Dee SA. Pathogenesis and immune response of nonporcine arteriviruses versus porcine arteriviruses. *Swine Health and Production*. 1998;6(2):73–77.

Pathogenesis and immune response of nonporcine arteriviruses versus porcine arteriviruses

Scott A. Dee, DVM, PhD, Diplomate; ACVM

Summary

The pathogenesis and immune response of pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) are not completely understood. PRRSV, along with equine viral arteritis (EAV), lactate dehydrogenase elevating virus of mice (LDV), and simian hemorrhagic fever virus (SHFV), are members of the genus *Arteriviridae*. This review summarizes the similarities and the differences found in the pathogenesis and immune response of nonporcine and porcine arteriviruses.

Keywords: swine, porcine reproductive and respiratory syndrome virus, PRRSV, Arteriviridae, equine arteritis virus, simian hemorrhagic fever virus, lactate dehydrogenase elevating virus

Received: September 11, 1996
Accepted: September 11, 1997

The pathogenesis and immune response of pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) are not completely understood. PRRSV is a member of the genus *Arteriviridae*, and an understanding of the other viruses in this genus can contribute to our understanding of pathogenic and immune mechanisms in pigs infected with PRRSV.

The genus *Arteriviridae* includes four members:

- equine viral arteritis virus (EAV);
- lactate dehydrogenase elevating virus (LDV) of mice;
- simian hemorrhagic fever virus (SHFV); and
- the newest member: porcine reproductive and respiratory syndrome virus (PRRSV).[1,2]

These viruses all share some common characteristics,[1] including:

- a size of 50- to 65-nanometers in diameter,
- an envelope with glycosylated surface projections,
- a 25- to 35-nanometer icosahedral nucleocapsid,
- single-stranded positive-sense RNA, and
- replication via budding of the nucleocapsid through cellular cytoplasmic membranes.

The taxonomy of the Arteriviruses has recently been changed. Based on common features of the *Coronaviridae* and *Arteriviridae*, they have been placed together in the order *Nidovirales*.[2] The taxonomic category of "order" is defined as a classification to include families of viruses with similar genomic organization and replication strategies.

Viruses are classified in the order *Nidovirales* if they have the following characteristics:

- linear, nonsegmented, positive-sense, single-stranded RNA;
- genome organization: 5'-replicase (polymerase) gene structural proteins-3';
- a 3' coterminal nested set of four or more subgenomic RNAs;
- the genomic RNA functions as the mRNA for translation of gene 1 (replicase); and
- only the 5' unique regions of the mRNAs are translated.

This report reviews the literature on the nonporcine *Arteriviridae* in hopes of elucidating the pathogenic and immune mechanisms in pigs infected with PRRSV.

Pathogenesis of equine viral arteritis virus (EAV)

Equine viral arteritis is an equine disease of low prevalence that has assumed increased significance since a 1984 epidemic in Kentucky thoroughbreds.[3] In the United States, one serotype of EAV has been reported; however, American and European isolates differ genetically.[4,5,6] Infection can be transmitted through the respiratory and venereal routes.[3,7] This virus has been isolated from buffy coat and lung tissue of healthy horses, and renal tissue from experimentally infected animals.[8]

After infection by the respiratory route, the virus is disseminated via alveolar macrophages to regional lymph nodes.[3] Viremia can be detected by 3 days postinfection (PI). The incubation period is 7 days PI and virus can be detected 7 days PI in respiratory secretions, 14 days PI in urine, and 28 days PI from vaginal swabs.[9] The primary sequel of EAV infection is a severe arteritis.[10] Viral replication occurs in vascular endothelial cells, which stimulates the influx of neutrophils into the internal elastic lamina.[10] This results in degenerative changes, furthering the migration of cellular products, viruses, and inflammatory cells into the tunica media. The final outcome is end-stage fibrinoid necrosis.[10]

Infection with EAV causes abortion in pregnant mares, while stallions can be long-term carriers.[11] Equine viral arteritis virus localizes in the reproductive tract of the stallion and can be shed in semen for 1–2 years PI.[8] The carrier status appears to be testosterone dependent: a long-term carrier state has not been demonstrated in prepubertal

SAD: Swine Health Center, 621 Pacific Avenue, Morris, Minnesota 56267; email: sss1dvm@rea-alp.com

