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Enzootic Bovine Rotavirus is Not a Source of Infection in Panamanian Cattle Ranchers and Their Families

R. W. Ryder, R. H. Yolken, W. C. Reeves, and R. B. Sack

From the Section of Epidemiology and Biostatistics, Boston University School of Public Health, Boston, Massachusetts; the Department of Pediatrics and the Division of Geographic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; and the Gorgas Memorial Laboratory, Panama City, Panama

Vaccination of humans against rotavirus (RV) diarrhea may be accomplished by oral immunization with attenuated animal strains known to be antigenically very similar to human strains. To define better the degree of infectivity in nature of these animal strains for humans, we conducted surveillance for RV infection/diarrhea in 180 farm workers, their 161 family contacts, and the 566 animals (512 cattle, 35 pigs, and 19 sheep) on 14 farms in rural Panama. No correlation between the high infection rates in farm workers (72%) and their family contacts (78%) and in cattle (56%) could be demonstrated. Heads of families with four or more children with RV infection experienced a twofold greater rate of RV infection compared with heads of families of similar size without RV infection. Despite the close similarity between human and bovine RV, in Panama intrafamilial (particularly child-to-child or child-to-parent) rather than interspecies transmission appeared to be the most important route for the spread of this highly infectious virus.

Rotaviruses are important worldwide causes of acute diarrhea in humans and in many domestic animals, in particular pigs and cattle [1-6]. Effective, safe vaccines will therefore have an important impact on morbidity and mortality in humans and animals. Development of the candidate vaccine strains has taken advantage of the antigenic similarity but varying pathogenicity of rotaviruses from many different species. Strains of rotavirus originally isolated from monkeys and cattle have now been shown to induce cross-species protective immunity in several challenge studies [7-11]. All of these studies have been performed under carefully controlled circumstances—none has sought to determine whether cross-species infection occurs in nature and, if it does, to quantitate the frequency with which it occurs. Therefore for an eight-month period we prospectively conducted surveillance for rotavirus infection/diarrhea in farm workers, their family contacts, and their animals on 14 farms (10 bovine, 3 porcine, and 1 ovine) in rural Panama.

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Informed consent was obtained from each of the farm owners and from all of the farm workers and their family contacts.

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Please address requests for reprints to Dr. R. B. Sack at his present address: Department of International Health, Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, Maryland 21205.

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Materials and Methods

Epidemiology. Subjects. Through the assistance of the Panamanian Veterinary Association (Panama City, Panama), representative animal farms were selected from throughout the country. Because of the possibility suggested in the literature [12] that rotavirus infection/diarrhea is most prevalent in young animals, farms specializing in production of young animals were heavily represented in our selection process. All 14 farms initially chosen agreed to participate in the study. Each farm occupied at least 2 km² and was located in a rural surrounding (population density, less than five persons per km²) at least 30 km from the capital, Panama City. Farm workers lived with their immediate family members either on the farm where they worked, in quarters provided by the owner, or in owned/rented facilities located nearby (<5 km) the farm. Housing on each of the farms was located various distances (but not >1 km) from the animal confinement areas; in several cases it consisted of the same or was immediately continguous with the structure(s) used to contain the animals.

Surveillance methods. We conducted a door-to-door census of all workers and their family contacts in August 1979. During this census a standard questionnaire was completed for each family and sought the following information: number of years working/living on the farm; number of years working with animals; if living off the farm, the distance from the farm; the job description of the farm workers; how often they actually had contact with the farm animals; the age and names of family members living with them; the number of rooms and the size of these rooms; and the availability of running water, electricity, and a toilet. All of the animals on the selected farms were marked either with ear tags or by branding.

For the next eight months, we conducted surveillance for diarrheal disease in farm workers, their family members, and the farm animals. Each week a trained epidemiology technician visited each farm and sought out the head of each household to inquire about diarrhea in his family. The foreman of each farm was also questioned about diarrheal disease in any of the animals. Diarrhea in humans was defined as the passage in a 24-hr period of more than three stools that took the shape of a container. In animals the judgment of the foreman was relied on to determine when an animal had diarrhea. Of the 419 people originally enrolled, 78 moved away (30 workers and 48 family contacts) and were unavailable for either the second and/or third venipunctures. There were no human deaths during the study period. Of the 823 animals included in the original August venipuncture cohort, 257 had been sold or slaughtered by the end of the study. No animals died of diarrhea while the study was being conducted.

