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In vitro studies on the use of clay, clay minerals and charcoal to adsorb bovine rotavirus and bovine coronavirus

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Abstract

Rotaviruses are the leading cause and coronaviruses are the major contributors of acute gastroenteritis in the young of various mammalian and avian species. Despite numerous trials and decades of research, vaccines have limited efficacy particularly for calves. As an alternative method of controlling infection, we have investigated broad spectrum antiviral agents that are not discriminatory among various viruses. This report involves testing a variety of adsorbent agents including charcoal, clay, and clay minerals to adsorb rotavirus and coronavirus in vitro. Results revealed that all the adsorbent agents had good to excellent capability of adsorbing rotavirus and excellent capability of adsorbing coronavirus. Percent adsorptions ranged from 78.74% to 99.89% for rotavirus and 99.99% for coronavirus; while sand (negative control) was <0.01%. A high affinity binding was present as determined by a low percent desorption (0.06–3.09%). However, the adsorbent bound virus complex retained, and may have actually enhanced, infectivity.

Keywords: Clay; Charcoal; Viral adsorbers; Rotavirus; Coronavirus

1. Introduction

Gastroenteritis is a clinical term referring to an acute diarrheal disease; in practice however, enteritis is a more correct term because infections are usually limited to the

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small and large intestines. Gastroenteritis primarily effects neonatal but also adult animals, birds and humans and is a major cause of disease and often death worldwide. Viruses, bacteria, protozoa, toxins and heavy metals are among the unrelated causes of the disease but environmental, immunological, and nutritional factors play an important role as well. To date, viruses that are implicated in this disease complex include adenoviruses, astroviruses, calicivirus-like agents, coronaviruses, parvoviruses, rotaviruses, and toroviruses. It has been shown that viruses are among the most common agents associated with diarrhea of children, calves, (House, 1978; Woode and Crouch, 1978; Tzipori, 1981, 1985; Bern and Glass, 1994) and foals (Dwyer et al., 1991).

Currently, rotaviruses are the leading cause and coronaviruses are major contributors of acute gastroenteritis in the young of various mammalian and avian species. The livestock industry carries a great financial burden due to the animal deaths and costs associated with prevention and treatment of disease as a result of infections by these two viruses. In epizootic infections of rotavirus, morbidity rates can be as high as 80–100% and although mortality rates are usually in the range of 0–5% they can be as high as 60%. Mortality rates are probably dependent upon the virulence of the virus strain, other pathogens present, and the host's phenotype and genotype. Implementation of fluid replacement therapy can greatly reduce or eliminate death rates (Bywater and Woode, 1980; Black et al., 1981; Bywater, 1983).

Gastroenteritis due primarily to rotavirus and coronavirus infections but also to other viruses has a significant economic impact on agriculture. Yet, despite the numerous trials and decades of research, effective vaccines are still not available for these or other viral agents that cause gastroenteritis. Although, research efforts have appeared promising, vaccine field trials continue to produce inadequate results or fail entirely (de Leeuw et al., 1980; Myers and Snodgrass, 1982; Snodgrass et al., 1982; Waltner-Toews et al., 1985; Yuan et al., 1998). The inadequacy of rotavirus vaccines may be explained, in part, by the complex antigenic diversity of the group A rotaviruses (Woode et al., 1983, 1987; Zheng et al., 1989; Hardy et al., 1991; Parwani et al., 1993; Xu and Woode, 1993), and by doubts concerning which of the two main antigens, VP4 or VP7, is the most important antigen to induce immune protection (Ward et al., 1993; Xu et al., 1993). In addition, immunizing neonates with modified live rotavirus or coronavirus vaccine is difficult because of interference by colostral antibodies which contain neutralizing activity and effectively block the host's immune response (Waltner-Toews et al., 1985). Post infection, the only treatment available is supportive therapy such as fluid replacement. Thus, viral gastroenteritis is still largely uncontrolled at present.

