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PSEUDORABIES: EXPERIMENTAL STUDIES IN RACCOONS WITH DIFFERENT VIRUS STRAINS

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ABSTRACT: Raccoons (*Procyon lotor*) were infected by the nasal/oral route with as little as 10^2 plaque forming units (PFU) of pseudorabies virus (PrV). There was no apparent difference in the susceptibility of raccoons to infection with either of two virulent field strains or with the naturally avirulent K strain which has been used in modified live virus vaccines. Each of these three viruses was transmitted by contact to uninfected raccoons. All raccoons that were infected with virulent field strains died; however only two of 11 (18%) raccoons that were infected with the K strain died. One of four raccoons that survived infection with the K strain survived superinfection with virulent virus. This finding was significant because it could be a mechanism by which virulent PrV can be introduced and persist in the raccoon population. The possibility of this event occurring is increasing because of the widespread prevalence of PrV and the use of modified live virus vaccines for controlling clinical pseudorabies in swine. Virus neutralizing activity was found in five of 47 serums collected from raccoons that were trapped in PrV endemic areas. This observation implies that a herpesvirus, possibly PrV, may be present in the wild raccoon population.

INTRODUCTION

In recent years there has been considerable interst in the possibility of raccoons becoming a natural reservoir of pseudorabies virus (PrV). For this event to occur, raccoons (a) must have access to the virus, (b) must survive PrV infection, and (c) must transmit the virus between members of their own species. Earlier studies have demonstrated that raccoons have access to PrV through PrV-infected pigs (Kirkpatrick et al., 1980; Wright and Thawley, 1980). However, there have not been any reports of raccoons surviving PrV infection or of PrV-infected raccoons transmitting the virus to other raccoons.

In the following study the susceptibility of raccoons to PrV strains of different virulence is compared. Contact transmission of PrV between raccoons is demonstrated and data are presented that suggest that raccoons which survive infection with a PrV strain of low virulence can be superinfected with strains of higher virulence. Results of serologic surveys of raccoons that were trapped in PrV endemic areas are also presented. The findings in this study indicate that the raccoon has the potential for becoming a significant natural reservoir of pseudorabies virus.

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MATERIALS AND METHODS

Cell medium, virus isolation medium and diluents

Growth medium (GM) and maintenance medium (MM) consisted of Eagle's minimum essential medium with Earle's salts containing 10% and 2% fetal bovine serum (FBS), respectively. Virus isolation medium consisted of MM containing 1,000 IU/ml penicillin G, 1,000 μg/ml streptomycin and 20 μg/ml amphoterocin B. Physiological salt-dextrose (saline G), pH 6.9, consisted of 8.0 g NaCl, 0.4 g KCl, 0.15 g KH_2PO_4, 15 g Na_2HPO_4 and 1.2 mg phenol red in deionized water q.s. to 1 liter.

Viruses and experimental animals

The viruses used in this study included the virulent field viruses Be, S62/26, 429; and the European vaccine strains BUK and K. The origin and history of each strain has been described (Platt et al., 1979). The K strain was originally isolated as a naturally occurring avirulent virus (Bartha, 1961). The field viruses were plaque cloned three times and serially passaged no more than 11 times in MDBK cells before use in the raccoon studies. The BUK and K strains were plaque cloned one time and passaged seven times in MDBK cells before use in raccoons.

Raccoons used in the study were trapped in the vicinity of Ames, Iowa, and varied in age from young to mature adults. Raccoons were immobilized to facilitate sampling and inoculation procedures by injecting them intramuscularly with 1–2 ml of a solution containing 9 mg/ml ketamine hydrochloride and 0.9 mg/ml acepromazine.

Virus isolation

Specimens for virus isolation were collected with cotton swabs. Tissue and secretory material were eluted from the swabs in 2 ml blanks of virus isolation medium. The eluent was then used as inoculum

for MDBK cell monolayers that were grown in glass tissue culture tubes. The cell monolayers were examined daily for the presence of viral cytopathic effects (CPE) over a 5 day period. If CPE were not present at the end of this period the cell cultures were blind passaged at least once. The identity of isolated virus was confirmed by serum virus neutralization.

