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RESPONSE OF WHITE-TAILED DEER TO INFECTION WITH PESTE DES PETITS RUMINANTS VIRUS

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Abstract: White-tailed deer (*Odocoileus virginianus*) were infected experimentally with two strains of peste des petits ruminants virus. The response varied from fatal consequence to subclinical infection. The clinical signs and gross lesions were similar to those in goats. Virus was recovered from all the infected deer, and survivors developed specific antibodies demonstrated by complement fixation and virus neutralization tests. Survivors also resisted challenge with virulent rinderpest virus that was lethal to a control deer.

INTRODUCTION

Peste des petits ruminants (PPR) or pest of small ruminants is an acute or subacute disease of goats and sheep, characterized by necrotic stomatitis, bronchopneumonia and a mucosal and lymphoid tissue reactional syndrome resembling rinderpest (RP) in cattle. The disease was first observed in previously French-occupied territories of West Africa and was causing severe losses.[1] Field observations showed that cattle did not develop clinical signs of the disease when kept in close contact with ill goats and sheep. Mornet *et al.*[7] proposed that the causal agent was a strain of rinderpest virus that had lost its capacity to infect cattle by the natural route but spread quite readily in small ruminants, specifically goats and sheep. Hamdy *et al.*[2] demonstrated that RP and PPR viruses are closely related, sharing some antigenic components, but are readily differentiated by virus neutralization tests. Because white-tailed deer (*Odocoileus virginianus*) are prevalent in North America and are susceptible to infection with rinderpest virus,[3] their response to infection with PPR virus was of concern and is the subject of this report.

MATERIALS AND METHODS

Vero Cell Culture

The growth and maintenance of Vero cell cultures were as described earlier.[3]

PPR Virus

The Sénégal and Nigerian strains of PPR virus were used. The first strain was originally obtained from the Laboratoire Nationale de l'Elevage et des Recherches Veterinaires, Dakar-Han (Senegal) as a lyophilized lung extract. After the virus was injected into susceptible goats, buffy-coat cells (BCC) were collected during the peak of temperature elevation. The virus was subsequently grown in bovine embryonic spleen and in Vero cell cultures. The Nigerian strain of PPR virus was isolated at Plum Island Animal Disease Center from tissues of five goats that were involved in outbreaks of stomatitis pneumoenteritis complex in Nigeria.[4]

RP Viruses

Virulent and attenuated forms of the Kabete "O" strains of RP virus were obtained from the East African Veterinary

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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Research Organization Laboratory, Muguga, Kenya, as a lyophilized Vero cell culture passage. For this experiment, the virulent virus was passaged once in Vero cell culture and used to challenge PPR recovered deer to assess their resistance to RP. The attenuated RP virus strain was used in the cross virus neutralization test between PPR and RP viruses.

Exposure of Deer to PPR Virus Infection

Deer were supplied by Dr. Frank A. Hayes, Director, Southeastern Cooperative Wildlife Disease Study, Athens, Georgia. Six deer were used in the experimental infection (Table 1) as follows: a) A buck (#1) and a fawn (#2) were inoculated intramuscularly with 2 ml of the Sénégal strain of PPR virus suspension containing $10^{4.8}$ $TCID_{50}$; b) A doe (#3) was housed in contact with the deer inoculated with the Sénégal strain (explained in a); c) Two bucks (#4 and #5) inoculated intramuscularly with 10% suspension of goat tissues infected with the Nigerian strain and housed in a separate animal isolation room; d) Uninoculated buck (#6) served as a RP virus challenge control.

Animals were observed daily; heparinized blood was collected 3 and 5 days post-exposure (DPE) and BCC fraction was prepared for virus recovery. Dead animals were necropsied, and lymph nodes (LN) were collected for virus recovery and complement fixation test.

Virus Isolation

Two-tenths ml of BCC fraction from each specimen was inoculated in Vero cell cultures grown in 50-ml Falcon plastic flasks. Ten percent (wt/vol) lymph node tissue suspensions from the two necropsied deer were prepared and inoculated in the same way as the blood samples.

Cultures were incubated for 1 hr at 37 C, after which the fluid maintenance medium was added. Medium was changed after 2 days and then at 3 day intervals until cytopathogenicity was observed, or for 14 days.

TABLE 1. Response of White-tailed deer to infection with Peste des Petits ruminants (PPR) virus.

