as a possible reservoir by Shope[22] who postulated a rat-pig-cattle-rat cycle of infection. Although some[16] concluded that rats are not likely reservoirs of the virus nor do they play an important role in its spread, other studies[17,18,19] did demonstrate carriers in free-ranging rats. Survivors of experimental infection harbored the virus up to 131 days after infection, and naturally infected rats remained carriers for 100 days. Laboratory mice were found more resistant than laboratory rats[6] to experimental Pr infection. Although rats and mice *(Mus musculus)* may play some role in the dissemination of the virus, their resistance to infection with Pr would seemingly minimize their importance.

Raccoons *(Procyon lotor)* and opossums *(Didelphis marsupialis)* also have been suggested as possible vectors.[8,30] Transmission experiments dealing with Pr and its interaction with wildlife have shown that intraspecific transmission of Pr occurs in foxes *(Vulpes* sp.), raccoons and rats.[2,13,25] In other studies swine have contracted Pr by consuming carcasses of infected rats,[22] although it has not been shown that wild rats can contract Pr from swine.[16] Furthermore, numerous ac-

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[2] Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907, USA.

[3] Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907, USA.

counts of Pr transmission to mink *(Mustela vison)* and foxes on fur farms in Europe have been attributed to the feeding of virus-infected pig offal,[10,11,12,14,26,29] and Americans[25] similarly infected 2 raccoons and a fox.

In the present study we postulated that wild mammals could play a role in Pr transmission if susceptible wild mammals become infected from swine, and in turn serve as sources of infection for other susceptible animals. To test the hypothesis, we made a three-part study: 1) experimentally induced Pr infections in various species of wild mammals to obtain information on relative susceptibility to infection, course of the disease, and most efficacious approach to laboratory confirmation of infection in each species; an earlier paper summarized findings from this part;[8] 2) a thorough laboratory examination of all wild mammals obtainable from the Carroll County, Indiana, Pr endemic area for information on the occurrence of natural infections; and 3) a controlled study of transmission of Pr between raccoons (presumed natural vectors) and swine.

EXPERIMENTAL APPROACH

Control Studies

Mammals used in the control studies and transmission experiments were live-trapped from an area in Tippecanoe County, Indiana, where Pr infection had not been reported from farms for at least 2 years. One group of these animals served as negative controls and provided normal tissue specimens for the fluorescent antibody tissue section (FATS) test. The others inoculated with Pr virus, served as positive controls. Serum samples were taken from these animals and tested for neutralizing antibodies to assure no exposure to the virus. Each animal was then inoculated with the stock virus and observed periodically for signs of disease. Those resisting infection by oral inoculation were tested for neutralizing antibody and then either killed for tissue examination or challenged by injection of 0.5 ml of the stock virus into the rump.

Positive control animals provided specimens of infected tissues for the FATS tests. Tissues, which were characteristically positive in the inoculated animals, were chosen for testing in the surveillance animals from Carroll County.

To compare the reliability of the fluorescent antibody tissue section and virus isolation (VI) tests, all tissues of the positive control animals were subjected to both tests.

Surveillance Studies

To study the possible role of indigenous wild mammals in herd-to-herd spread of Pr during epizootics of the disease, we chose Carroll County as the survey area because 1) historically, Pr has been endemic there in some intense swine production operations; 2) there had been an increase in Pr cases reported during 1974 and 1975; and 3) the county led Indiana in number of recent outbreaks of Pr. Survey animals were live-trapped or found dead on and around swine production farms with histories of Pr outbreaks. Trapped animals were tested for infection with the FATS, VI, and serum virus neutralization (VSN) tests. Detection of any natural infections would indicate possible vectors for Pr infections.

Transmission Experiments

Experiment 1. Raccoons to Raccoons

This experiment tested the possibility of transmitting Pr from infected raccoons to uninfected raccoons by contact.

Trial 1 was conducted so that limited contact existed between inoculated and uninoculated raccoons. Seven raccoons were used. Two raccoons, in primate cages, were inoculated orally with Pr virus. Five raccoons were allowed free range over and around the cages within

the isolation unit. Nasal and oral swabs were taken daily from each infected raccoon and again after death. After the infected raccoons died, the free-ranging raccoons were allowed access to the contaminated food and water containers which were left in place.