Because of the above problems and the number of enteric pathogens, alternative methods to control infection should be investigated. One of our goals has been to investigate broad spectrum methods which are not discriminatory among various serotypes and strains of viruses.

Anecdotal data showed that clays have been incorporated into diets for centuries by many different societies to prevent gastroenteric diseases and clays have been used in the treatment of a variety of illnesses including abscesses, acne, allergies, arthritis, headaches, and shingles (Abehsera, 1979). More recently, a new approach has been directed towards the use of clays and clay minerals to alter the bio-availability of toxins. A hydrated sodium calcium aluminosilicate clay (HSCAS) has been found to bind

aflatoxins and when added to the food greatly reduces the adverse effects of aflatoxin in Leghorn and broiler chicks (Phillips et al., 1988). Various clays have also been shown to adsorb and inactivate the thermo-labile (LT) enterotoxins of *Escherichia coli* and the cholera toxin (CT) enterotoxins of *Vibrio cholerae* which has been reviewed recently (Ramu et al., 1997). There is also evidence of virus adsorption to clays; the viruses most studied include poliovirus, encephalomyocarditis virus and reovirus (Kelly et al., 1961; Schaub and Sagik, 1975; Taylor et al., 1981; Lipson and Stotzky, 1983; Preston and Farrah, 1988).

This paper is the first of the two investigating the use of non-specific methods to inhibit the infectivity of rotavirus and coronavirus.

2. Materials and methods

2.1. Cell cultures and viruses

The viruses used in these experiments include bovine rotavirus, NCDV-Lincoln strain (Fernelius et al., 1972) and bovine coronavirus, BCV ATCC P2 (Mebus et al., 1973; Sharpee et al., 1976). Culturing and assaying for infectivity were performed in the BSC-1 cell line for rotavirus and the HRT-18 cell line for coronavirus. These methods have been described previously (Woode et al., 1987; Storz et al., 1991).

2.2. Adsorbent materials

The various adsorbent materials that were tested included: alumina-boehmite (alumina oxyhydroxide), attapulgite (palygorskite), charcoal, kaolinite, manu, muscuvite (mica), various smectites: acid activated clay (X-4994-H), acid activated polkville, hydrated sodium calcium aluminosilicate (HSCAS I, HSCAS II, and HSCAS III), and sodium-bentonite and two zeolites: clinoptilolite and mordenite. Sand has low ion exchange ratios and a low surface area which results in low or no adsorption of viruses and thus, was used as a negative control for all experiments. Sand was washed prior to use and sand as well as all of the adsorbent materials were autoclaved prior to use.

2.3. Adsorption assays

Experiments were designed to test the ability of various adsorbent materials to tightly bind bovine rotavirus and coronavirus. Bovine rotavirus was chosen to be the model for the experimental assays and as such was tested multiple times with all the adsorbent materials. Bovine coronavirus was tested with selected materials; various sorbents were screened while HSCAS I and II were repeatedly tested for their ability to bind BCV. HSCAS I and II were tested more extensively because of their chemical selectivity which has been well documented (Phillips et al., 1995).

For each adsorption assay, 0.1 g of clay was weighed in a centrifuge tube, 5 ml of media was added and then equilibrated for 5 min. The virus (at a titer of 10^5–10^6 immunofluorescent focus forming units/ml) was added to each clay sample to result in a

final 1% w/v concentration of clay. Two controls were used in each experiment: (1) media plus virus and (2) washed sand plus virus. The samples were incubated for 15 min at room temperature on a Glas-col rotator at 70 rpm. Afterwards, all samples were centrifuged at relative centrifugal force (RCF) of $700\times g$ for 10 min. Supernatants of each sample were then transferred into snap cap tubes (one sample per tube). The clay sample was reserved for desorption and infectivity studies. All supernatants were titrated from 10^{-1} to 10^{-3} in serum free (SF) media. The original supernatants and each of their dilutions were assayed for infectivity.