Deer #	PPR virus exposure	Viral Strain	Clinical Response	Virus Recovery	CF Antibody	VN Antibody	Result after Infection	Response to RP Virus Challenge
1	Inoc. IM	Sénégal	+	+	+	+	Recovery	Resist
2	Inoc. IM	Sénégal	+	+	NA	NA	Death 6 DPI	NA
3	Contact	Sénégal	+	+	NA	NA	Death 11 DPE	NA
4	Inoc. IM	Nigeria	—	+	+	+	Recovery	Resist
5	Inoc. IM	Nigeria	+	+	+	+	Recovery	Resist
6	*Normal control		—	—	—	—	—	Death 5 DPC

CF: Complement Fixing; VN: virus neutralizing; RP: rinderpest

Inoc. IM—Inoculated intramuscularly.

DPI: days post inoculation; DPE: days post exposure; DPC: days post challenge; NA: not applied.

* used as RP virus controlled challenge.

Deer #2 and 3 are fawn and doe.

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Virus Neutralization

Viral isolates recovered from the five deer were identified by virus neutralization with PPR virus immune goat serum. Virus suspensions were harvested at a stage of approximately 50% cell destruction and clarified by centrifugation. PPR virus immune and normal goat sera were diluted 1:5 in phosphate buffered saline and inactivated at 56 C for 30 min. They were then chilled, dispensed in test tubes, and mixed with equal volumes of a series of tenfold dilutions of each of the recovered virus suspensions. The virus serum mixtures, as well as the virus and serum controls, were incubated at 37 C for 30 min., after which they were chilled, and 0.2 ml of each mixture was inoculated in triplicate Vero cell cultures grown in 50 ml Falcon plastic flasks. A few Vero cell cultures were not inoculated and served as cell controls. All cultures were incubated at 37 C and examined by light microscope for the development of cytopathic effects (CPE). Titers were recorded as neutralization indices representing the numbers of $\log_{10}$ of viruses neutralized by the serum.

For demonstrations of virus neutralizing antibody in the sera of convalescent deer at 14 and 21 DPE, serial twofold dilutions of the deer sera were mixed with equal volumes of RP virus in one set and PPR virus in another set appropriately diluted to contain 100 $TCID_{50}$/0.1 ml (as predetermined by previous virus titrations). The rest of the procedure was as described above, and the titers were considered as the highest serum dilutions that neutralized the cytopathogenicity of the virus in more than one-half of the cultures.

Complement Fixation (CF) Test

Antigens were prepared from LN of the two deer that succumbed to PPR virus infection. Ten percent (wt/vol) suspension in veronal buffer diluent[5] was subjected to three cycles of freezing (—70 C) and thawing (37 C) and clarified by centrifugation at 2000 x g for 1 hr.

Control antigen prepared from normal fawn LN served as a negative control. Antigens were tested against PPR viral immune goat serum and RP immune goat serum in a dilution that contained four CF antibody units. The CF method was essentially the same as that of the Laboratory Branch Task Force,[5] except that C′ titration was performed in the presence of the test antigen. Five $C'H_{50}$ units were used.

For demonstrations of CF antibody in convalescent deer sera, a series of twofold dilutions of sera were tested against PPR virus infected caprine LN and RP virus infected bovine LN antigen at dilutions that gave optimal activity (as determined by previous block titrations).

Electron Microscopy (EM)

Vero cell cultures inoculated with deer BCC revealed CPE characteristic of PPR virus and were repassed in Vero cells and processed for EM. Infected Vero cell cultures and corresponding controls were fixed in glutaraldehyde-osmium, dehydrated, and embedded in Epon 812[6] as described before.[3] Thin sections were cut on a Porter Blum MT-2 ultramicrotome[2] and stained with uranyl acetate and lead citrate.[8] Electron micrographs were taken with an RCA-EMU-3G electron microscope.

RESULTS

Clinical picture

Deer that were experimentally infected with PPR virus varied in their response from a fatal sequence with typical signs and lesions (similar to those produced in goats and sheep) to subclinical infection with no visible signs of the disease. The observed clinical signs were fever, nasal mucopurulent discharge, conjunctivitis, stomatitis with erosions and diarrhea at a later stage. The response of five deer to infection with the two strains of PPR virus is shown in Table 1. Each of the five exposed deer, including the contact control, developed viremia. Fawn

[2] Sorvall, Incorporated, Norwalk, Connecticut 06854 USA.

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#2 and doe #3 succumbed 6 and 11 DPE, and necropsy revealed necrotic stomatitis, abomasal congestion, catarrhal enteritis, bronchopneumonia and subendocardial hemorrhages. Bucks #1, 4 and 5 survived the infection after transient viremia.

Virus Isolation

PPR virus was recovered from blood samples taken 3 and 5 DPI and from LN from two deer after necropsy. Vero cells inoculated with the BCC fraction or LN suspension developed CPE 7-11 DPI. The CPE consisted of cell rounding and polykaryocyte formation. It started as primary foci that spread outward to become more generalized. Figure 1 is a photomicrograph of Vero cells at 9 DPI. The inoculum was BCC preparation from deer blood 5 DPI.