This article is available online at http://www.aasp.org/shap/issues/v6n2/index.html

colts, but experimental infection of peripubertal colts resulted in viral shedding for up to 15 months PI.[12] Carrier stallions only shed virus via the venereal route.[13] Virus appears to localize in the ampulla of the vas deferens and is only shed in the sperm-rich fraction of the ejaculate.[8] There has been no evidence of intermittent shedding or the existence of a latent state.[14]

Abortion in pregnant mares occurs after they are exposed to contaminated semen via natural service or artificial insemination, or after they are exposed to respiratory secretions.[13–15] Clinical signs prior to abortion are anorexia, lameness, fever (41°C), conjunctivitis, and nasal discharge.[3] Abortion appears to result from a diffuse vacuolation of endometrial endothelium and a necrotizing vasculitis.[16] No consistent lesions have been reported in the fetus, but pulmonary edema has been described.[17] However, it is difficult to identify microscopic lesions because of autolysis. Although rare, transplacental transmission can occur if the mare is exposed to the virus in the third trimester.[3] No reports of teratological abnormalities exist; however, infected foals may develop interstitial pneumonia and fibronecrotic enteritis shortly after birth.[3]

Immune response

Immunity after natural infection with EAV has been reported to endure for up to 7 years.[7] After infection, the host produces complement fixing (CF) and serum neutralizing (SN) antibodies.[7] Neutralizing antibodies peak within 1–2 months and last for up to 3 years or more.[7] The CF antibodies peak 2–3 weeks PI and persist for 8 months.[7] Numerous studies have documented the importance of CF antibody.[2,7,18–20] Equine viral arteritis virus and CF antibody produce infectious complexes. Complement components C1423 bind to virus : antibody complexes and block attachment of EAV to host cell receptor sites. Then, the lipoprotein envelope lyses by enzymatic action of components C5–9.

Immunization with modified-live virus (MLV) and killed virus (KV) preparations have been described.[21–25] Vaccination with MLV is safe in stallions and open mares. It is not recommended in pregnant mares, particularly during late gestation, or in foals less than 6 weeks old.[21–23] Foals born to mares that have been immunized with MLV vaccines are protected up to 6 weeks via colostral antibodies.[7] Modified-live virus vaccination appears to protect against challenge with wild-type EAV in the majority of immunized horses.[15,24] A protective immune response appears within 8 days post vaccination and lasts for 1–3 years.[15] The vaccine does not prevent infection, but does reduce the duration of shedding. Although shedding of wild-type virus occurs after vaccination, the amount of virus does not appear to be sufficient to infect susceptible contact controls. While prior vaccination with MLV does prevent the carrier state, it has not been beneficial in eliminating the carrier state from infected stallions, and can cause a temporary increase in sperm cell abnormalities.

The use of killed preparations have also been reported.[25] Multiple (3–4) doses were required to observe SN titers lasting 6 months. However, not all vaccinated horses were protected after challenge, and a 50% protective level was not demonstrated with SN titers of < 1:43. Titers after vaccination peaked at 1:5120 and then declined rapidly. Follow up boosters stabilized titers for 6 months at 1:80–1:320.[25]

Pathogenesis of lactate dehydrogenase elevating virus (LDV) of mice

Lactate dehydrogenase elevating virus causes lifelong asymptotic infections in mice characterized by persistent viremia.[2] Virus is spread primarily via saliva and bite wounds.[2] Transmission via the vaginal route requires 4–6 times the concentration of virus necessary for infection via other routes. Transplacental infection of LDV has been reported, but it is limited to a short period of time (1–4 days) PI.[26]

High titers (10^{10} ID_{50} per mL) of virus are observed 1 day PI.[2] Lactate dehydrogenase elevating virus replicates in certain permissive subpopulations of peritoneal macrophages. One- to 2-week-old mice are highly susceptible to infection and up to 80% of the macrophages from mice of this age are reported to be infected.[2] In contrast, only 5%–15% of macrophages harvested from mice from 2–5 weeks of age are susceptible to infection. Persistent infection is maintained by new susceptible cells that are produced and then infected over time. These cells may contain a yet-undefined receptor on the cell-surface membranes that enhances viral attachment and subsequent replication.[2] Persistently infected macrophages may reside in lymph nodes, spleen, thymus, liver, and the lepto-meninges. Replication occurs in the perinuclear region, destrogying macrophages, elevating the plasma lactate dehydrogenase.[2,27]