Virus and serum antibody assay

Virus activity was assayed by the plaque method. Serial dilutions of virus were made in cold saline G containing 2% FCS. Three-quarters of a ml of each virus dilution was inoculated in duplicate onto confluent MDBK-cell monolayers in eight-well Lux tissue culture plates. Inocula were removed after 90 min incubation at 37 C and replaced with MM containing 1% carboxymethylcellulose. Virus infected cell sheets were then incubated 48 hr at 37 C, fixed with formalin and stained with a 5% solution of crystal violet prepared in 20% ethanol.

Serum antibody titers were determined by the plaque reduction method. Two-fold serial dilutions of normal and suspect PrV antibody positive serums were made in saline G. One ml of each dilution of serum was mixed with an equal volume of saline G containing 30,000 plaque forming units (PFU) of virus. The serum/virus mixture was incubated for 45 min at 37 C and then assayed for virus activity as previously described. The titer of a given serum was considered to be the highest serum dilution that reduced virus activity by at least 50%.

Susceptibility of raccoons to different strains of pseudorabies virus

The susceptibility of raccoons to strains Be, S62/26 and K was determined on three separate occasions. Four 10-fold dilutions of S62/26 were made which yielded 6.8 through 6,800 PFU/ml. One raccoon was inoculated with each virus dilution by dropping 0.5 ml of virus suspension into each nostril and 0.5 ml onto the tongue. Similarly 10-fold dilutions of virus Be were made that yielded 105, 1,050, and 10,500 PFU/ml. One raccoon was inoculated as above with the dilution containing the highest concentration of virus and two raccoons were similarly inoculated with each of the remaining virus dilutions. Dilutions of the K strain yielded 29, 290 and 580 PFU/ml. Two raccoons were inoculated as above with each dilution.

Nasal/tonsillar swabs were taken daily for the first week postinoculation (PI) and assayed for the presence of virus. Brain stem, lung and heart blood of dead raccoons were also assayed for the presence of virus. Serums of surviving raccoons were collected at 2 to 3 wk PI and assayed for PrV neutralizing antibody.

Transmission of pseudorabies virus between raccoons

Pseudorabies virus strains Be, S62/26, BUK and K were studied separately in a series of experiments to determine if they could be transmitted between raccoons by contact. The format of each experiment was essentially identical. Raccoons were inoculated intranasally with 1 ml of virus suspension diluted to yield 10^4 PFU. Twenty-four hr later a single previously unexposed contact raccoon was placed in the same cage with one of the PrV-infected raccoons. All cages were thoroughly cleaned prior to the introduction of the unexposed contact raccoons to eliminate the possibility of infection by residual PrV in the cage environment. This protocol was used for all transmission experiments with the exception of one experiment. In this experiment single contact raccoons were placed in two cages containing two raccoons each that were previously inoculated with the K strain. Nasal/tonsillar swabs were taken daily for up to 8 days from raccoons following exposure to strains Be, S62/26, and BUK and assayed for the presence of virus. Raccoons exposed to or inoculated with the K strain were sampled daily for 14 days then at 2 or 3 day intervals until day 30 following exposure. Brain stem and heart blood of raccoons that died following exposure to virus were also assayed for the presence of virus. Serums were collected from surviving raccoons 20 days after exposure to strains S62/26, Be and BUK and 30 days after exposure to strain K.

Superinfection studies

Three raccoons were infected with the K strain of PrV as determined by virus isolation from nasal/tonsillar swabs and seroconversion. All three raccoons were challenged with the virulent PrV strain 429 by inoculating both nostrils with 1 ml of virus suspension containing 10^3, 10^4, and 10^7 PFU virus on days 55, 76 and 97, respectively, following infection with the K strain. None of the three raccoons were superinfected as determined by failure to isolate virulent virus from the nasal passage after challenge and by the absence of an anamnestic serum antibody response represented by at least a four-fold increase in titer.

The above raccoons were again challenged with PrV strain 429, 300 days following infection with the K strain. Two of these raccoons were inoculated as described above with 10^4 PFU of virus. The third raccoon and a new raccoon that was infected with the K strain 41 days earlier were similarly challenged with 10^7 PFU of strain 429. A control raccoon was also inoculated nasally with 10^4 PFU of strain 429 to verify the virulence of the strain. Serums were collected from all raccoons 1 wk prior to challenge, on the day of challenge and at 8 and 14 days following challenge. Nasal swabs were collected from all raccoons on days 2 and 4 following challenge and examined for the presence of virus.

