Pathogenicity and Persistence of Pleural Effusion Disease Virus Isolates in Rabbits

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SUMMARY

Nine isolates of pleural effusion disease agent or virus (PEDV) from treponema-infected rabbits in various countries were examined for pathogenicity and persistence in rabbits. The isolates showed a wide range of pathogenicity and were categorized into three groups according to the severity of the acute infection. Group 1 comprised isolates causing more than 50% mortality, group 2 isolates causing mortality below 50%, while group 3 comprised isolates causing almost subclinical infections. The range between group 1 and group 3 was similar to that observed with virulent and avirulent progeny of the original PEDV isolate. Infection by each of the nine isolates resulted in a chronic low level viraemia which persisted for up to 2 years or more. Viral progeny of pathogenic isolates obtained in serum after the 2nd month of infection failed to induce clinical disease on rabbit inoculation. The chronic, subclinical infection was associated with a moderate, continued increase in serum IgG, but circulating immune complexes could not be demonstrated. Two years after infection slight histopathological changes were present in lymph nodes, spleen, liver, heart and lung. Evidence of immune complex disease could not be demonstrated.

INTRODUCTION

Pleural effusion disease virus (PEDV) is found as a passenger agent in testicular suspensions from rabbits infected with Treponema pallidum and causes intercurrent subclinical to fatal disease in rabbits (Gudjönnsson et al., 1970; Vaisman et al., 1973; Fennestad et al., 1980; Menke et al., 1980). As yet, the agent has not been identified, but it is considered to be a host-specific, enveloped virus, measuring 25 to 50 nm by filtration (Fennestad & MacNaughton, 1983). Observations on the intercurrent disease and experimental studies with the original PEDV isolate suggest that the emergence of PEDV infection as a clinical disease of rabbits is the result of a specific selective pressure on a benign virus population. This selection for virulence may require years of rabbit passages at intervals of 1 to 2 weeks, such as is commonly practised for the propagation of treponemes to be used in the T. pallidum immobilization (TPI) test (Fennestad et al., 1975; Fennestad, 1985).

PEDV infection may result in long-lasting viraemia and a characteristic feature is that circulating virus in the rabbit host may readily lose its capacity to induce clinical disease. The loss of this property is gradual and occurs during the chronic, subclinical phase of infection. The property of avirulence of isolates obtained during chronic infection also seems to become more fixed as viraemia continues, i.e. an increasing number of rabbit passages is required before virulence is restored (Fennestad et al., 1981; Fennestad, 1985).

In this study, the pathogenicity of PEDV isolates from various countries is examined and compared with the pathogenicity of two derivatives, representing extremes of virulence and avirulence, of the original PEDV isolate. The persistence of viraemia and the long-term effects of PEDV infection are also investigated.
METHODS

History of isolates. All isolates examined came from rabbits inoculated with *T. pallidum* (Table 1). The material for isolation of eight of the nine isolates consisted of samples of serum obtained 48 h after inoculating rabbits with treponemes in selected laboratories, some of which had experienced serious intercurrent mortality among the treponema-inoculated rabbits (Fennestad *et al.*, 1980). One laboratory was represented by two isolates: Paris I was isolated in 1978 in connection with intercurrent rabbit mortality. Paris II had been passaged separately from Paris I for some months and caused no remarkable mortality; our isolate was obtained from rabbits inoculated in the Paris laboratory in 1979 with a treponemal suspension prepared from rabbit testes which had been stored in liquid nitrogen since 1974 (Fennestad *et al.*, 1982). The Stockholm agent was provided by Dr J. D. Small (NIH, Bethesda, Md., U.S.A.) as infectious serum 73-015. The agent was originally shipped to Baltimore from Stockholm in January 1970 as a contaminant of rabbit testicular suspensions of treponemes and used for several experiments (Gudjönsson *et al.*, 1970, 1972; Small *et al.*, 1979). The detailed history of the Stockholm agent since isolation is not known. All nine isolates are considered serologically closely related or identical with PEDV on the basis of cross-protection studies (Fennestad *et al.*, 1980; Fennestad & MacNaughton, 1983).

The virulent PEDV was isolated on 22 October 1970 from a freeze-dried rabbit testicular suspension of *T. pallidum*. The lyophilization causes death of treponemes, but not of the virus (Fennestad *et al.*, 1975). Before preparing a stock, the virus was passed serially, at intervals of 3 to 10 days, through more than 124 rabbits. These passages augmented virulence. The stock consisted of pooled rabbit serum obtained 48 h after inoculation with blood or pleural fluid.