Only certain species of rodents can be infected with LDV. Wild house mice (*Mus musculus domesticus*) from Australia, Germany, the United States, and England are susceptible to infection.[28] Infection has not been demonstrated in mice of *Peromyscus* spp., nor has it been possible to demonstrate infection in rats, guinea pigs, or rabbits.[28] The resulting clinical disease following LDV infection is an age-related poliomyelitis; however, numerous factors must be present for this to occur.[29] Certain genetic lines of mice (AKR and C58) demonstrate clinical disease when infected with neurovirulent variants of LDV. C58 and AKR strains of mice are also permissive for induction and replication of murine leukemia virus (MuLV), and the expression of MuLV in glial cells renders neurons of the anterior horn susceptible to infection by neurovirulent strains of LDV. Lactate dehyodrogenase elevating virus is released into the cerebral spinal fluid and is disseminated into the CNS, resulting in lysis of motor neurons with subsequent paralysis. However, paralysis does not develop unless the immune response is suppressed or until the mouse reaches 12 months of age.[30,31] The immune response can be suppressed by administering radiation or cyclophosphamide.

Immune response

Initially after infection with LDV, mature macrophages are destroyed. Over time, susceptible macrophage subpopulations are regenerated, resulting in persistent infection. After infection, high concentrations of nonneutralizing antibodies are generated (IgG_{2a} and IgG_{2b}),[32] which form immune complexes.[2] Two theories have been presented to

explain the poor neutralizing response:

- the immune system lacks the ability to recognize an essential neutralizing epitope on the virus glycoprotein, or
- immune complex formation may shield the virus from the body's defense system.[1]

Despite the presence of antigen-antibody complexes, glomerular disease is not seen.[33] Lactate dehydrogenase elevating virus has other effects on the immune system, including decreased autoantibody production, transient thymic necrosis, lymphopenia, and suppression of cell-mediated immune responses.[33]

Eventually, a specific anti-LDV antibody is formed in the host animal that can recognize a different epitope on the VP-3 glycoprotein.[34] The binding of the multiple antibodies to each virion is believed to neutralize the virus, resulting in sloughing of the viral envelope, exposure of the nucleocapsid, and enzymatic degradation of viral RNA.[34] High antibody:virus ratios are required to obtain neutralization activity.[2,34] The older mouse may be unable to produce the required concentration of antibody, resulting in the observed relationship between aging and the incidence of poliomyelitis.[34]

Pathogenesis of simian hemorrhagic fever virus (SHFV)

Simian hemorrhagic fever virus (SHFV) was first identified as the etiologic agent responsible for outbreaks of severe hemorrhagic disease with high mortality in primate centers in the former Soviet Union and the United States.[33] Simian hemorrhagic fever virus replicates in peritoneal macrophages of rhesus and patas monkeys and the kidney cells of African green monkeys.[32,35,36] These species of African monkeys carry the virus in an asymptomatic state and are persistently infected for life. In one serologic survey, approximately 50% of patas monkeys were positive for SHFV antibodies.

In contrast, infection of Asian macaques results in acute fatal hemorrhagic disease.[35,36] The cause of death is shock secondary to vasodilation, statis, and venous thrombosis.[35,36] The route of virus transmission is thought to be salivary, secondary to bite wounds.[33,35,36] Transmission via contact with infected nasal and aerosol secretions, and spread via contaminated needles has been reported.[33,35,36] Insect vectors have not been demonstrated. The high lethality of SHFV in Asian macaque monkeys is hypothesized to be due to the extreme sensitivity of their macrophage population to cytocidal infection.