2.4. Desorption assays

The pellet of clay that was reserved for the desorption experiment was resuspended in SF-media at a final 1% w/v concentration of clay. The sample was mixed well and incubated at room temperature for 1 h on the rotator at 70 rpm. (If an infectivity study was to be run on the clay sample, an aliquot of clay suspended in media was removed post incubation.) The sample was centrifuged at RCF $700\times g$ for 10 min. The supernatant was then transferred to a snap cap tube and titrated from 10^{-1} to 10^{-3} in SF/media and assayed for infectivity.

2.5. Infectivity assays of clay/virus complex

The suspended samples of clay bound virus or clay/virus complex retained from the adsorption and desorption experiments were used for infectivity testing. An aliquot of the clay sample was removed and placed in a snap cap tube and then titrated from 10^{-1} to 10^{-3} in SF/media. These clay samples were assayed for infectivity.

Virus dilutions were inoculated (100 μl/well, 3 wells/dilution) into microtiter plates of 5–7 days old BSC-1 cells. Microtiter plate(s) were incubated at 37°C for 20 h and then fixed with 80% acetone in water (−20°C) for 10 min; the acetone was removed and the plates were dried for approximately 3 h. The plates were rehydrated in PBS prior to a two part staining procedure. Plates were stained for 1 h with approximately 0.03 ml/well of monoclonal antibody B223 E4 (Mab E4) which was made to an epitope on VP6 of B223 rotavirus. (Mab E4 was diluted 1:25 in PBS, pH 7.2.) Plates were washed with PBS and then stained with approximately 0.03 ml/well of Fluorescein-labeled affinity purified antibody-goat anti-mouse IgG (GAM) for 1 h at room temperature and then washed with PBS. (GAM was diluted 1:50 in PBS.) The fluorescein-tagged cells were viewed by a Leitz inverted UV microscope. Titers were calculated by counting fluorescent cells/well or immunofluorescent focus forming units. The methodology described by Reed and Muench was used to determine the 50% endpoint (Reed and Muench, 1938).

2.6. Plaque assay

Virus dilutions were inoculated (1 ml/well, 2 wells/dilution) into six well plates of 5-day-old BSC-1 cells. After a 60 min absorption time at 37°C, the virus dilutions were removed and 2 ml of nutrient agar (equal volumes of 2% agar and double strength MEM) was added to each well. The plates were then incubated at 37°C for 3–4 days. Post

incubation, plates were fixed with 10% formalin for at least 1 h. The formalin and agar was removed and the cells were immediately stained with crystal violet. Titers were calculated by counting the number of plaques at each dilution.

2.7. Calculations

Percent of adsorbed virus, desorbed virus, and infectivity of clay bound virus were calculated. The following symbols were used in the formulas listed below:

T_v Titer of virus
T_a Titer of supernatant from adsorption study
T_d Titer of supernatant from desorption study $\times 10$ (dilution factor)

T_i Titer of clay/virus complex from infectivity study

$$\%\text{Adsorbed virus} = T_v - \frac{T_a}{T_v} \times 100$$

$$\%\text{Desorbed virus} = \frac{T_d}{T_v} - T_a \times 100$$

$$\%\text{Infectivity of clay/virus complex} = \frac{T_i}{T_v} - T_a \times 100$$

3. Results

3.1. Adsorption and desorption assays

A variety of adsorptive materials were tested extensively using bovine rotavirus. Charcoal, sodium bentonite, attapulgite, kaolinite, and HSCAS III were found to adsorb greater than 99.0% of bovine rotavirus while the other materials assayed ranged in their adsorptive capabilities from less than 99% to greater than 78% (Table 1). Charcoal, sodium bentonite, attapulgite, kaolinite, mordenite, mica, HSCAS III were screened for their adsorptive capability of bovine coronavirus; their mean percent adsorption was 99.99%. HSCAS I and HSCAS II tested more extensively due to their chemiselectivity and their mean percent adsorption was found to be $99.99 \pm 0.0\%$ and $99.98 \pm 0.01\%$, respectively.