Laboratory procedures. Collection of sera. One month after the August census, baseline sera were obtained by venipuncture from all of the farm workers and their families and from all of the previously identified animals on each farm. Follow-up serum samples were obtained from the original cohort of people and animals in November and the following March, three and eight months, respectively, after the first venipuncture. All serum samples were collected by aseptic technique, centrifuged within 6 hr of collection, and kept at 4 C for <48 hr before they were entered into the Gorgas Memorial Laboratory Serum Bank (Panama City), where they were stored at −20 C before being shipped frozen to Baltimore. Titers of IgG antibodies to rotavirus were measured by ELISA techniques [13]. Human rotavirus antigen used in these assays was obtained from gnotobiotic calf stools from an animal experimentally inoculated with a stool from an infected infant. The present report includes only those people who participated in the surveillance activities and from whom three serum samples were collected. At least two consecutive serum samples (August–November or November–March) had to have been obtained before an animal was included in the study.

Collection of stool samples. When a case of diarrhea was detected in a farm worker, a family member, or an animal, a stool sample was obtained as early as possible in the course of the illness and frozen at −20 C in a designated location on each farm pending weekly transport to our central laboratory. In Panama City the specimens were maintained at −20 C until testing. ELISA techniques were used to detect rotavirus antigen [13].

Definition of rotavirus infection. We defined rotavirus infection by a fourfold or greater rise in titer of serum antibody in one of the two collection periods (T_1 = August–November; T_2 = November–March).

Definition of rotavirus diarrhea. Rotavirus diarrhea was defined as a diarrheal episode in which rotavirus antigen was detected in at least one stool specimen.

Data analyses were conducted with the CLINFO Data Management System (Bolt, Beranicam, and Newman, Cambridge, Mass).

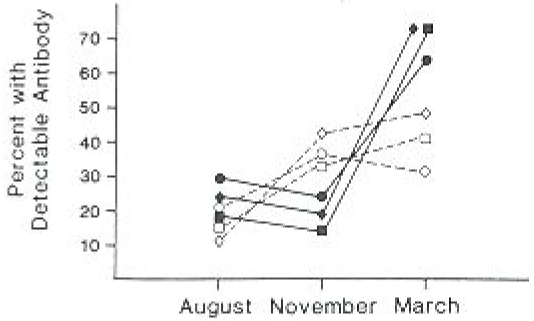

Figure 1. Prevalence of serum IgG antibodies to rotavirus at three study times in Panamanian farm workers, their families, and domestic cattle on 10 cattle ranches in rural Panama. The cohorts were divided by age. The humans were categorized as <5 years old (●), 5 up to 20 years old (■), and ≥20 years old (◆); the cattle were categorized as <0.25 years old (○), 0.25 up to 1 year old (□), and ≥1 year old (◇).

Table 1. Incidence of rotavirus infection and diarrhea in humans and cattle in Panama during two consecutive surveillance periods.

Cohort, illness	Surveillance period T_1 Rate	T_1 Interfarm range (%)	T_2 Rate	T_2 Interfarm range (%)
Humans				
Rotavirus-associated diarrhea	23/341 (7)	0–18	41/341 (12)	7–24
Rotavirus infection	43/341 (13)	0–37	204/341 (60)	18–84
Infection:case ratio	1.9		5.0	
Cattle				
Rotavirus-associated diarrhea	19/483 (4)	0–9	24/472 (5)	2–11
Rotavirus infection	164/483 (34)	6–86	104/472 (22)	15–75
Infection:case ratio	8.6		4.3	

NOTE. Data are no. with RV diarrhea/total no. of subjects included in surveillance activities (%). See text for a description of T_1 and T_2.

Results

At the start of the study <30% of the people on all of the farms had detectable IgG antibody to rotavirus (figure 1). Baseline prevalence rates of antibody to rotavirus were consistently low on all of the farms and did not vary by age group or the job description of a farm worker. Workers who had daily exposure to animals were as likely to lack baseline titers of antibodies to rotavirus as were women and children, who rarely had contact with farm animals. Baseline prevalence rates of antibody to rotavirus in animals did not vary by age or farm location. Antibodies to rotavirus were not found in any of the sera obtained from pigs or goats. In both humans and cattle, the prevalence of IgG antibodies to rotavirus increased during the study (figure 1). By March ~70% of the humans of all ages and nearly 40% of the cattle had developed detectable antibodies to rotavirus.

Seroconversion rates in cattle were similar in both surveillance periods (34% in T_1 and 22% in T_2), whereas human rates were low in T_1 (13%) but high in T_2 (60%; table 1). No humans or animals experienced rotavirus infection during each of the two consecutive surveillance periods. Seroconversion rates in humans did vary from farm to farm but were consistently low during T_1 and consistently high during T_2. No correlation could be demonstrated between rates of rotavirus infection in humans and animals on the same farm either during the same surveillance period or during the preceding one. Farms with particularly low rates of rotavirus infection in cattle during T_2 had rotavirus infection in humans during T_2 as high as farms with high rates of rotavirus infection in cattle during T_2. Rates of rotavirus infection in humans during T_1 and T_2 on swine and ovine farms (where no rotavirus infections among animals were documented) were similar to the rates of rotavirus infection in humans on the cattle farms during the same interval. Parallel but lower rates of rotavirus diarrhea compared with rates of rotavirus infection during each of the two surveillance periods were found in cattle and in humans (table 1).