The avirulent derivative of PEDV was obtained from blood of a rabbit 6 months after experimental neonatal infection with the above-mentioned stock virus. This isolate was passed serially through 61 rabbits at weekly intervals by inoculation of 0.2 ml serum mixed with 0.8 ml phosphate-buffered saline (PBS) pH 7.0. During these passages no mortality occurred and typical clinical signs of PED were seen in only two rabbits (passage no. 46 and 55). The stock pool of the isolate consisted of serum from the 11th rabbit passage obtained 72 h after inoculation. The ID<sub>50</sub> of the two stocks was 10<sup>6.2</sup> and 10<sup>4.0</sup>, respectively using eight rabbits per 10-fold dilution (Fennestad, 1985).

Preparation and infectivities of virus stocks. Stocks of the nine isolates consisted of serum harvested 48 h after subcutaneous (s.c.) inoculation of rabbits with 0.2 ml of the infectious material received. Eight of the stocks represented the first rabbit passage in Copenhagen whereas the stock of the Stockholm agent was from our second rabbit passage of infectious serum 73-015. The number of rabbit-infectious doses (RID) per ml of the stocks was estimated by inoculating 10-fold dilutions in PBS, using two to five rabbits per dilution. The term RID refers to a dose capable of inducing clinical signs of PED and/or clinical protection against virulent challenge. The highest dilution producing these responses in more than half of the animals was taken as the endpoint titre of the virus. All stocks were stored at −70 °C until use.

Virus assay. For demonstration and quantitation of the virus the rabbit test was used as described (Fennestad *et al.*, 1980). Briefly, the inoculum to be examined was given s.c. to a rabbit. Fever together with uveitis (ocular inflammation with iris hyperaemia and episcleral injection) or death with necropsy findings characteristic of PED, or both, were taken as evidence of PEDV in the inoculum. Animals failing to show these signs were challenged 30 days after inoculation with 10<sup>4</sup> RID of the virulent PEDV. Clinical protection following challenge was taken as evidence of virus in the inoculum.

Rabbits. New Zealand White rabbits, aged 3 to 5 months, were used and all inoculations were made by the s.c. route in 1 ml amounts, using PBS as diluent. Most of the rabbits were females which had been employed once for pyrogen testing of protein fractions of human blood. All rabbits came from the same closed colony (stock code name: Ssc : CPH) which had supplied rabbits for our previous experiments on PED. During the experiments the rabbits, including uninfected control rabbits, were housed under the same conditions.

Infection experiments. For examination of pathogenicity of the isolates, groups of rabbits were inoculated with virus stocks diluted to contain 10 RID. The rabbits were then observed twice daily for typical clinical signs of PED. A rectal temperature of 40 °C or more was considered to represent fever (normal temperature about 39 °C), and the time interval between inoculation and the onset of fever was considered to be the incubation period. Animals that died were examined for gross lesions and bacteriologically as described (Fennestad *et al.*, 1975); the time interval between inoculation and death was calculated as death time. Surviving rabbits were challenged with virulent PEDV. In Table 2 are included the results of previous comparable infection experiments with a highly virulent derivative and an avirulent derivative of the original PEDV isolate from 1970 (Fennestad, 1985).

In another experiment, single, male rabbits were inoculated with 0.2 ml of the original infectious material received from the laboratories. These rabbits represented the first passage of the isolates in Copenhagen, except for the Stockholm agent where the inoculum consisted of 72 h serum from our second rabbit passage of infectious serum 73-015. The nine rabbits were monitored by serum sampling from post-inoculation day 30. All sera were stored at −70 °C until examined. To demonstrate presence or absence of circulating virus, 0.2 ml of serum was used for inoculation. Virus concentration in selected serum samples was determined as for the virus stocks. At the
Pleural effusion disease virus in rabbits