Sand was used as a negative control in all adsorption assays. Because sand did not adsorb either virus, it was not assayed for desorption or infectivity. All adsorbents tested had a high affinity binding to both viruses as determined by a low percent desorption. Mean percent desorptions ranged from 0.06% to 3.09% for bovine rotavirus with charcoal and attapulgite having the lowest percent desorptions (Table 1). Desorption assays of bovine coronavirus were performed on the chemiselective substances, HSCAS I and HSCAS II; no desorption (0.0%) was detected by our assay.

Table 1
Results of NCDV adsorption, desorption, and infectivity assays

Sample	Adsorption			Desorption			Infectivity		
	Mean (%)	*n*	Standard error	Mean (%)	*n*	Standard error	Mean (%)	*n*	Standard error
Charcoal	99.89	6	0.04	0.06	5	0.04	21.24	3	6.46
Sodium bentonite	99.83	6	0.05	0.23	4	0.18	276.17	3	91.04
Attapulgite	99.76	7	0.04	0.07	5	0.04	221.25	3	59.42
Kaolinite	99.16	7	0.23	0.62	5	0.35	394.94	3	39.89
X-4994-D (HSCAS III)	99.05	7	0.62	0.81	5	0.61	66.59	3	5.86
Acid activated clay (X-4994-H)	98.22	4	0.13	2.34	4	0.36	626.78	3	75.23
Muscuvite	98.14	7	0.63	3.09	5	1.05	536.55	3	156.92
Clinoptilolite	97.97	6	0.73	2.72	4	1.67	573.51	3	24.18
HSCASI	96.41	10	1.88	0.57	6	0.4	476.77	3	49.72
Peru Clay	96.25	3	0.56	1.83	3	0.44	382.4	3	42.37
HSCAS II	94.95	11	1.85	0.61	6	0.21	299.77	3	91.42
Acid activated polkville	94.87	3	1.3	2.76	3	0.44	300.78	3	11.31
Mordenite	94.61	6	3.06	2.74	4	1.42	498.31	3	33.25
Alumina boehmite	78.74	7	8.16	2.17	5	0.57	512.54	3	97.18

3.2. Infectivity studies of adsorbent agent/virus complex

As both bovine rotavirus and coronavirus bound to various adsorbent materials, including HSCAS I and II, with high affinity, both were tested for infectivity when in the bound state. However, as indicated by the infectivity assays, neither virus was inactivated by the adsorbent materials. According to the microtiter assay method at least some infectivity remained in all samples of both viruses. Mean percent infectivity of clay/rotavirus complex ranged from 21.24% to 626.78% with charcoal and HSCAS III having the lowest percent infectivity (Table 1). As with the other assays using coronavirus, only the chemiselective adsorbents (HSCAS I and HSCAS II) were tested. Complexes of HSCAS I/coronavirus and HSCAS II/coronavirus both retained 100% infectivity.

The plaque assay method was used to compare the infectivity results of rotavirus and HSCAS II to the results in the microtiter assay. The percent infectivity of HSCAS II in the plaque assay was 337.62% compared to a mean of 299.77% in the microtiter assay. The plaque assay results confirmed the accuracy of the data from the microtiter assays.

4. Discussion

We have looked at a variety of inorganic adsorbent materials and have found that all adsorbent agents tested had good (70–90% adsorption) to excellent (>90% adsorption) capability of adsorbing bovine rotavirus and excellent capability of adsorbing bovine coronavirus, both with a high affinity. We have characterized a high affinity binding as an adsorbent material that has a high percent adsorption of virus and a low percent desorption. The structure of the adsorbent materials is very complex and the relationship of binding is not completely understood. However, the diameter of rotavirus and coronavirus particles are 60–80 and 60–220 nm, respectively which are significantly larger than the pores of the adsorbent material particles. Therefore, the virions must be attached to the outer surface of each adsorbent particle. Furthermore, we believe this interaction to be a non-specific binding of protein. This is supported by the binding of viruses including rotavirus, coronavirus, poliovirus, reovirus, and enterovirus, CT and LT enterotoxins, and casein which was found to block the adsorption of CT and LT enterotoxins which has been reviewed previously (Ramu et al., 1997).