Immune response

Infection of African monkeys with SHFV variants of low pathogenicity results in persistent viremia with minimal anti-SHFV antibody response.[33,35,36] In contrast, infection with a highly virulent strain resulted in a rapid immune response with clearance of virus from the body within 21 days PI. Coinfection of persistently infected patas monkeys with virulent isolates (superinfection) of SHFV also results in clearance of virus.[36] Transplacental transmission does not occur despite persistent infection of pregnant females and it is hypothesized that blockage is due to anti-SHFV antibodies.[35,36]

Discussion

Numerous similarities exist between the nonporcine Arteriviruses and PRRSV. PRRSV infection takes place through the respiratory and the venereal route, with virus being shed intermittently via the urine, feces, saliva, and semen.[37] The alveolar macrophage is the primary host cell of PRRSV, and the virus replicates within and destroys mature alveolar macrophages.[37] Replication can also take place in vascular endothelial cells and necrosis of vascular tissue occurs in a number of tissues PI.[38] After PRRSV infection, a prolonged viremia exists in the presence of circulating antibodies and persistent infection of macrophages can occur.[39] Persistently infected cells can be detected in the lung, lymphoid tissue, the male reproductive tract, and the brain.[40] Transplacental infection of PRRSV occurs during the third trimester of gestation, and piglets can be born viremic and remain persistently infected for extended periods.[41]

The rapid spread of PRRSV throughout the international swine industry has raised the question of its origin. There are clearly variants of PRRSV; however, the greatest diversity exists between the North American and European genotypes.[42] While there is a 40% nucleotide divergence between the two strains, the ORF5 envelope glycoproteins within each variant are closely related to each other and to LDV.[1] While EAV is distantly related to PRRSV, certain amino acids in all ORFs are conserved between both viruses.[1] Finally, PRRSV, SHFV, and EAV all readily replicate in African green monkey kidney cell lines (MA-104).[43] In contrast, EAV replicates in numerous cell lines of related and unrelated species, including baby hamster kidney cells, rabbit kidney cells, and kidney cells of rhesus monkeys.[43,44]

In the case of PRRS and EAV, cellular immunity is critical for protection. Modified-live virus vaccines are more effective than killed preparations. Passive immunity after natural infection to PRRSV exists for up to 4–6 weeks postpartum. While serum neutralizing antibodies clear PRRSV from the bloodstream, animals may remain persistently infected for up to 3 months PI, even in the presence of neutralizing antibodies.[39]

Attempts to extrapolate current knowledge of the nonporcine Arteriviruses to PRRSV raise the following questions:

- Is the carrier state of PRRSV testosterone-dependent as seen in the case of EAV? Will vaccination of male pigs prior to puberty reduce the chances of developing such a carrier state?
- Will modified-live vaccination against PRRSV assist in clearing the carrier state in boars?
- Will vaccination with a killed product containing PRRSV result in transient protective immunity similar to that seen with EAV?
- What is a protective antibody titer after vaccination with killed virus compared to MLV vaccines?
- What is the interval between vaccinations required to induce a protective response?
- Will "superinfection" with another strain of PRRSV enhance elimination of infection as it does in SHFV? Can commercially available

MLV vaccines fulfill the role of "superinfection"?

- Are genetic relationships similar to those observed in SHFV and LDV important for PRRSV-related disease problems? Variants of PRRSV exist, differing in virulence and molecular structure; what, then, is the role of genetics? Are certain lines of pigs more susceptible to PRRSV infection and does infection of a genetic line of swine with a specific variant of the virus result in variations in clinical presentation?
- Immune complexes have been described as playing a significant role in the perpetuation of LDV and EAV survival and replication. Antibody-dependent viral enhancement has been demonstrated as an in vitro mechanism to enhance PRRSV replication. Is this phenomenon demonstrable in vivo? Does it play a significant role in maintaining viral infection in chronically infected swine farms?

At this time, it is unknown whether data specific to the nonporcine members of the Arterivirus genus will assist in providing the answers to future questions on PRRSV. However, attempts to understand PRRSV in terms of LDV, EAV, and SHFV will be far more effective than attempting to design control measures that are modeled after characteristics of unrelated viruses, such as Aujeszky's disease virus (pseudorabies virus) or swine influenza virus.

Implications

- Porcine arteriviruses have both similarities and dissimilarities with nonporcine arteriviruses.
- A heightened understanding of the nonporcine arteriviruses can enhance efforts to elucidate the pathogenesis and immune response of pigs infected with PRRSV.

References

1. Plagemann PGW. Lactate dehydrogenase-elevating, virus and related viruses. In Fields BN, Kimpe PM, Hawley PM, eds. *Virology* 3rd ed. Philadelphia, Pennsylvania: Lippencott-Raven Publishers. 1996.