The viruses recovered from deer blood and LN obtained at necropsy were identified by neutralization test in Vero cell cultures. Goat PPR immune serum at tenfold dilution neutralized 4.8-5.7 $\log_{10}$ of virus whereas neither normal goat serum nor RP immune serum had any significant neutralizing activity.

The three deer that survived the PPR virus infection (bucks #1, 4 and 5) developed virus neutralizing antibodies in their sera (Table 2).



Photomicrograph of Vero cell inoculated with buffy coat cell blood fraction from PPR virus-inoculated deer. Note the CPE. Bar = 100 μm.

TABLE 2. Levels of antibodies in sera of convalescent deer infected with peste des petits ruminants virus against peste des petits ruminants and rinderpest viruses.

Deer #	Preinoculation Sera				*Convalescent Sera				Post RP virus challenge Sera			
	CF[a]		SN[b]		CF[a]		SN[b]		CF[a]		SN[b]	
	PPR	RP	PPR	RP	PPR	RP	PPR	RP	PPR	RP	PPR	RP
1	<4	<4	<10	<10	64	32	2560	<10	64	64	2560	1280
4	<4	<4	<10	<10	32	64	640	<10	64	128	640	1280
5	<4	<4	<10	<10	8	16	320	<10	16	64	640	640
Normal Control fawn												
	<4	<4	<10	<10								
#6 control deer												
	<4	<4	<10	<10								

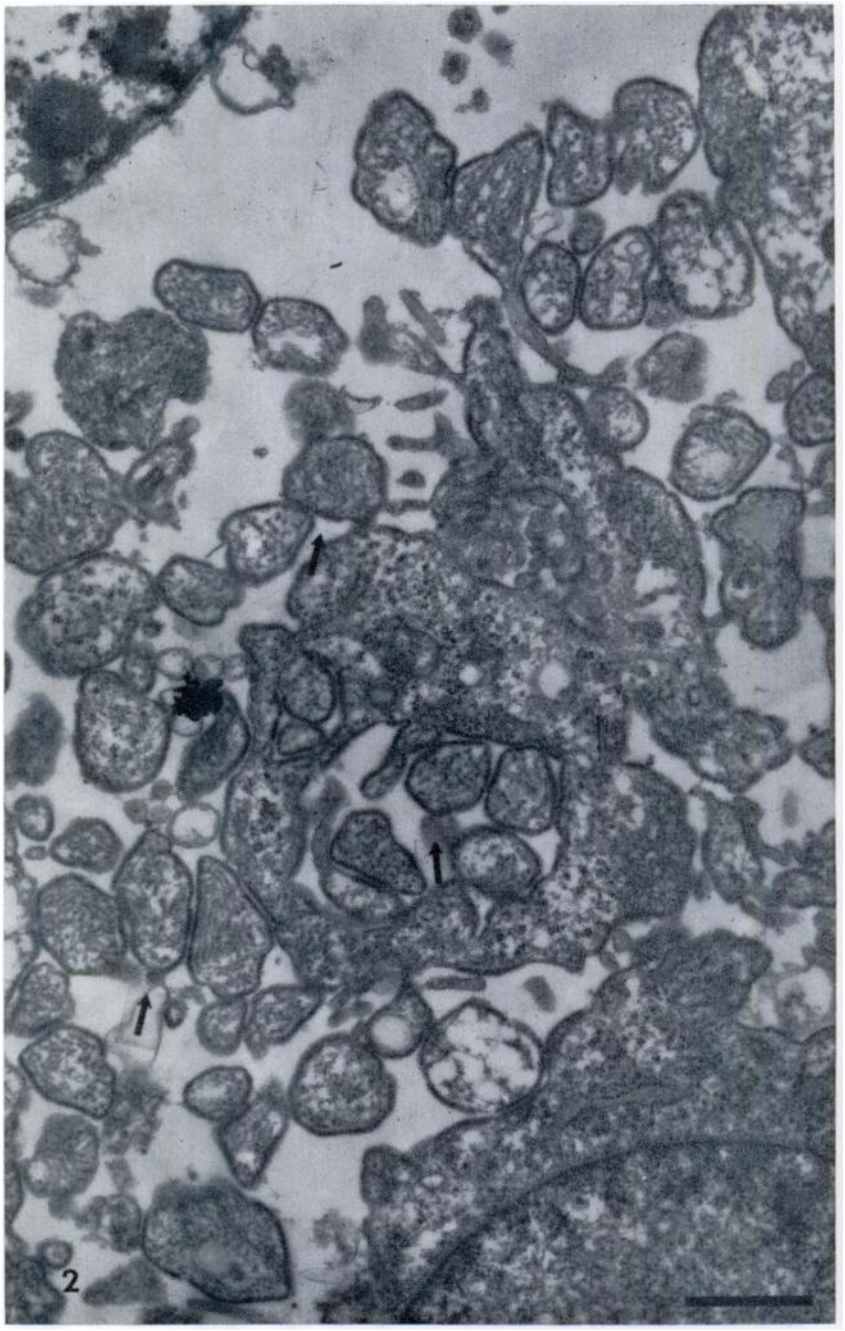
CF = Complement Fixation: SN = Serum Neutralization

[a] Numbers represent the highest serum dilution that fix complement specifically by ≧ 70%.