Adsorbent materials appear promising as a method of adsorbing virus from solution and may be useful as such in filtering aqueous systems. They may have a distinct advantage in prevention of infection and/or disease because most agents that cause gastroenteritis, including rotavirus and coronavirus, are generally contracted orally, usually via contaminated food and water. According to our studies, adsorbent materials will allow between 1% and 10% of virions to remain in solution. As a result they may not eliminate infection but may eliminate water borne disease by effectively reducing the infectious dose by greater than 90%. It was our hypothesis that the prophylactic use of adsorbent agents (feeding adsorbent agents daily in grain) could decrease enteric viral infections by binding with the viral pathogens in the gut and thus reducing the infectious dose. However, after completing the infectivity studies we lack support of this hypothesis. The infectivity data of the adsorbent material/virus complex (over virus alone) indicates

that the complexes are not only infectious but many complexes are more infectious than the virus was originally. These results correspond with prior investigations which found that the persistence of reoviruses and enteroviruses is maintained and often enhanced when associated with clay and clay minerals (Schaub and Sagik, 1975; Lipson and Stotzky, 1984). There is a potential error of titrating a solid in a liquid; however, the infectivity data is consistent and reproducible. The plaque assay is accepted as a more sensitive assay method (0.5–1 log dilution) than the microtiter assay. The microtiter assay was used primarily in these studies because it is efficient and conserves time and cost over the plaque assay method. Results from the microtiter assay were confirmed by plaque assay using bovine rotavirus. It is our opinion that the adsorbent materials have enhanced the infectivity of the virus. One possibility to explain this observation is an improved presentation of virus to cells. A previous study (Bridger and Woode, 1976) indicates that there are two particle types of calf rotavirus: infectious and non-(or low) infectious particles. The complete rotavirus virion is composed of both inner and outer protein shells and is highly infectious as opposed to an incomplete virion which is missing its outer protein shell and is non-infectious. The outer protein shell is composed of viral proteins 7 and 4 (VP7 and VP4) which are responsible for the binding of virion to cell which is necessary for infection and thus replication. Bridger and Woode found that the ratios of particle number to infectivity were 7×10^2:1 for the complete virion vs. 1×10^6:1 for the incomplete virion. Our hypothesis for a more efficient presentation of virus is that an increased fraction of these incomplete particles are entering cells as a result of adsorbent materials being engulfed/phagocytosed and thus carrying virus into the cell with them. If this is the case, it may prove to be a method of culturing viruses that are difficult to propagate because they are comprised of a large proportion of incomplete virions. It is difficult of course to speculate what effect this will have in vivo but it is possible that diet may enhance infection. Future work includes separating complete and incomplete virions and repeating the infectivity studies, exploring both the reasons for infectivity of the complexed virus/adsorbent materials and the methods to significantly reduce the infectivity levels, and investigating the mechanisms of viral concentration within aqueous systems.

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References

Abehsera, M., 1979. Clay cures for modern ailments. In: Anonymous (Ed.), The Healing Clay vol. 2. Swan House Brooklyn, NY, 2, pp. 83–109.

Bern, C., Glass, R.I., 1994. Impact of Diarrheal Diseases Worldwide. In: Kapikian, A.Z. (Ed.), Viral Infections of the Gastrointestinal Tract. Marcel Dekker, New York, vol. 2, pp. 1-26.

Black, R.E., Merson, M.H., Taylor, P.R., Yolken, R.H., Sack, D.A., 1981. Glucose vs. sucrose in oral rehydration solutions for infants and young children with rotavirus-associated diarrhea. Pediatrics 67, 79–83.

Bridger, J.C., Woode, G.N., 1976. Characterization of two particle types of calf rotavirus. J. Gen. Virol. 31, 245–250.