Table 1. PEDV isolates from rabbits used for propagation of T. pallidum

<table>
<thead>
<tr>
<th>Isolate designation*</th>
<th>History of intercurrent mortality</th>
<th>Date isolated</th>
<th>Virus stock from rabbit passage no.</th>
<th>Titre of stock virus†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stockholm agent‡</td>
<td>+</td>
<td>1970</td>
<td>2</td>
<td>10⁶</td>
</tr>
<tr>
<td>Oslo</td>
<td>+</td>
<td>17/10/78</td>
<td>1</td>
<td>10⁵</td>
</tr>
<tr>
<td>Utrecht</td>
<td>+</td>
<td>1/11/78</td>
<td>1</td>
<td>10⁶</td>
</tr>
<tr>
<td>Paris I</td>
<td>+</td>
<td>8/12/78</td>
<td>1</td>
<td>10³</td>
</tr>
<tr>
<td>Minneapolis</td>
<td>0</td>
<td>19/3/79</td>
<td>1</td>
<td>10⁴</td>
</tr>
<tr>
<td>Wroclaw</td>
<td>0</td>
<td>9/5/79</td>
<td>1</td>
<td>10³</td>
</tr>
<tr>
<td>Paris II</td>
<td>0</td>
<td>27/6/79</td>
<td>1</td>
<td>10⁴</td>
</tr>
<tr>
<td>Tokyo</td>
<td>0</td>
<td>21/7/79</td>
<td>1</td>
<td>10³</td>
</tr>
<tr>
<td>Tel Aviv</td>
<td>+</td>
<td>7/5/80</td>
<td>1</td>
<td>10⁶</td>
</tr>
</tbody>
</table>

* Designation of isolate according to geographical origin.
† Rabbit-infectious doses per ml.
‡ Received 16 June 1980 from U.S.A. as infectious rabbit serum 73-015.

end of the observation period the rabbits were killed for examination. Two groups of uninfected rabbits of the same age as the infected animals served as controls for serological and histopathological examinations.

Protein, IgG and immune complexes. Protein concentrations in sera from the rabbits observed for chronic viraemia were determined by means of a clinical refractometer (American Optical) and the IgG concentration was determined by the single radial immunodiffusion method (Mancini et al., 1965). For detection of complement-fixing circulating immune complexes the polyethylene glycol–complement consumption (PEG–CC) assay (Brandslund et al., 1981) was used.

Light and immunofluorescence microscopy. Rabbits were killed 2 years after infection with the isolates and examined for gross and microscopic lesions. The kidneys were specifically examined for evidence of glomerulonephritis and deposits of immunoglobulins and complement factor C3.

All tissue specimens were fixed in Lillie's buffered formalin. Paraaffin-embedded sections, cut at 6 to 7 μm, were routinely stained with haematoxylin and eosin (H & E), periodate–Schiff's reagent (PAS) and van Gieson–Alcian blue. Sections from lymphoreticular tissues were stained with methyl green pyronine and Perl's prussian blue and sections from the central nervous system were stained by the gallocyanin method. For staining of microorganisms the Gram, Ziehl–Neelsen and Grocott stains were used. Sections from the following organs or tissues were examined: cerebrum, cerebellum, medulla oblongata, thymus, lung, myocardium, liver, spleen, kidney, adrenal gland, popliteal lymph node, duodenum, ileum and colon.

Tissue blocks from the kidneys were embedded in Paraplast® and cut at 2 and 3 μm and stained with H & E, PAS + H, and silver methamine + H & E.

For immunofluorescence microscopy (IFM), kidney tissue frozen in a dry ice–alcohol mixture was embedded in Tissue Teck®-gelatine mixture (Ames Laboratories) and cut into 1 μm sections at −24 °C. The details of preparation and the IFM procedures have been described previously (Larsen, 1978). Fluorescein isothiocyanate-conjugated antisera (Nordic Immunological Laboratories) from goat, specific for rabbit immunoglobulins (IgG1, IgG2, IgA, IgM, Fc + Fab) and rabbit complement factor C3, and sera from swine specific for rabbit IgG (Fc + Fab), were used at a 1:20 dilution.

RESULTS

Pathogenicity of isolates

The isolates differed considerably in their ability to cause disease and death in rabbits. In Table 2 the nine isolates are shown in order of decreasing pathogenicity and the results of comparable infection experiments with the virulent and avirulent derivatives of the original PEDV isolate are also included.