Trial 2 was conducted in a manner which allowed closer physical contact between the raccoons. Four raccoons, of five surviving Trial 1, were first observed for 2 weeks to preclude the possibility of previous infections with Pr. One raccoon was then removed from the isolation unit, inoculated orally with the stock virus, and returned to the isolation unit to mingle with three other raccoons. Observations were taken on the same schedule as in Trial 1 and specimens were collected from the experimentally infected raccoon following its death. The remaining raccoons were observed an additional 2 weeks before the experiment was terminated.

Trial 3 replicated Trial 2, with the exception that three raccoons were used. The three raccoons which survived Trial 2 were observed for 2 more weeks after which one raccoon was removed, inoculated orally with Pr virus, and returned to share the isolation unit with the two uninoculated raccoons. Tissues were collected from the infected raccoon following its death. Two weeks after the infected raccoon died the experiment was terminated and the surviving raccoons were used for another experiment.

Experiment 2. Raccoons to Swine

This experiment investigated the transmission of Pr from raccoons to swine through contact between the species and through consumption of infected raccoon carrion by pigs. We used raccoons because they are susceptible to experimental Pr infection, they were the only wild species found with natural infection confirmed by both FATS and VI tests, and their habits are conducive to contracting and spreading the disease.

Trial 1 allowed free contact between inoculated raccoons and uninoculated swine. Two raccoons, one a survivor of Experiment 1, Trial 1, and two pigs, each weighing approximately 11.4 kg were housed together in an isolation unit. Serum samples collected from both pigs before the trial began were free of neutralizing antibody.

The raccoons were inoculated orally with Pr virus and then allowed to mingle with the pigs. All shared the same food and water troughs. The floor was washed down only every other day to allow the swine some contact with the feces, urine, and nasal discharges of the infected raccoons. A build-up of feces was avoided since feces had been shown detrimental to survival of the virus.[28]

The infected raccoons were observed periodically for signs of the disease. After their deaths, one raccoon was frozen at −30 C for later use in Experiment 2, Trial 2, and the other raccoon was given a postmortem examination.

Serum samples were collected from the pigs 1 week and 3 weeks after the second raccoon died and tested for Pr virus antibodies. After 3 weeks, tissues from the pigs' lungs and tonsils were examined by the FATS and VI tests.

Trial 2 tested the hypothesis that swine contract Pr by consuming infected raccoon carrion. Food was withheld for 2 days from two 11.4-kg pigs whose serums had previously been tested and found free of Pr virus antibodies. On the third day, the partially skinned carcass of an infected raccoon was placed in the unit with the pigs. The animals were observed daily. After 2 days, the remains of the carcass were removed from the unit.

Beginning 2 days after the removal of the carcass, rectal temperatures and nasal swabs were taken daily from each pig for 1 week. Serum samples for serum virus neutralization studies were collected from the pigs 2 weeks and 3 weeks post exposure. Both pigs were then killed and specimens of lung and tonsil

tissue subjected to the FATS and VI tests.

Experiment 3. Swine to Raccoons

This experiment investigated the transmission of Pr from swine to raccoons through contact between the species and through consumption of infected pig carrion by raccoons.

Trial 1, Exposure by Contact. Two raccoons which survived Experiment 1, Trial 3, and two pigs were used in this trial. Serum samples collected from all the animals were tested and found free of neutralizing antibody prior to start of the experiment.

The pigs were inoculated intranasally with the stock virus and housed together with two free-ranging raccoons. All shared the water and food troughs.

Rectal temperatures and nasal swabs were taken from the pigs the day following their inoculation and daily thereafter for 1 week. When the raccoons died, their tissues were collected as described below (Materials and Methods). Following death of the second raccoon, serum samples were collected from each pig for 2 weeks. The experiment was then terminated.

Trial 2, Exposure by Ingestion. Two piglets, experimentally infected with a field strain of Pr virus, developed typical clinical signs of pseudorabies and died. Their carcasses were frozen and stored at −30 C for use in this trial.