Clearance of virus from the nasal cavity of raccoons

It was important to demonstrate that virus recovered from raccoons on days 2 and 4 was newly replicated and not residual virus from the inoculum. For this reason the clearance rate of PrV strain 429 from the nasal passages of two raccoons was determined. The right and left nasal passage of each raccoon was inoculated with 1 ml of virus suspension containing 10^7 PFU/ml. Nasal swabs were taken at 2, 4, 8, 12, 16, 20, 24 and 48 hr after inoculation.

Virus was eluted from swabs into 2 ml blanks of virus isolation medium. All samples were stored at −70 C and then assayed for virus activity on the same day.

Field studies

Serums were collected in the spring of 1978 from 47 raccoons that were live trapped in three different PrV endemic areas in central Iowa. Twenty serums were collected from area 1 which experienced a severe outbreak of pseudorabies in 1974. Nine serums were collected from area 2 which suffered a similar outbreak in the winter of 1976. Eighteen serums were collected from the third area which incurred a pseudorabies outbreak in the winter of 1977. The serums were assayed for the presence of virus neutralizing antibody by the plaque reduction test. Nasal and tonsillar swabs were also collected from each raccoon and assayed for the presence of PrV.

RESULTS

Virus isolations

Virus was consistently isolated from the brain stem of raccoons that died following PrV infection with the exception of two raccoons that died following infection with the K strain of PrV. No virus was found in the heart blood of dead raccoons. Attempts were also made to isolate virus from heart blood, lung, urinary bladder and rectum of seven raccoons that died between 3 and 5 days following infection with virulent field strains of PrV. Virus was isolated from the lungs of four (57%) of the seven raccoons, but not from any of the other organs sampled.

Studies on the susceptibility of raccoons to different pseudorabies virus strains

Susceptibility studies indicated that there was no marked difference in the susceptibility of raccoons to infection with wild and vaccine strains of PrV (Table 1) and that raccoons could be infected with as little as $10^{2.0}$ PFU of virus. These studies also suggested that the period of virus shedding was influenced by the amount of virus in the infecting dose. Virus was recovered 2 to 3 days prior to death from the nasal passage of raccoons that were infected with $10^{2.8}$ PFU of strain S62/26, and with 10^{3} PFU of strain Be. No virus was recovered from the nasal/oral passages of raccoons that were infected with $10^{2.0}$ PFU of strain Be or with $10^{2.5}$ PFU or less of strain K. However, in the transmission studies described below, raccoons infected with 10^{4} PFU of strain K shed virus for up to 13 days.

TABLE 1. Susceptibility of raccoons to infection by different pseudorabies virus strains.

Virus strain	Dose (log_{10} plaque forming units)	Number of raccoons	Virus recovered from brain stem	Number[a] of seroconversions	Day of death
S62/26	0.8	1	ND[b]	0	S[c]
S62/26	1.8	1	ND	0	S
S62/26	2.8	1	Yes	ND	5
S62/26	3.8	1	Yes	ND	5
Be	2.0	2	Yes	ND	4
Be	3.0	2	Yes	ND	3, 4
Be	4.0	1	Yes	ND	3
K	1.5	2	ND	0	S
K	2.5	2	ND	2	S
K	2.8	2	ND	1	S

[a] Determined by serum neutralization/plaque reduction 3 to 5 wk PI
[b] ND = not done.
[c] S = survived.

Transmission studies

Transmission studies demonstrated that wild and vaccine strains of PrV can be transmitted between raccoons. The results of these studies are summarized in Table 2. Virus was transmitted by contact in all experiments involving the PrV strains K and Be. Transmission of strain S62/26 also occurred in one of two experiments. Transmission of the vaccine strain BUK was not demonstrated.

Raccoons that were experimentally infected with the K strain of PrV shed virus continuously from the nasal/oral cavities for 8 to 10 days PI and intermittently thereafter until day 13 PI. Virus was recovered from nasal/oral cavities of contact raccoons between 2 and 4 days postexposure (PE). These raccoons continued to shed virus intermittently for up to 14 days PE. Deaths

TABLE 2. Transmission of pseudorabies virus strains between raccoons.