Typical clinical signs of PED, i.e. fever, uveitis and death with pleural effusion (up to 50 ml plasma-like fluid), were observed after infection with seven of the isolates whereas a subclinical course, except for transient fever in some of the animals, was observed after infection with two of the isolates. The incubation period for these two groups of isolates was about 3 and 4 days, respectively. Pleural effusion was observed in most of the fatal infections, but several of the rabbits infected with the Tel Aviv or the Stockholm agent did not show this characteristic of PED. This suggests that pleural effusion per se was not the cause of death.
Table 2. Comparative rabbit pathogenicity of PEDV isolates

| Isolate            | Inoculum (RID)* | Number of rabbits | Incubation period (mean)† | Died | Survivors showing | Result of challenge|||
|--------------------|----------------|------------------|---------------------------|------|------------------|-------------------|
|                    |                |                  |                           | Number | P.i. day | MDT‡ | PE$ | Fever | Uveitis |               |
| Tel Aviv           | 10             | 8                | 2-4 (2-7)                 | 7     | 5-11   | 7-3  | 2   | 1     | 1       | 1/1            |
| Stockholm agent    | 10             | 8                | 2-5 (3-2)                 | 5     | 4-16   | 8-6  | 3   | 3     | 2       | 3/3            |
| Utrecht            | 10             | 8                | 3 (3-0)                   | 2     | 5-6    | 5-5  | 2   | 5     | 4       | 6/6            |
| Paris I            | 10             | 8                | 3-4 (3-1)                 | 2     | 11     | 11   | 2   | 6     | 3       | 6/6            |
| Oslo               | 10             | 8                | 3-7 (3-6)                 | 2     | 4-5    | 4-5  | 2   | 4     | 6       | 6/6            |
| Wroclaw            | 10             | 8                | 2-5 (2-7)                 | 1     | 4      | 4    | 1   | 7     | 7       | 7/7            |
| Tokyo              | 10             | 8                | 3 (3-0)                   | 1     | 4      | 4    | 1   | 6     | 6       | 7/7            |
| Paris II           | 10             | 8                | 3-6 (3-6)                 | 0     |        |      |     | 5     | 0       | 8/8            |
| Minneapolis        | 10             | 8                | 4 (4-0)                   | 0     |        |      |     | 2     | 0       | 8/8            |
| PEDV               | 10             | 8                | 3 (3-0)                   | 5     | 4-5    | 4-4  | 5   | 3     | 3       | ND‡            |
| PEDV, avirulent    | 10             | 16               | 3-6 (4-3)                 | 0     |        |      |     | 3     | 0       | 16/16          |

* Number of rabbit-infectious doses.
† Time in days from inoculation until onset of fever.
‡ Mean death time as average time in days after inoculation.
§ Number of dead animals with pleural effusion.
¶ Numerator equals number of protected and denominator number of rabbits challenged with PEDV.
K. L. Fennestad and others
Table 3. Presence of viraemia and virus concentration at various times after rabbit infection with PEDV isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Examination of serum months after infection*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 9 12 15 18 21 24</td>
</tr>
<tr>
<td>Tel Aviv</td>
<td>10^{+} + 10^{0} + + + 10^{2} + + + 10^{2}</td>
</tr>
<tr>
<td>Stockholm agent</td>
<td>10^{1} + 10^{1} + 0 &lt; 10^{1} + 10^{1} + + + &lt; 10^{1}</td>
</tr>
<tr>
<td>Utrecht</td>
<td>10^{+} + 10^{1} + + + 10^{2} + 10^{2} 0 + 0 10^{2}</td>
</tr>
<tr>
<td>Paris I</td>
<td>10^{2} ND 10^{5} + ND 10^{2} + 10^{2} + + + 10^{2}</td>
</tr>
<tr>
<td>Oslo</td>
<td>ND ND 10^{1} + + 10^{5} + 10^{1} + + + 10^{1}</td>
</tr>
<tr>
<td>Wroclaw</td>
<td>10^{1} + 10^{1} + + + 10^{2} + 10^{2} + + + 10^{2}</td>
</tr>
<tr>
<td>Tokyo</td>
<td>10^{3} + 10^{3} + + + 10^{3} + 10^{3} + + + 10^{3}</td>
</tr>
<tr>
<td>Paris II§</td>
<td>10^{+} + &lt; 10^{1} 0 + 10^{2} 0 &lt; 10^{1} 0 0 0 &lt; 10^{1}</td>
</tr>
<tr>
<td>Minneapolis</td>
<td>10^{2} + &lt; 10^{1} + 0 10^{1} + 10^{1} 0 + + 10^{1}</td>
</tr>
</tbody>
</table>

* Lower limit of detection for absence of virus 10^{-1} or 2 \times 10^{-1} for untitrated samples.
† Number of rabbit-infectious doses per ml.
‡ ND, Not done.
§ Paris II represents a period in the continuous rabbit propagation of *T. pallidum* 5 years earlier than the date of isolation.