To determine whether farm workers who had daily contact with animals had an increased risk of rotavirus infection, we looked at rates of rotavirus infection by the number of years of direct contact each worker on the bovine farms had had with cattle and

Table 2. Relation between presence of antibody to rotavirus at the start of two surveillance periods and risk of rotavirus infection in the subsequent four months in humans by age on 14 Panamanian farms.

Age*	Antibody in August 1979: Absent	Antibody in August 1979: Present	Antibody in November 1979: Absent	Antibody in November 1979: Present
<5	8/36 (22)	0/13 (0)	19/32 (59)	4/12 (33)
5 up to 20	13/95 (14)	1/24 (4)	74/103 (72)	3/22 (14)
≥20	21/132 (16)	0/41 (0)	101/145 (70)	4/27 (15)
All ages	42/263 (16)	1/78 (1)	194/280 (69)	11/61 (18)

NOTE. Infection was defined as a fourfold or greater rise in titer of antibody in sera collected during August 1979, November 1979, and March 1980. Data are no. of subjects in that age group with a fourfold or greater rise in titer of antibody/total no. of subjects in that age group with paired sera collected in August and November (% seroconverting).

* Age in years in August 1979.

Table 3. Relation between presence of antibody to rotavirus at the start of two surveillance periods and the risk of rotavirus infection in the subsequent four months in cattle by age on 10 Panamanian cattle farms.

	Antibody in August 1979		Antibody in November 1980	
Age*	Absent	Present	Absent	Present
<0.25	16/56 (28)	0/6 (0)	41/46 (88)	0/14 (0)
0.25 up to 1	101/240 (42)	2/61 (3)	67/204 (33)	0/111 (0)
≥1	47/104 (45)	0/16 (0)	24/54 (44)	0/43 (0)
All ages	164/400 (41)	2/83 (3)	132/304 (43)	0/168 (0)

NOTE. Infection was defined as a fourfold or greater rise in titer of antibody in sera collected during August 1979, November 1979, and March 1980. Data are no. of cattle in that age group with a fourfold or greater rise in titer of antibody/total no. of cattle in that age group with sera collected in August and November (% seroconverting).

* Age in years in August 1979.

by his job description. Infection rates were similarly high in all workers regardless of the interval they had worked with animals. Farm workers who had worked less than one year with cattle had an infection rate of 76% during T_2, whereas workers with >20 years of working with cattle had an infection rate of 73% during T_2. Similar patterns and rates of rotavirus infection were found in office workers who rarely had contact with animals (T_2 infection rate, 58%), farm worker family members who never had contact with animals (T_2 infection rate, 60%), and cattle handlers who had daily close contact with aimals (T_2 infection rate, 60%).

Evidence of immunity induced by naturally acquired rotavirus infection was found in both humans and cattle of all ages. In humans only one rotavirus infection occurred during T_1 in the 78 persons with detectable baseline antibody to rotavirus, whereas 42 (16%) of 263 persons with no baseline titer developed rotavirus infection ($P < .001$; table 2). During T_2, 69% of the 280 humans without any detectable antibody to rotavirus in their November serum sample acquired rotavirus infection compared with 18% of 62 persons who had detectable antibody to rotavirus in their November sera ($P < .001$). Forty-one percent of 400 cattle with no detectable baseline antibody in August 1979 had rotavirus infection during the ensuing four months (T_1) compared with none of 168 cattle who had antibody to rotavirus in November ($P < .001$; table 3). During T_2, 43% of 304 cattle without antibody to rotavirus in November 1979 subsequently had rotavirus infection compared with none of 168 cattle who had antibody to rotavirus in November ($P < .001$).

To determine the role that intrafamilial spread of rotavirus played in transmission and possibly to define the source of the high T_2 infection rates in humans, we looked at the T_2 infection rates in children according to their parents' status for rotavirus infection during T_2. Rates of rotavirus infection in children were similar regardless of the status for rotavirus infection of their mother or father (table 4). We also looked at rates of rotavirus infection in parents of children with known rotavirus infection during T_2. Both maternal and paternal infection rates were highest in families with the largest number of children experiencing rotavirus infection (table 5). We were unable to demonstrate any association between increased rates of rotavirus infection and monthly earnings of the farm worker, dwelling size, number of rooms in a dwelling, and access to a flush toilet or electricity and/or piped water in the home.