Bywater, R.J., 1983. Diarrhoea treatments: fluid replacement and alternatives. Ann. Rech. Vet. 14, 556–560.

Bywater, R.J., Woode, G.N., 1980. Oral fluid replacement by a glucose glycine electrolyte formulation in *E. coli* and rotavirus diarrhoea in pigs. Vet. Rec. 106, 75–78.

de Leeuw, P.W., Ellens, D.J., Talmon, F.P., Zimmer, G.N., Kommerij, R., 1980. Rotavirus infections in calves: efficacy of oral vaccination in endemically infected herds. Res. Vet. Sci. 29, 142–147.

Dwyer, R.M., Powell, D.G., Roberts, W., Donahue, M., Lyons, E.T., Osborne, M., Woode, G., 1991. A study of the etiology and control of infectious diarrhea among foals in central Kentucky. Proc. 36th Annual Meeting. AAEP, pp. 337–355.

Fernelius, A.L., Ritchie, A.E., Classick, L.G., Norman, J.O., Mebus, C.A., 1972. Cell culture adaptation and propagation of a reovirus-like agent of calf diarrhea from a field outbreak in Nebraska. Arch. Virusforsch. 37, 114–130.

Hardy, M.E., Woode, G.N., Xu, Z., Gorziglia, M., 1991. Comparative amino acid sequence analysis of VP4 for VP7 serotype 6 bovine rotavirus strains NCDV, B641, and UK. J. Virol. 65, 5535–5538.

House, J.A., 1978. Economic impact of rotavirus and other neonatal disease agents of animals. J. Am. Vet. Med. Assoc. 173, 573–576.

Kelly, S., Sanderson, W.W., Reidl, C., 1961. Removal of enteroviruses from sewage by activated sludge. J. Water Pollut. Control Fed. 33, 1056–1062.

Lipson, S.M., Stotzky, G., 1983. Adsorption of reovirus to clay minerals: effects of cation-exchange capacity, cation saturation, and surface area. Appl. Environ. Microbiol. 46, 673–682.

Lipson, S.M., Stotzky, G., 1984. Effect of proteins on reovirus adsorption to clay minerals. Appl. Environ. Microbiol. 48, 525–530.

Mebus, C.A., Stair, E.L., Rhodes, M.B., Twiehaus, M.J., 1973. Neonatal calf diarrhea: propagation, attentuation, and characteristics of a coronavirus-like agent. Am. J. Vet. Res. 34, 145–150.

Myers, L.L., Snodgrass, D.R., 1982. Colostral and milk antibody titers in cows vaccinated with a modified live–rotavirus–coronavirus vaccine. J. Am. Vet. Med. Assoc. 181, 486–488.

Parwani, A.V., Hussein, H.A., Rosen, B.I., Lucchelli, A., Navarro, L., Saif, L.J., 1993. Characterization of field strains of group A bovine rotaviruses by using polymerase chain reaction-generated G and P type-specific cDNA probes. J. Clin. Microbiol. 31, 2010–2015.

Phillips, T.D., Kubena, L.F., Harvey, R.B., Taylor, D.R., Heidelbaugh, N.D., 1988. Hydrated sodium calcium aluminosilicate: a high affinity sorbent for aflatoxin. Poult. Sci. 67, 243–247.

Phillips, T.D., Sarr, A.B., Grant, P.G., 1995. Selective chemisorption and detoxification of aflotoxins by phyllosilicate clay. Natural Toxins 3, 204–213.

Preston, D.R., Farrah, S.R., 1988. Activation thermodynamics of virus adsorption to solids. Appl. Environ. Microbiol. 54, 2650–2654.

Ramu, J., Clark, K., Woode, G.N., Sarr, A.B., Phillips, T.D., 1997. Adsorption of cholera and heat-labile *Escherichia coli* enterotoxins by various adsorbents: an in vitro study. J. Food Protection 60, 1–5.