The Paris I and Paris II isolates came from the same laboratory, but were separated by a period of 5 years of rabbit propagation of treponemes. Assuming that the earlier isolate, Paris II, continued to be a passenger in treponemal suspensions of rabbit testes, the higher pathogenicity of Paris I as compared with Paris II could be explained by an increase in virulence of the virus during the 5 year period of continuous rabbit passages.

By assessment of the pathogenicity of the isolates in terms of their ability to kill, the isolates could be divided into three groups, one causing mortality of more than 50%, a second causing mortality of less than 50%, and a third causing no mortality. Comparing these three groups of isolates with the virulent and avirulent PEDV in terms of mortality, the pathogenicity of virulent PEDV corresponds to group 1 while that of avirulent PEDV corresponds to group 3. However, if pathogenicity is also measured by the rapidity by which death occurs after inoculation, i.e. mean death time (MDT) as shown in Table 2, virulent PEDV would appear to be more pathogenic than the group 1 isolates.

When the surviving rabbits were challenged with virulent PEDV there were no differences between the ability of isolates to establish clinical protection. This suggests that the observed differences in pathogenicity of the isolates were not associated with differences in their immunogenicity.

**Persistence of viraemia**

Nine rabbits, each infected with one isolate, were observed for viraemia during the chronic phase of infection, i.e. from post-inoculation day 30 until 2 years after inoculation (Table 3). During this period viraemia could be demonstrated regularly in most of the rabbits, the only exception being the Paris II-infected rabbit in which viraemia was not demonstrable after the 6th month. The virus concentration in the serum of the rabbits ranged from 10^{1} to 10^{4} RID/ml 1 month after infection. At 3 months these titres had declined slightly and from the 6th month until the end of the period of observation the virus titres ranged from less than 10^{1} to 10^{2} RID/ml. This indicates that the isolates, independent of their pathogenicity, induced a similar chronic low level viraemia.

In the *in vivo* assays for chronic viraemia, group 3 isolates were demonstrable only by induction of protection to challenge. Circulating virus from rabbits inoculated with group 1 or 2 isolates caused clinical disease (but not death) when obtained 1 to 2 months after inoculation. By contrast, group 1 or 2 isolates obtained after the 3rd month and later on resulted in subclinical infections demonstrable by immunity to challenge. This suggests that progeny of the pathogenic isolates with continued viraemia gradually lost their capacity to cause disease and that all isolates obtained from the 3rd month of infection were equally low- or non-pathogenic on primary rabbit inoculation.
Fig. 1. Arithmetic mean concentrations of serum IgG in nine rabbits with chronic viraemia after PEDV infection (●) as compared with four normal rabbits (○).

**Concentration of serum proteins and IgG during chronic infection**

The group of rabbits with chronic viraemia listed in Table 3 and the control group of uninfected rabbits showed similar levels of serum protein during the observation period. The range of protein concentrations for the two groups was 42.5 to 74.0 and 47.0 to 72.5 mg/ml, respectively.

The level of IgG for the two groups differed distinctly (Fig. 1). The infected rabbits showed a rise in IgG from the 3rd month and this rise continued almost until the end of the observation period, whereas the control group showed no rise. The range of IgG concentrations for the two groups was 3.51 to 17.99 and 3.26 to 7.91 mg/ml, respectively. Values above 10 mg IgG per ml were seen only in one rabbit during the last quarter of the observation period. Analyses of the differences between IgG levels of the two groups during the first 2 months (post-inoculation months 1 and 2) and the last 6 months (post-inoculation months 18 and 24) of the observation period showed a significant increase for the viraemic rabbits, whereas there was no increase for the control group (P 0.0014, Wilcoxon test for two samples).

Grouping the viraemic rabbits according to the pathogenicity of the isolates they received did not reveal any differences in IgG concentrations among the three pathogenicity groups. This may be attributed to the small number of rabbits used and individual differences among rabbits in response to the same antigenic stimulus.