Discussion

The purpose of this investigation was to determine whether an exchange of species-specific rotavirus occurs between farm workers (and their family contacts) and domestic farm animals coexisting under sometimes suboptimal conditions of animal husbandry. Rotavirus infection was common during the eight-month study, with over half of the humans and cattle experiencing illness. However, for several reasons cross-species infection appeared not to be an important causal factor in these high rates. First, there was no concordance between farms with high infection rates in humans and those with high infection rates in cattle. Second, there was no temporal association between the periods of greatest rotavi-

Table 4. Rates of rotavirus infection between November 1979 and March 1980 in children according to status for rotavirus infection of their parents during the same interval.

Maternal rotavirus infection status	Paternal rotavirus infection status: Positive	Negative
Positive	38/47 (81)	26/36 (72)
Negative	17/29 (59)	13/21 (62)

NOTE. Data are no. of children with rotavirus infection/total no. of children of parents with the specified combination of rotavirus infection (% seroconverting).

rus infection in cattle and humans, regardless of age. Indeed, cattle had the highest infection rate in T_1, whereas most human infections occurred during T_2. The short incubation period for rotavirus infection (<72 hr) and the four-month period between peak rotavirus infection in animals and humans make it unlikely that cross-species transmission was an important factor in these high infection rates. Third, we were unable to demonstrate an association between infection rates in farm workers and their exposure to animals. Workers who cleaned animal feces from animal pens had infection rates similar to those of women and children who lived off the farm and who only rarely had contact with the animals. Fourth, workers on swine farms, where no rotavirus infection was documented, had patterns and rates of infection similar to those of workers on bovine farms. Finally, our data strongly suggest that for rotavirus infections in humans, children are the most important reservoir. Rates of rotavirus infection were higher in parents of children with rotavirus infection than in parents of children who did not have such illness. Conversely, rates of rotavirus infection in children were the same regardless of whether their parents had had rotavirus infection. Our data suggesting that children are the most important reservoir for intrafamilial rotavirus confirm the findings of Wenman et al. [2] and Kim et al. [14]. We were unable to obtain large enough quantities of stool from ill humans and animals to compare the electrophoretic mobility of rotavirus RNA from animal and human isolates.

Large longitudinal studies of rotavirus infection have not often been performed in the developing world. The extensive rates of rotavirus seroconversion without much evidence of associated diarrhea documented in our study suggest that repeated episodes of rotavirus infection occur in the same individual, perhaps at intervals as short as a year. Each episode appears to be accompanied by a transient rise in titer of antibodies, which in the present study appeared to be associated with protection but which also waned quite rapidly. The role in inducing or maintaining clinical protection that these repeated reinfections provides is unclear. Previous studies in young children have shown that an initial rotavirus infection is likely to produce symptomatic illness, whereas recurrent episodes of exposure most often result in asymptomatic infection [15]. Future studies of rotavirus vaccine, when vaccine response and efficacy are assessed, must consider these high background rates of seroconversion. Earlier studies in humans have demonstrated prevalence rates of antibody to rotavirus of >50% in persons older than two years of age [3]. The relatively low prevalence of antibody to rotavirus in sera collected from people in August and November may imply a diminished sensitivity in our assays for antibody to rotavirus.

Table 5. Relation between number of children infected in a family with rotavirus infection between November 1979 and March 1980 and rotavirus infection in their parents during the same interval.

	No. of children in family with rotavirus infection			
Parents (*n*)	0	1–2	3–4	≥4
Mothers (41)	3/6 (50)	7/16 (38)	6/10 (60)	7/9 (78)*
Fathers (41)	2/6 (33)	9/16 (56)	7/10 (70)	6/9 (67)*

NOTE. Data are no. of mothers (or fathers) with rotavirus infection between November 1979 and March 1980/total no. of mothers (or fathers) with the specified no. of children having rotavirus infection during the same interval (% seroconverting).
* χ^2 for trend, $P < .05$.

The major modes of transmission of rotavirus among animals and among humans in our study remain conjectural. Children appeared to be reservoirs for human infection. Fecal-oral transmission of these viruses, which can induce infection with a relatively small inoculum size, probably promotes person-to-person (and animal-to-animal) spread. The extraordinary high infection rates in humans during T_2 suggest that a transmission mechanism allowing effective exposure to large numbers of individuals, such as airborne transmission, might also play an important role [16]. Our data provide strong epidemiological evidence against frequent cross-species transmission of rotavirus in a population that clearly was at heightened risk of experiencing this event.

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