Two raccoons, without previous contact with Pr, were not fed for 4 days. On the fifth evening, the partially-thawed carcasses of the infected piglets were placed on the floor in the isolation unit with the raccoons. The carcasses were refrigerated during the day and placed in the unit only during the evenings. After 4 days, remains of the pig carcasses were discarded. The raccoons were observed daily until clinical signs of the disease appeared (see Control Animal Results), after which they were observed every 2 h. After death, they were autopsied as described below.

MATERIALS AND METHODS

Swine

The swine for the transmission experiments were normal pigs, farrowed and raised in the research facilities of the Purdue University School of Veterinary Medicine.

Isolation Facilities

Two isolation units housed the experimental animals. Each unit had an inner animal room (2.7 × 3.3 × 2.7 m) and an outer supply room. The inner room of each unit was equipped with a floor drain into which all animal waste materials were flushed. Filtered air entered through a ceiling duct and exhausted through another to the outdoors. Water was provided by automatic devices, and food was supplied in metal troughs on the floor.

Primate cages placed in isolation units housed the larger wild animals, which were fed and watered in glazed pottery bowls. Wastes of caged animals fell to the floor beneath the cages for transmission experiments.

Smaller animals were held in 37.5 × 37.5 × 25-cm stainless steel cages supplied with feeding trays and water bottles.

Anesthesia Procedures

Drugs used to anesthetize the wild animals for handling and collecting blood samples are detailed elsewhere.[14]

Collection of Tissues

Serum samples for antibody determination were taken from control and surveillance animals prior to death, and from transmission experiment animals prior to and following each experiment.

Tissues routinely collected at necropsy included cerebrum, cerebellum, medulla, spinal cord (cervical, thoracic, and lum-

bar), lung, spleen, pancreas, adrenal, kidney, liver, tonsil, parotid and mandibular lymph nodes, and salivary glands. Tissue specimens were either immediately examined by the (FATS) and virus isolation (VI) tests, or transferred to plastic bags and frozen for later work.

One oral and three nasal swabs were taken from each transmission experiment animal. Two of the nasal swabs were taken at the nares, the third from mucosa deep in the nasal cavity. Swabs were extracted for VI studies or stored frozen at −70 C for later work.

Fluorescent Antibody Procedures

The Pr virus fluorescent antibody (PrV-FA) conjugate used in this study was a diagnostic reagent prepared by CLK by labeling the globulin fraction of Pr virus immune swine serum with fluorescein isothiocyanate.

For the FATS test, tissue blocks were trimmed while frozen and mounted on metal carriers with a carbowax embedding medium. Sections of 8 μm were cut in a cryostat at −20 C, picked up on labeled slides, and dried. The sections were fixed in reagent grade acetone for 10 min at room temperature, removed, drained, and dried. Slides were then dipped in a phosphate buffered saline (PBS) solution containing 3% Tween-80 and 0.02% sodium azide and dried. The slides were flooded with PrV-FA and incubated in a moist chamber at 37 C for 30 min, then removed, rinsed in distilled water, and washed in PBS for 10 min. They were again rinsed in distilled water, air-dried, cover-slipped with glycerin-PBS (50:50, pH 7.5), and examined for specific immuno-fluorescence with a Leitz Orthoplan ultraviolet microscope.[14]

Cell Culture Methods

Basic media for cell culture were Eagle's minimum essential medium (MEM) with non-essential amino acids in Earle's salt solution containing 10mM $NaHCO_3$ and 15mM HEPES (N-2 hydroxyethylpiperazine -N'-ethanesulfonic acid), or a highly modified MEM containing 0.25% lactalbumin hydrolysate and 15mM HEPES. Penicillin, streptomycin sulfate, and fungizone were added to all media to retard microbial growth. The media were sterilized by filtration and stored in 500-ml aliquots at 4 C. Fetal bovine serum was added to the media as a serum supplement at a 10% level for use as a growth medium and at a 2% level for use as a maintenance medium.

A bovine turbinate cell line, BT/5705, was used for virus isolation and serum neutralization studies. This cell line had been established from bovine turbinate tissues by CLK.

Cell cultures were grown in plastic 250-ml tissue culture flasks. Medium changes were made twice a week. MEM or KSM (special medium prepared by CLK),[14] supplemented with fetal bovine serum, was used as the nutrient for the cell line.