**Immune complexes during chronic infection**

Consecutive serum samples obtained at 0, 30, 90, 180 days and 1 and 2 years after infection of the rabbits which developed chronic viraemia were tested in the PEG–CC assay. Of the 52 samples examined, only one showed a weak positive reaction for circulating immune complexes. This serum sample was collected 2 years after the rabbit had been inoculated with the virus.

**Histological findings**

The rabbits killed 2 years after infection appeared normal at necropsy. Examination for bacteria by aerobic cultivation and for other microorganisms by histological staining gave negative results.

As compared with the controls, notable histological changes were found only in lymph nodes,
spleen, liver and lung. These changes could not be correlated with pathogenicity of the isolates. The lymph nodes showed dilated sinusoidal vessels, moderate proliferation of histiocytes and slight T cell hyperplasia. Similar changes, but also with phagocytic cells in the sinusoids were found in the spleens. In addition, the spleens showed extreme deposits of iron in the sinusoidal reticulum cells (siderosis). Slight portal inflammation and scattered parenchymal necrosis could be seen in the livers. The myocardial changes were variable, but slight interstitial fibrosis with a few mononuclear cells were often seen. In the interstitial tissue of the lungs there was an increase in aggregates of lymphocytes as compared with the controls.

No pathological changes were found in the kidney glomeruli and vessels, and immune deposits could not be demonstrated in the glomeruli. In three of nine rabbits with persisting viraemia and in two of the three controls a small amount of complement factor C3 was found in a segmental pattern solely along the basement membrane in a few of the capillary loops in some of the glomeruli.

DISCUSSION

The wide range in pathogenicity of the isolates corresponds fairly well to observations made in the laboratories supplying the isolates. Thus, group I isolates came from laboratories having experienced serious intercurrent rabbit mortality, whereas group 3 isolates were from laboratories with no mortality. The group 2 isolates were intermediate with respect to mortality, or caused no mortality (Fennestad et al., 1980, 1982). This indicates that commonly used laboratory rabbits are probably equally susceptible to disease caused by PEDV.

The occurrence of low-pathogenic or non-pathogenic PEDV isolates in treponema-infected rabbits, loss of pathogenicity of circulating virus during the chronic phase of infection, and perhaps life-long persistence of viraemia, suggests that PEDV may exist in nature as a low- or non-pathogenic rabbit virus. At present, clinical disease resembling PED has not been described as a naturally occurring rabbit disease, and there is no information about the subclinical occurrence of PEDV infections outside laboratories studying treponematoses (Fennestad et al., 1975). This situation resembles the early stage in the study of lactate dehydrogenase-elevating virus (LDV) infection in mice when the presence of this passenger virus was linked to experimental mouse neoplasms; later on, LDV infection was found in both mouse colonies and wild mice (Riley et al., 1960; Rowson & Mahy, 1975).

The enduring rise in IgG concentration in the group of viraemic rabbits is considered to be evidence of continued antigenic stimulation. This is compatible with our observation of a significantly higher IgG concentration in 6-month-old viraemic rabbits after neonatal infection with PEDV as compared with control age-matched cage mates. Serum from these viraemic rabbits, rendered non-infectious by ether treatment, had a protective effect. This indicates that the increased level of IgG was due in part to PEDV antibodies (Fennestad et al., 1981). In a clinical study of PED it was found that electrophoretic gamma globulin increased significantly during the 2nd month after virulent infection, i.e. after development of clinical protection to challenge (Fennestad et al., 1975). The histopathological changes after chronic infection differ from those observed in the acute phase (Christensen et al., 1978) by a return to the normal state of the thymus and partial recovery of the lymph nodes. The T cell hyperplasia and the siderosis of the spleen appear to be features of the chronic infection. Inflammatory changes of the liver and myocardium were also present in animals examined 5 to 48 days after PEDV infection and seem to persist to a milder degree during chronic infection.

Lack of demonstrable circulating immune complexes or histopathological evidence of immune complex disease is at variance with the findings in well-studied animal models of persisting viral infections (Oldstone & Dixon, 1975; Porter et al., 1984; Cafruny & Plagemann, 1982). It should be pointed out that the assay used here for immune complex detection is complement-dependent and only registers complexes containing complement-fixing antibodies. The detection limit of this assay is approximately 25 µg immune aggregates/ml serum. Thus, it can not be excluded that low levels of certain types of immune complexes may be present in rabbits with chronic PEDV infection. In further studies, attempts will be made to demonstrate PED virus in material precipitated by antibodies to rabbit IgG.
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REFERENCES


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