Experiment	Virus strain[a]	Number of raccoons inoculated	Number of contact raccoons	Number of contact raccoons infected[b]
1	K	4	2	2
2	K	1	1	1
3	S62/26	2	2	0
4	S62/26	1	1	1
5	Be	1	1	1
6	BUK	2	2	0

[a] All inoculated raccoons received 1 ml virus suspension in each nostril. All virus inoculums were diluted to yield 10^{4} plaque forming units/ml.
[b] Virus infection was confirmed by serology, and/or recovering virus from the nasal/oral cavity of living raccoons 2 days or longer after inoculation and from the brain stem of dead raccoons.

TABLE 3. Clearance of pseudorabies virus from the nasal passage of the raccoon.

Time (hr)	Virus titer (log_{10} PFU/ml eluent)	
	Raccoon A*	Raccoon B
0	0.00	0.00
2	1.00	2.57
4	1.48	2.23
8	0.00	1.41
12	0.00	1.04
16	1.40	0.00
20	2.04	0.40
24	1.00	2.04
48	4.40	4.68

* Each raccoon was inoculated nasally with 10^7 plaque forming units of virus strain 429 contained in 1 ml of diluent.

occurred in one of five inoculated raccoons and in one of three contact raccoons at 13 and 22 days PE, respectively. Pruritus as indicated by a traumatized area behind the right ear was observed in the contact raccoon on the day prior to death. The experimentally infected raccoon suffered from partial posterior paralysis for 2 to 3 days prior to death. All other raccoons that were infected with strain K were maintained for at least 90 days PE without showing clinical signs of PrV infection. All surviving raccoons remained serologically positive during this period.

Raccoons that were experimentally infected with strain S62/26 shed virus for 1 to 3 days prior to death on days 4 and 5 PI. The contact raccoon shed virus for 1 day prior to death 7 days PE. The raccoon inoculated with strain Be shed virus for the first 2 days PI and died on day 3. The corresponding contact raccoon shed virus for one day prior to its death on day 6 PE. Strain BUK-infected raccoons died 8 and 9 days after inoculation while virus was recovered from the nasal/oral cavities only on day 2 PI. Virus was not recovered from the corresponding contact raccoons which also remained serologically negative for PrV infection.

Clearance of virus from the nasal cavity

The clearance of PrV from the nasal cavity of raccoons is summarized in Table 3. No virus could be recovered from raccoon A at 8 or 12 hr PI, nor from raccoon B, at 16 hr PI. Thereafter the amount of virus that was recovered from both raccoons increased. Consequently virus which is isolated from nasal passages at 2 days or longer after inoculation can be considered newly replicated and not residual virus from the original inoculum.

Superinfection studies

The data summarized in Table 4 indicate that raccoon 1 and possibly raccoon 3 were superinfected with the virulent PrV strain 429. Virus was recovered from the nasal passages of both raccoons 2 days after challenge. However, an anamnestic serological response only occurred in raccoon 1 whose serum neutralizing antibody titer rose four-fold by 7 days after inoculation. No anamnestic response occurred in raccoon 3. Virus was not recovered from raccoon 4 which survived the lower level of virus challenge and did not respond serologically. The virulence of strain 429 for raccoons was confirmed by the deaths of raccoon 2 and the control.

Field studies

Three of 20 (15.0%) serums collected in area 1 were positive for PrV neutralizing antibody.

TABLE 4. Superinfection of raccoons previously infected with pseudorabies virus strain K.

Raccoon	Days since infection with the K strain	Challenge dose (log_{10} plaque forming units)	Serum titer on day of challenge	Status following challenge	Virus shedding	Anamnestic response
1	41	10^7	16	Survived	Yes[c]	Yes[d]
2	300[a]	10^7	16	Died[b]	Yes	ND[e]
3	300	10^8	16	Survived	Yes	No
4	300	10^8	32	Survived	No	No
Control	—	10^8	0	Died	Yes	ND

[a] Raccoons 2, 3, and 4 were previously challenged with 10^6, 10^8 and 10^7 PFU of strain 429 at 55, 76 and 97 days, respectively, following infection with the K strain. No evidence of superinfection was detected.

[b] Raccoon 2 died 7 days postchallenge, the control raccoon died 3 days postchallenge.