Cell transfer was accomplished by washing the monolayer cell sheet with 10 ml of alkaline chelating solution (ACS) for a few seconds at room temperature. This solution was then discarded and replaced by 4.5 ml of ACS and 0.5 ml of a commercial trypsin solution, mixed and in contact with the cells until cell detachment occurred. The fluids were removed and the flask was incubated at 37 C for approximately 30 min. The cells were then suspended in 9 ml of nutrient medium and the suspension was either split in a 1:3 ratio and used to plant new cultures or further diluted for use in serum neutralization or virus isolation studies. For virus isolation, 1-ml aliquots were pipetted into Leighton tubes containing glass coverslips. Cells were allowed to settle and adhere to the flat surfaces of the coverslips. These tubes were incubated at 37 C for 3 days, then the medium was changed and they were ready for use.

Virus Isolation Procedures

Five classes of specimens were subjected to virus isolation studies: 1) tissues

from positive control animals; 2) tissues from clinically suspicious Pr surveillance animals which were Pr negative by the FATS test; 3) tissues of surveillance animals Pr positive by the FATS test; 4) tissues of swine and raccoons from transmission experiments; and 5) oral and nasal swabs collected from animals in transmission experiments.[14]

Tissue samples were homogenized in tissue culture medium (KSM or MEM) without serum in laboratory blenders for two minutes, and swab specimens were extracted in 2 1/2 ml of MEM. Homogenates were centrifuged at 3000 × g for 15 min. Supernatant fluids of tissue and swab samples were filtered through 0.45-μm membrane filters. Filtrates were inoculated onto coverslip cultures of BT/5705 cells, 0.2 ml per tube, and incubated at 37 C. The tubes were examined daily for approximately one week for cytopathic effects (CPE). If the specimen proved toxic to the cells, the inoculation was repeated but the inoculum was allowed to absorb onto the cell monolayer for only 30 min, then the supernatant fluid was decanted and fresh medium added. If Pr virus CPE was observed, the cultures were stained by the fluorescent antibody staining method. Coverslip cultures were stained essentially as described for the FATS test.

Serum Virus Neutralization Procedures

Serum virus neutralization (SVN) tests were performed in a microtiter system. Serums were first diluted 1:2 in PBS and inactivated by heating at 60 C for 20 min to destroy nonspecific inhibitors of viral infectivity. Tissue culture medium, KSM with 10% fetal bovine serum, was used as diluent for serum dilutions and for virus and cell suspensions. Two-fold serum dilutions were prepared in triplicate in 96-well, flat-bottom microtiter plates in 0.025-ml volumes. A 0.025-ml drop of suspension containing 100-1000 $TCID_{50}$ was added to each of 2 of the 3 serum dilution series to give a final serum dilution scheme of 1:4 to 1:512. An equal drop of diluent was added to each well of the third series which served as a serum toxicity control. Plates were covered and incubated at room temperature for 1 h. Following incubation, a 0.050-ml drop of BT/5705 cell suspension was added to each well and the plates were sealed with adhesive film. The plates were incubated at 37 C and examined for CPE after 2 or 3 days. Serum antibody titers were recorded as the highest dilution of serum completely inhibiting CPE.

Inoculation Procedures

A field strain of Pr virus, designated PrV-P8251, was used to infect positive control animals and the virus source animals in the transmission experiments. This virus was isolated in BT/5705 cell cultures from a tonsil of a pig submitted to the Purdue Animal Disease Diagnostic Laboratory. The culture had been passed one time in PK-15 cells to prepare a stock of virus.

Another field isolate of virus was used to infect young piglets in Experiment 3, Trial 2.

The initially infected raccoons in the transmission experiments and all positive control animals, rats excepted, were inoculated orally with 2 ml of virus suspension containing *ca.* 10^8 $TCID_{50}$ of PrV-P8251. The inoculum was drawn into a 2.5-ml syringe via an 18-ga 38-mm needle fitted with a 100-mm length of flexible tubing. Inoculum was introduced into the buccal cavity of the animal while it was in an upright position in the transfer unit of a primate cage. Oral inoculation of the rats was done by feeding 1/3 of a 50-g portion of commercial dog food mixed with 2.5-ml of virus suspension.

Natural oral exposure to Pr was simulated in the transmission experiments by placing partially-thawed infected carcasses of raccoons or pigs in the units housing other uninfected animals.