2. Cavanagh D. Nidovirales: A new order comprising *Coronaviridae* and *Arteriviridae*. *Arch Virol.* 1997; 142(3):629–633.

3. Timoney PJ, McCollum WH. Equine viral arteritis. *Vet Clinics of North America: Equine Practice*. 1993;9(2):295–309.

4. Fukunaga Y, McCollum WH. Complement fixation reactions in equine viral arteritis. *Am J Vet Res*. 1977; 38:2043.

5. Murphy TW, Timoney PJ, McCollum WH. Analysis of genetic variation among strains of equine arteritis virus. In Powell DG (ed). *Proc 5th Int Conf*, Lexington, Kentucky: University Press of Kentucky. 1987; p 3.

6. Murphy TW, Timoney PJ, McCollum WH, et al. Genomic variability among globally distributed isolates of equine arteritis virus. *Vet Microbiol*. 1992;32:101.

7. Chirnside ED. Equine arteritis virus: An overview. *Br Vet J*. 1992; 148:181–197.

8. Neu SM, Timoney PJ, McCollum WH. Persistent infection of the reproductive tract in stallions experimentally infected with equine arteritis virus. In Powell DG (ed): *Equine Infectious Diseases V*: Proc 5th Int Conf. Lexington, Kentucky: University Press of Kentucky, 1987; p. 149.

9. McCollum WH, Prickett ME, Bryans JT. Temporal distribution of equine arteritis virus in respiratory mucosa, tissues and body fluids of horses infected by inhalation. *Res Vet Sci*. 1971; 2:459.

10. Jones TC, Doll ER, Bryans JT. The lesions of equine viral arteritis. *Cornell Vet*. 1957; 47:52.

11. Timoney PJ, McCollum WH, Roberts AW, et al. Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. *Res Vet Sci*. 1986; 41:279.

12. Little TV, Holyoak GR, McCollum WH, et al. Output of equine arteritis virus from persistently infected stallions is testosterone-dependent. In *Equine Infectious Diseases VI*: Proc 6th Int Conf. Cambridge, United Kingdom: University of Cambridge Press. 1991;225.

13. Timoney PJ. The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of virus transmission. *J Reprod Fertil Suppl*. 1987;35:95.

14. Timoney PJ, McCollum WH, Murphy TW, et al. A longitudinal study of equine arteritis virus infection in Standardbred stallions with special reference to occurrence of the carrier state. In: *Equine Infectious Diseases VI*: Proc 6th Int Conf, Cambridge, United Kingdom: University of Cambridge Press. 1991

15. McCollum WH, Timoney PJ, Roberts AW, et al. Response of vaccinated and nonvaccinated mares to artificial insemination with semen from stallions persistently infected with equine arteritis virus. In: *Equine Infectious Diseases V*: Proc 5th Int Conf, 1987, Lexington, Kentucky: University Press of Kentucky, 13.

16. Coignoul FL, Cheville NF. Pathology of the maternal genital tract, placenta and fetus in equine viral arteritis. *Vet Pathol.* 1984; 21:333.

17. Johnson B, Baldwin C, Timoney P, et al. Arteritis in equine fetuses aborted due to equine viral arteritis. *Vet Pathol.* 1991; 28:248.

18. Radwan AI, Crawford TB. The mechanisms of neutralization of sensitized equine arteritis virus by complement components. *J Gen Virol.* 1974; 25:229–237.

19. Radwan AI, Burger D. The complement-requiring neutralization of equine arteritis virus of late antisera. *Virology*. 1973; 51:71–77.

20. Radwan AI, Burger D, Davis WC. The fate of sensitized equine arteritis virus following neutralization by complement or anti-IgG serum. *Virology.* 1973; 53:372–378.

21. Doll ER, Bryans JT, Wilson JC, et al. Immunization against equine viral arteritis using modified live virus propagated in cell cultures of rabbit kidney. *Cornell Vet*. 1968; 48:497.

22. McKinnon AO, Colbern GT, Collins JK, et al. Vaccination of stallions with a modified live equine arteritis virus vaccine. *J Equine Vet Sci*. 1986; 6:66.

23. Timoney PJ, Umphenour NW, McCollum WH. Safety evaluation of a commercial live equine arteritis virus for use in stallions. In Powell DG (ed): *Equine Infectious Diseases V*, Proc 5th Int Conf. Lexington, Kentucky: University Press of Kentucky. 1987;19.