[c] Yes = virus recovered from nasal cavity on day 2 following challenge.

[d] Yes = a four-fold increase in serum antibody titer at 7 days postchallenge.

[e] ND = not done.

Two of these serums were positive at a serum dilution of 1:2 and one serum was positive at a 1:4 dilution. One of nine (11.1%) area 2 serums was positive at 1:2 and one of 18 (5.5%) area 3 serums was positive at 1:2. Pseudorabies virus was not isolated from any of the 47 trapped raccoons.

DISCUSSION

The preceding study has shown that (a) raccoons can be infected with lower doses of virus than previously reported, (b) PrV-infected raccoons can transmit the virus by contact to uninfected raccoons, and (c) raccoons can survive infection with the naturally avirulent K strain and be superinfected with virulent PrV. These findings indicate that raccoons have the potential of becoming a significant natural reservoir of PrV and should not necessarily be considered a "dead end" host.

Virus given via the nasal/oral route appears to be 10 to 100 times more likely to infect raccoons than if given solely via the oral route. This conclusion is based partially on the observation of Wright and Thawley (1980) who reported that nine raccoons were refractory to oral inoculation with 10^3 $TCID_{50}$ and that three of nine raccoons resisted challenge with 10^4 $TCID_{50}$. Because 1 $TCID_{50}$ can contain 1 to 9 PFU of virus, it follows that raccoons would be refractory to oral inoculation with 10^3 to 10^4 PFU of virus. In our study raccoons were infected with as little as 10^2 PFU of virus. The lower infectivity of the virus dose used in Wright and Thawley's study could have been due to a strain difference, however, no differences in infectivity were found among three of the strains used in our study (Table 1).

Survival of raccoons following experimental inoculation and contact infection with the K strain is significant because the strain is a naturally occurring avirulent virus (Bartha, 1961). The increasing prevalence of pseudorabies in the USA means that there will be a concurrent increase in the exposure of raccoons to PrV strains of different virulence. Some of these strains may be avirulent. The fact that superinfection of raccoons with virulent virus can occur is important because it provides a mechanism by which virulent PrV may be introduced and maintained in the raccoon population. The possibility of these events occurring is increasing because of the widespread use of modified live virus vaccines for controlling clinical pseudorabies in swine.

The presence of low levels of virus neutralizing activity in five of 47 (10.6%) raccoon serums could mean that PrV was circulating in the feral raccoon population. This interpretation would be especially attractive considering that the largest number of positive serums were collected in area 1 where PrV has been endemic for apparently the longest period of time. However, some mammalian herpesviruses do share common neutralizing antigens (Killington et al., 1977). Consequently PrV may also share a common neutralizing antigen with a naturally occurring herpesvirus of raccoons. The presence of such a common antigen could account for the low virus neutralizing titers found in the five raccoons. If the serum titers were greater than 1:4 we would be inclined to believe that the raccoon population had been infected with PrV. Unfortunately sufficient amounts of the positive serums were not available to study the specificity of the neutralizing activity in more detail.

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LITERATURE CITED

BARTHA, A. 1961. Kiserletek az Aujeszky-fele virus virulenciajanak szeliditesere. Magy. Allatorv. Lapja 16: 42–45.

KILLINGTON, R. A., J. YEO, R. W. HONESS, D. H. WATSON, B. E. DUNCAN, I. W. HALLIBURTON, AND J. MUMFORD. 1977. Comparative analyses of the proteins and antigens of five herpesviruses. J. Gen. Virol. 37: 297–310.

KIRKPATRICK, C. M., C. L. KANITZ, AND S. M. MCCROCKLIN. 1980. Possible role of wild mammals in transmission of pseudorabies to swine. J. Wildl. Dis. 16: 601–614.

PLATT, K. B., C. J. MARÉ, AND P. N. HINTZ. 1979. Differentiation of vaccine strains and field isolates of pseudorabies (Aujezky's disease) virus: Thermal sensitivity and rabbit virulence markers. Arch. Virol. 60: 13–23.

WRIGHT, J. C., AND D. G. THAWLEY. 1980. Role of the raccoon in the transmission of pseudorabies: A field and laboratory investigation. Am. J. Vet. Res. 41: 581–583.