Contact was the other kind of natural exposure simulated in the transmission experiments. In one trial, infected raccoons were held in wire-bottom primate cages so that their feces, urine, and mucous discharges dropped to the isolation unit floor where uninfected animals ranged freely. In another trial, direct contact occurred when infected and uninfected animals ranged freely together within the isolation unit and ate and drank from the same troughs.

Pigs in Experiment 3, Trial 1 were exposed intranasally with 1 ml of the stock Pr virus. The inoculum was drawn into a syringe as previously described and introduced into the nasal cavity of the pig while it was restrained upright.

Observation Schedules

Observations of inoculated animals were made one to three times daily post inoculation (PI) and were increased to every 2 h once clinical signs of the disease appeared.

RESULTS

Control Animals

The 26 wild mammals collected for control studies included 4 opossums, 6 raccoons, 4 woodchucks *(Marmota monax),* 8 muskrats *(Ondatra zibethica),* and 4 Norway rats. None of the tissues from this group reacted positively to the FATS test, and the serums contained no Pr virus antibodies. Twelve of these animals served as negative controls and provided normal tissues. The remaining 14, inoculated orally with Pr virus, included 2 opossums, 3 raccoons, 2 woodchucks, 4 muskrats, and 3 Norway rats.

Not all of the animals inoculated developed the disease. One raccoon died without showing signs of the disease. The other two showed signs of CNS disturbance including excessive salivation, tooth grinding, equilibrium derangement, anorexia, convulsions and pruritus.

Gross changes most often noted in positive control animals were moderate congestion of lungs, occasional petechiation of the heart, and engorged meningeal vessels.

Virus was isolated from 19 of the 51 FATS positive tissues from animals which died following inoculation with Pr virus and from 4 FATS negative tissues. Virus was most often detected by both tests in the cerebrum, cerebellum, and medulla oblongata of the brain, and in the tonsil. Neutralizing antibody was not detected in any serums collected from the positive control animals.[14]

Surveillance Animals

From July 1974 through June 1975, 54 mammals were trapped, 2 were found dead on or near Pr infected premises by McCrocklin, and 17 were collected by local practitioners (see Acknowledgments). The total mammals collected and examined included 17 opossums, 22 raccoons, 1 red fox *(Vulpes fulva),* 1 striped skunk *(Mephitis mephitis),* 2 muskrats, 21 Norway rats, 6 domestic cats, 2 domestic dogs, and 1 calf.

The 56 animals trapped or found dead by McCrocklin were from 7 of the 9 Carroll County swine farms surveyed. One farm had an outbreak of Pr early in 1974. All others had Pr in the swine herds when trapping was initiated. In these 56 mammals, natural infections with Pr were confirmed in 6 raccoons and 2 cats, and a suspicious reaction was seen in the FATS test of 1 rat. All positive animals, except one sick raccoon submitted alive for examination, were found dead on or near farms where Pr had previously been diagnosed in swine. In addition, at the same time of this study, seven positive Pr cases (six domestic dogs and one calf) from the endemic area were diagnosed incidentally at the Animal Disease Diagnostic Laboratory. Of this total of 16 positive cases in wildlife and other species, 8 animals were taken from farms where Pr had previously been diagnosed in swine. The remaining eight were from farms with no history of Pr infections.

Tissues of the survey animals examined by the FATS test included brain, spinal cord, parotid and mandibular lymph nodes, parotid and mandibular salivary glands, tonsil, adrenal gland, and lung. Of the nine mammals confirmed positive for Pr by the authors, infections were detected in only four by the FATS test. Pseudorabies virus was most often detected in the brain, spinal cord, and tonsil of the naturally infected surveillance animals.[14]

Virus isolation confirmed infection in three of the positive FATS test animals, and, also, detected Pr virus in the CNS tissues of three additional raccoons and two cats which had been negative on the FATS test.

Serum neutralization tests failed to detect neutralizing antibody in any of the serums of the surveillance animals.

Transmission Experiments

Experiment 1. Raccoons to Raccoons

Trial 1. None of the uninoculated raccoons developed Pr. Both inoculated raccoons died 3 days PI. One showed respiratory distress, equilibrium derangement, pharyngeal paralysis, vomiting, and immediately prior to death, excessive salivation. The other, in addition to equilibrium derangement, pharyngeal paralysis, and excessive salivation, also had severe pruritus.