Reed, I.J., Muench, H., 1938. A simple method in estimating fifty percent endpoints. Am. J. Hyg. 27, 493–497.

Schaub, S.A., Sagik, B.P., 1975. Association of enteroviruses with natural and artificially introduced colloidal solids in water and infectivity of solids-associated virions. Appl. Microbiol. 30, 212–222.

Sharpee, R.L., Mebus, C.A., Bass, E.P., 1976. Characterization of a calf diarrheal coronavirus. Am. J. Vet. Res. 37, 1031–1041.

Snodgrass, D.R., Nagy, L.K., Sherwood, D., Campbell, I., 1982. Passive immunity in calf diarrhea: vaccination with K99 antigen of enterotoxigenic *Escherichia coli* and rotavirus. Infect. Immun. 37, 586–591.

Storz, J., Herrler, G., Snodgrass, D.R., Hussain, K.A., Zhang, X.M., Clark, M.A., Rott, R., 1991. Monoclonal antibodies differentiate between the haemagglutinating and the receptor-destroying activities of bovine coronavirus. J. Gen. Virol. 72, 2817–2820.

Taylor, D.H., Moore, R.S., Sturman, L.S., 1981. Influence of pH and electrolyte composition on adsorption of poliovirus by soils and minerals. Appl. Environ. Microbiol. 42, 976–984.

Tzipori, S., 1981. The aetiology and diagnosis of calf diarrhoea. Vet. Rec. 108, 510–515.

Tzipori, S., 1985. The relative importance of enteric pathogens affecting neonates of domestic animals. Adv. Vet. Sci. Comp. Med. 29, 103–206.

Waltner-Toews, D., Martin, S.W., Meek, A.H., McMillan, I., Crouch, C.F., 1985. A field trial to evaluate the efficacy of a combined rotavirus–coronavirus/*Escherichia coli* vaccine in dairy cattle. Can. J. Comp. Med. 49, 1–9.

Ward, R.L., McNeal, M.C., Sander, D.S., Greenberg, H.B., Bernstein, D.I., 1993. Immunodominance of the VP4 neutralization protein of rotavirus in protective natural infections of young children. J. Virol. 67, 464–468.

Woode, G.N., Kelso, N.E., Simpson, T.F., Gaul, S.K., Evans, L.E., Babiuk, L., 1983. Antigenic relationships among some bovine rotaviruses: serum neutralization and cross-protection in gnotobiotic calves. J. Clin. Microbiol. 18, 358–364.

Woode, G.N., Zheng, S.L., Rosen, B.I., Knight, N., Gourley, N.E., Ramig, R.F., 1987. Protection between different serotypes of bovine rotavirus in gnotobiotic calves: specificity of serum antibody and coproantibody responses. J. Clin. Microbiol. 25, 1052–1058.

Woode, G.N., Crouch, C.F., 1978. Naturally occurring and experimentally induced rotaviral infections of domestic and laboratory animals. J. Am. Vet. Med. Assoc. 173, 522–526.

Xu, Z., Hardy, M.E., Woode, G.N., Ramig, R.F., 1993. Immunodominant neutralizing antigens depend on the virus strain during a primary immune response in calves to bovine rotaviruses. Vet. Microbiol. 35, 33–43.

Xu, Z., Woode, G.N., 1993. Studies on the role of VP4 of G serotype 10 rotavirus (B223) in the induction of the heterologous immune response in calves. Virology 196, 294–297.

Yuan, L., Kang, S.Y., Ward, L.A., To, T.L., Saif, L.J., 1998. Antibody-secreting cell responses and protective immunity assessed in gnotobiotic pigs inoculated orally or intramuscularly with inactivated human rotavirus. J. Virol. 72, 330–338.

Zheng, S.L., Woode, G.N., Melendy, D.R., Ramig, R.F., 1989. Comparative studies of the antigenic polypeptide species VP4, VP6, and VP7 of three strains of bovine rotavirus. J. Clin. Microbiol. 27, 1939–1945.