24. McCollum WH. Development of a modified virus strain and vaccine for equine viral arteritis. *JAVMA*. 1969; 155:318.

25. Fukunaga Y, Wada R, Matsumura T, Sugiura T, Imagawa H. Introduction of immune response and protection from equine viral arteritis by formalin inactivated virus vaccine for EAV in horses. *J Vet Med*. 1990; 37:135–141.

26. Haven TR, Rowland RRR, Plagemann PGW, et al. Regulation of transplacental virus infection by developmental and immunological factors: studies in lactate dehydrogenase elevating virus. *Virus Res*: 1996; 41:153–161.

27. Contag CH, Harty JT, Plagemann, PGW. Pathogenesis of age-dependent poliomyelitis. In: *Molecular Neurovirology*. Roos RP (ed). Totawa, New York: Humana Press. 1992;377–415.

28. Rowson KEK, Mahy EWS. Lactate dehydrogenase virus. *Viral* Manager: 1975;13.

29. Anderson GW, Palmer GA, Rowland RCC, et al. Infection of central nervous system cells by ecotropic murine leukemia virus in C58 and AKR mice and in utero-infected CE/J mice predisposes mice to paralytic infection by lactate dehydrogenase elevating virus. *J Virol.* 1996; 69:308–318.

30. Murphy WH, Mazur JJ, Fulton SA. Animal model for motor neuron disease. In Behan WMH, Behan PO, Aarli JA, eds. *Clinical Neuroimmunology*. Oxford, England: Blackwell Scientific. 1987; 135–155.

31. Martinez D, Briton M, Tachovsky TG, Phelps AH. Identification of LDV as the etiological agent of genetically restricted age-dependent polioencephalomyelitis of mice. *Infect Immun*. 1980; 27:979–987.

32. Li X, Hu B, Harty J, Even C, Plagemann PGW. Polyclonal B-cell activation of IgG_{2a} and IgG_{2b} production by infection of mice with lactate dehydrogenase-elevating virus is partly dependent on CD4+ lymphocytes. *Viral Immunol*. 1990; 3:273–288.

33. Fox JG, Cohen BJ, Loew FM: Laboratory Animal Medicine. London, England: Academic Press, Inc., 1984;59.

34. Harty JT, Plagemann PGW. Formalin inactivation of the lactate dehydrogenase-elevating virus reveals a major neutralizing epitope not recognized during natural infection. *J Virol.* 1988; 62:3210–3216.

35. Gravell M, Landon WT, Leon ME, et al. Differences among isolates of simian hemorrhagic fever virus. *Proc Soc Exp Biol Med*. 1986; 181:112–119.

36. Gravell M, Landon WT, Leon ME, et al. Elimination of persistent simian hemorrhagic fever virus infection in patas monkeys. *Proc Soc Exp Biol Med.* 1986; 181:219–225.

37. Done SM, Paton DJ, White MEC. Porcine reproductive and respiratory syndrome. *Br Vet J*. 1996; 152:153–174.

38. Lager KM, Halbur PG. Gross and microscopic lesions in porcine fetuses infected with PRRS virus. *J Vet Diagn Invest*. 1996; 8:275–282.

39. Meredith MJ. *PRRS.* 7th ed. Cambridge, England: University of Cambridge Press. 1993.

40. Shin J, Torrison J, Kang YB, Molitor TW. In situ localization of PRRSV in boar tissue. *Proc IPVS Cong*. Bologna, Italy. 1996; 57.

41. Benfield DA, Christopher-Hennings J, Nelson EA, et al. Persistent fetal infection of PRRS virus. *Proc AASP Ann Meet*. Quebec City, Quebec. 1997; 455–458.

42. Meulenberg JJM, Hulst MM, de Meijer EJ, et al. Lelystad virus, the causative agent of PEARS is related to LDV and EAV. *Virology.* 1993; 192:62–72.

43. Murtaugh M, Collins JE, Rossow KD, et al. PRRS. *Proc A.D. Leman Swine Conf,* Minneapolis, Minnesota. 1993; 43–53.

44. Horzinck MC. *Nonarthropod-borne Togaviruses*. London, England: Academic Press. 1981.