Pseudorabies virus was isolated from 75% of the oral swabs 1 day PI but not from any of the nasal orifice swabs. Virus was also present in swabs taken after death from the posterior nasal cavities.

Trial 2. Again, none of the uninoculated raccoons developed Pr. The infected raccoon died 3 days PI following a clinical syndrome similar to that observed in the inoculated raccoons in Trial 1.

Trial 3. The third attempt to transmit Pr infections between raccoons was also unsuccessful. The infected raccoon died 3 days PI. In addition to showing clinical signs described above, this raccoon consumed feces prior to its death.

Experiment 2. Raccoons to Swine

Trial 1, Exposure by Contact. Both inoculated raccoons developed clinical Pr, one dying 2 days and the other 3 days PI. Neither pig showed clinical signs of the disease. A low titer of neutralizing antibody was detected in the serum of one pig 1 week following its exposure to the inoculated raccoons. Serum samples from both pigs taken 3 weeks later contained higher titers of neutralizing antibody.[14] Lung and tonsil tissues from the pigs in this trial were confirmed Pr virus negative by the FATS and VI tests.

Trial 2, Exposure by Ingestion. The pigs consumed the raccoon's visceral organs and much muscle tissue. There was no evidence that the pigs had consumed any brain or spinal cord. The pigs showed clinical signs of the disease 4 days following their exposure to the infected raccoon carcass. Both showed anorexia and depression. Two days later their temperatures rose from the normal temperature of approximately 39 C to 41 C and 42 C. Pr virus was isolated from the nasal discharges of both pigs on the eighth day following their exposure to the infected carcass. Neutralizing antibody was detected in their serums 2 weeks and 3 weeks following their exposure to Pr. Lung and tonsil Tissues taken from the two pigs at 3 weeks PI was confirmed negative by the FATS and VI test.[14]

Experiment 3. Swine to Raccoons

Trial 1, Exposure by Contact. The inoculated pigs developed typical signs of Pr. Temperatures of each rose 3 days PI to highs of 42 C and 42+ C. Clinical signs included fever, depression, anorexia, and labored breathing. Virus was isolated every day for 1 week from nasal swabs of both pigs.

Both raccoons exposed to Pr through free contact with the inoculated pigs developed clinical signs of Pr. One died 5

days and the other 6 days after their exposure to the infected pigs. Only one raccoon developed pruritus.

Trial 2, Exposure by Ingestion. The two raccoons in this trial developed Pr infections. Both raccoons avoided the carrion for 2 nights, but later consumed some viscera from both piglets. These raccoons showed clinical signs of Pr previously noted in infected raccoons. One died 5 days and the other 9 days from the time their consumption of pig carrion was first noted. The raccoon dying at 9 days developed intense pruritus. A chin lesion from scratching was denuded, lacerated, edematous, and hemorrhagic.

SUMMARY OF FATS, VI, AND SVN TEST RESULTS

The FATS and VI tests performed on tissues of the nine raccoons used in the transmission experiments detected the virus most often in the medulla oblongata, tonsil, lung, and pancreas.[14] By VI tests, virus was also isolated from 100% of brain tissues, 100% of parotid salivary glands, 78% of mandibular salivary glands, and 56% of kidneys. Infection had not been detected in most of these tissues by the FATS test.

Virus was isolated from all of the oral swabs and from some of the nasal swabs taken after death from these nine raccoons.[14]

Serum neutralization tests failed to detect any neutralizing antibody in any of the postmortem serums from the nine raccoons, nor in serums taken from two raccoons prior to initiating Experiment 3, Trial 1.

DISCUSSION

The question of whether wild mammals spread Pr among closed swine herds in Carroll County, Indiana, can only be considered in light of several essential conditions. First, natural Pr infections in wild mammals must be present in the endemic area. Mammals infected with the virus must survive long enough to carry the virus from farm to farm, including the possibility that the infected wild mammal may die and be eaten by swine. Finally, proof must be shown that infected wild mammals could transmit the disease to swine through mutual contacts.