[b] Numbers represent the highest serum dilution that neutralize the virus in > 50% of the tested cultures.

* 21 DPI

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Photoelectronmicrograph of a 2nd passage of Vero cells inoculated with PPR virus infected deer blood. Note the extracellular virions (arrow), virus particles inside a cytoplasmic vacuole (V). Bar = 1 μm.

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Complement Fixation Test

The antigens prepared from LN of the two deer examined at necropsy had CF activity against RP and PPR goat antisera (Table 3). The normal control antigen tested simultaneously was negative at its lowest dilution (tenfold). Similarly, no CF activity was detected when LN antigens from deer infected with PPR virus were tested against normal control goat serum.

Sera from bucks 1, 4 and 5 developed CF antibody against both RP and PPR viral antigens (Table 2).

Effect of Virulent RP Virus Challenge on PPR Immune Deer

Bucks 1, 4 and 5 that survived PPR virus infection were resistant to virulent RP virus challenge while the control buck succumbed 5 DPI with typical signs and lesions characteristic of RP[3] (Table 1).

Ultrastructural Morphology

Thin sections of Vero cells inoculated with infected deer blood revealed virus morphogenesis similar to that of the closely related RP virus. Viral structures consisted of intracytoplasmic and, occasionally, intranuclear inclusions consisting of random arrays of fibrillar strands, pleomorphic particles in budding stages from the modified thickened plasma membrane of the infected cell, and mature virions extracellularly. The shape and size of the virus particles varied widely. On virus population basis, PPR virions assumed larger size than did RP virions. Some of the virions contained strands morphologically similar to those forming the cytoplasmic inclusions whereas other particles lacked these internal ultrastructures. Figure 2 is a photo electron micrograph showing virus particles in a 2nd passage of Vero cells inoculated with blood from a deer 5 DPI with PPR virus.

TABLE 3. Detection of Complement Fixing antigen in Peste des Petits ruminants virus infected deer lymph nodes.[a]

Deer #	Titer with PPR antiserum	Titer with RP antiserum
3	80	20
2	160	40
(Normal Control)	<10	<10

[a] Numbers represent the highest antigen dilutions that fix complement specifically by $\geq$ 70%.
PPR = Peste des petits ruminants; RP = Rinderpest.

DISCUSSION

The response of white-tailed deer to infection with PPR virus varied from a fatal sequence, to transient illness, to subclinical infection. The clinical picture resembled PPR virus infection in goats[2] and RP infection in white-tailed deer,[3] except that the morbidity and mortality rates are higher in RP than in PPR virus. The infection of PPR virus in deer was demonstrated both by parenteral route and by close contact. The clinical signs in white-tailed deer were similar to those reported with RP but in a milder form. The sera derived from convalescent deer at 21 DPI had CF antibodies that reacted with both PPR and RP viral antigens. However, the virus neutralizing antibodies were PPR type-specific and did not neutralize PR virus. In spite of lack of cross neutralization between PPR and RP viruses, the surviving deer resisted challenge with virulent RP virus that was lethal at 5 DPI to a normal control deer. This phenomenon also was observed in goats.[4]

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Recovery of virus from deer blood and LN from deer at necropsy, the identification of these isolates, plus demonstration of CF antibodies, PPR viral type-specific neutralizing antibodies, and the resistance of the subsequent challenge with virulent RP virus, leave no doubt of established infection. Additional evidence, however, was obtained when virus particles and virus related structures morphologically characteristic of PPR virus were demonstrated.[4] As with RP virus[3] we were able to demonstrate morphologic entities in the nucleus in addition to those in the cytoplasm, the budding viruses, and the extracellular virions.

The mechanism of resistance to subsequent RP virus challenge of PPR virus immune deer, in spite of the lack of humoral RP virus neutralizing antibody in their sera, was not clear and is similar to the same phenomenon in goats.[4] Cell-mediated immunity or biological antagonism with yet unknown mechanism may provide a hypothetical explanation.

Natural and experimental PPR disease was reported in goats and sheep.[1,7] As a result of this present work, the host range of the animal susceptibility to this disease was expanded to the American species of white-tailed deer.

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