Wildlife and the Spread of Pseudorabies

Control studies in the present research agreed with previous work[25] that several species of wild North American mammals are susceptible to experimental Pr infection. Of the wild animals inoculated orally, raccoons, opossums, and muskrats were highly susceptible to pseudorabies, but Norway rats were resistant. Only one rat developed pseudorabies. No evidence of a "silent" carrier state[17,18,19] was found in rats inoculated in this study.

Animals contracting the disease typically died within a matter of days, reducing the likelihood of their travel far enough to infect other herds of swine; however, the time between infection and onset of clinical signs is inversely related to amount of the infecting dose. The dose of virus used manually to infect positive control animals was unnaturally high; therefore, the time from infection to death was short (average 3.7 days PI). In the simulated natural exposure trials, the time from infection to death for raccoons ranged 5 to 9 days. This result suggests that incubation periods in natural environments may be longer than in experimental conditions. During incubation periods, exposed animals in enclosure units were mobile. From their behavior, we suspect that an infected wild raccoon would survive long enough to travel from one swine farm to another.

The surveillance results suggest that raccoons, dogs, cats, and possibly rats are in some way responsible for transmitting the disease. The fact that 8 of 16 natural Pr infections in wildlife and other species were found in animals from

farms with no known Pr history suggests that these 8 were infected on a Pr positive farm, presumably by contact with swine or other Pr positive animals present there, and then moved to an uninfected farm during the incubation period. In spite of this suggestive evidence, no prior study has demonstrated that a wildlife species can contract the disease from swine and in turn transmit the disease to other susceptible swine, or that swine can contract the disease from wildlife and serve as sources of infection for other wildlife.

During winter months, when epizootics of Pr tend to occur, wild and domestic animals commonly associate with swine herds as the former inhabit farm premises seeking food and refuge. All of the wild species found susceptible to experimental PrV infection, with exception of muskrats, are commonly observed around farmsteads. Muskrats sometimes do seek winter refuge in farm buildings, and dispersing young muskrats in late summer, fall, and early spring may come into close contact with swine.[5]

The species suspected as possible Pr vectors are either omnivorous or carnivorous, with the exception of muskrats that are primarily herbivorous. Nevertheless, muskrats do feed on fish, frogs, clams, and carcasses of dead muskrats when aquatic vegetation is difficult to obtain.[3] Conceivably, starving winter muskrats would feed on pig carcasses. Hogs will scavenge muskrat carcasses.[5] Some trappers undoubtedly dispose of muskrat carcasses where hogs find them.

The diets of opossums and raccoons are determined by what is available. Opossums scavenge foods disdained by other animals.[3] Raccoons are opportunists, but more fastidious than opossums. In east-central Illinois, movements of three raccoons radiotracked in late spring were affected by available sources of corn. Each raccoon made intensive use of corn in hog feeders.[4] In the surveillance area of this study, one farmer reported that raccoons fed from a hog feeder. We also observed raccoon tracks on and around feeders. In this situation, an opportunity again exists for transfer of Pr between species if the shed virus remains viable.

For the virus to be spread, the range of an infected raccoon must include more than a single farm. The average home range diameter of adult male raccoons in Michigan was 1.6 km (range 0.48-3.2).[24] In Indiana, most female raccoons are bred during late January and in February. Adult males often travel long distances at night in search of females.[3] Their ranges likely increase during the same time period of increased incidence of Pr outbreaks. The average home range diameter of female raccoons in Michigan was 1.1 km (range 0.3-2.2).[24] Since the average density of hog production farms in Carroll County is greater than one per 2.59 km^2, a raccoon of either sex could include more than one farm in its home range.

Data from the raccoon-to-raccoon transmission trials, and the few natural Pr infections detected in wild raccoons, suggest that the disease is not endemic in the raccoon population in Carroll County. Rather, it is likely that an occasional animal could contract the disease from infected swine and spread Pr by wandering onto a disease-free farm. Recovery of virus from various tissues of the raccoons used in the transmission experiments suggests that the virus is shed in saliva, nasal discharges, and urine, all possible sources of viral contamination.

Failure in our study to transmit Pr between raccoons may have resulted from several factors. Because the virus is heat labile,[23,27,28] temperatures around 30 C in the raccoon isolation unit when the virus was shed possibly inactivated virus of low titers. Raccoons, which are not considered natural hosts for the virus, could require exposure to relatively large amounts of infective virus to establish infection. Raccoons also shed

lesser amounts of virus than pigs as shown by virus isolation from nasal swabs.[14]

Our experiments demonstrated that raccoons can contract Pr through contact with infected pigs or by feeding on infected pig carrion, and that swine can be infected through similar contacts with infected raccoons. These facts increase the possibility that raccoons play an epidemiological role in spread of the disease in the natural environment.

The transmission of Pr from raccoons to swine in the "contact" trials likely resulted from swine contact with excretions of the infected raccoons. Likewise, the isolation of virus from nasal and oral secretions and from the urine of pigs in previous studies,[15,20,21] and from nasal secretion of pigs in the present study suggests a means by which the disease is transmitted by contact to raccoons, along with interspecific scavenging of carrion, supports the idea of interspecific transmission of Pr by consumption of infected carrion. Opportunity for this interaction comes from wild animals inhabiting the premises with swine during the winter months when the disease is more prevalent. Producers sometimes accumulate pig carcasses by piling them outdoors until collection by an animal refuse service. This method of carcass disposal was used on six of the nine positive farms surveyed, and may have been employed by two other positive farms for which the authors had no data. Infected carcasses serve as possible sources of infection for both domestic and wild animals. Any hungry carnivore or omnivore may find such available food attractive.

Comparison of the FATS and VI Tests

The control studies showed that CNS tissues and the tonsil are best for diagnosis for Pr infections in wild mammals by the FATS test and by VI.[8,14] They also suggested that the FATS test was more sensitive and efficient than the VI test in detecting Pr. High titers of infectious virus are not always produced in Pr infected tissues and may be lost to postmortem autolysis. Virus was isolated from only 37% of the FATS positive tissues. Thus, if diagnosis depended upon the isolation of viable virus, many Pr infections would pass undetected, especially if specimens were not handled properly.

In the surveillance studies and transmission experiments, however, virus was isolated from brain and spinal cord composites of five FATS negative wild animals from Carroll County and from a large percentage of FATS negative tissues of the nine raccoons used in transmission experiments. The apparent higher sensitivity of the virus isolation test may be explained, in part, by changes in methodology. The majority of tissues from the positive control animals subjected to VI were stored at 32 C in a standard freezer. Upon learning that optimal storage temperatures for specimens were either at +4 C or below −30 C,[31] we stored tissues properly thereafter. Proper storage, coupled with larger amounts of tissues, enabled the detection of infections by VI which had been missed by the FATS tests. These findings suggest that the FATS test is best employed when used in conjunction with the standard virus isolation test. If the clinical diagnosis is suggestive of Pr infection and the FATS test is negative, virus isolation should be attempted, especially when large amounts of central nervous tissues are available.

Limitations of This Research

One limitation in methodology of the present research was the small number of species sampled in the control and surveillance studies. Although this research focused on those species most often associating with swine, other species indigenous to endemic areas, that may be capable of contracting Pr and spreading the disease, include striped skunk, red fox, gray fox *(Urocyon cinereoargenteus)* and, perhaps, house mouse.

Another limitation of the present research is sample size. A larger number of each species collected would have increased the probability of finding animals with natural Pr infections. The intensity of our live-trapping was probably inadequate to detect occasional infected animals. Otherwise, small wild mammals, dead or in late stage of fatal disease, rarely are collected except in epidemic situations.

The transmission experiments demonstrated that Pr can be transmitted experimentally between swine and raccoons, but did not reveal the probability with which transmission might occur naturally; nor did they establish whether contact or consumption of infected carrion is more important in nature.

CONCLUSIONS

From this research, we conclude that a variety of wild and domestic animals can contract Pr. Not only has the disease been produced in several Indiana species of wild animals in the laboratory, but natural infections occurred in raccoons. The collection of diseased wild animals from farms with no known Pr history indicates that the animals contracted the disease elsewhere and traveled to their collection site. We have shown experimentally that raccoons and pigs can transmit the disease interspecifically, but intraspecific transmission in raccoons seems more difficult. However, these results provide only circumstantial evidence that wild mammals transmit Pr to swine.

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