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Comparison of the Host Ranges and Antigenicity of *Cryptosporidium parvum* and *Cryptosporidium wrairi* from Guinea Pigs

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ABSTRACT. Oocysts of a *Cryptosporidium* isolate from guinea pigs were not infectious for adult mice, but were infectious for two of three newborn calves and for suckling mice. However, oocysts isolated from calves or mice infected with guinea pig *Cryptosporidium* were not infectious for guinea pigs. Four isolates of *C. parvum* from calves were incapable of infecting weanling guinea pigs. Microscopic examination of tissue from the colon and cecum of suckling guinea pigs inoculated with *C. parvum* revealed sparse infection of some pups. These host range studies and previously described differences in ^{125}I-labeled oocyst surface protein profiles between *Cryptosporidium* sp. from guinea pigs and *C. parvum* suggest they are distinct species. We propose the name *Cryptosporidium wrairi* be retained. Studies with monoclonal antibodies indicate that *C. wrairi* and *C. parvum* are antigenically related.

Key words. Monoclonal antibodies, oocysts, sporozoites, transmission experiments.

We recently described transmission of a *Cryptosporidium* sp. from a spontaneous infection in guinea pigs to both adult and juvenile guinea pigs [5]. Guinea pigs from an infected colony occasionally died, with microscopic evidence of severe blunting and fusion of villi, most prominent in the ileum. Cryptosporidia were found only in the small intestine and were indistinguishable from *Cryptosporidium parvum*, a species reported in natural infections of numerous mammals, but especially in calves under 1 mo of age and in humans [8, 17]. Experimentally infected guinea pigs, recovered by 1 mo post-infection, had high titers of anticryptosporidial serum antibodies, and were resistant to reinfection. The aim of this study was to compare the host ranges of *Cryptosporidium* isolated from guinea pigs [5] and *C. parvum* and to determine if monoclonal antibodies raised against oocysts and sporozoites of *C. parvum* would react with *Cryptosporidium* from guinea pigs.

MATERIALS AND METHODS

Cryptosporidium oocysts originally isolated from a naturally infected guinea pig (*Cavia porcellus*) [5] were passaged by oral gavage in 3–4-wk-old specific pathogen-free female Hartley guinea pigs (Charles River Laboratories, Portage, MI). Seven days after inoculation of 1×10^5 oocysts, cecal and colon contents were removed and stored in 2.5% $K_2Cr_2O_7$ at 10° C. Oocysts were isolated from fecal debris as previously described [5], washed free of $K_2Cr_2O_7$, and resuspended in 0.9% NaCl before inoculation by gavage into weanling (3–4-wk-old) guinea pigs, 1-wk-old specific pathogen-free CD-1 mice, 11-wk-old pathogen-free C57/Bl mice (Charles River Laboratories, Portage, MI), and calves during the first week of age. Ten 1-wk-old mice, five 3–4-wk-old guinea pigs and one 4-day-old calf were inoculated with 0.9% NaCl as negative controls. Infection of mice and guinea pigs was determined by examination of tissue sections from the duodenum, ileum, cecum and colon of each animal for cryptosporidia 7 days post-inoculation (PI). Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with Giemsa.

Two calves (#593 and #596) were allowed to suckle their dams right after birth; they were then removed from their dams, and individually housed in concrete stalls in an isolation building at the Animal Parasitology Unit (APU), Agricultural Research Service, United States Department of Agriculture in Beltsville, MD. An additional Holstein calf was allowed to suckle, and then was taken from its dam and housed in an isolation building at Utah State University in Logan, UT. The APU and Utah calves were inoculated with 2.9×10^6 and 5×10^6 guinea pig isolate oocysts at 4 days and less than 1 day of age, respectively. Feces from the APU calves were collected daily and checked for oocysts beginning on the day of inoculation in order to rule out natural infection with *C. parvum*. Each mouse in a litter of 1-wk-old mice housed with their dam in shoebox cages at the University of Michigan was inoculated with 5×10^5 oocysts from the same inoculum. The viability and infectivity of the inoculum was confirmed by inoculating 5×10^4 oocysts into each of three weanling guinea pigs. Feces from each calf were examined for oocysts for 7 days PI. Litters containing six and 11 1-wk-old mice, respectively, were inoculated with 5×10^5 oocysts recovered from feces of each positive calf. Similarly, 5×10^4 and 1×10^6 oocysts from each calf were each inoculated into two weanling guinea pigs. The following experiment was conducted in order to determine if the guinea pig isolate recovered from infected 1-wk-old mice would be infectious for guinea pigs. One-week-old mice from litters containing five and six mice, respectively, were inoculated with 5×10^5 oocysts of the guinea pig isolate. Four weanling guinea pigs were gavaged with 1×10^5 oocysts of the same inoculum. Mice were killed 7 days PI and a sample of ileal tissue from each mouse was taken for histopathology. The remaining intestines were pooled, minced, and oocysts recovered by sugar flotation as described [2]. Two weanling guinea pigs were each inoculated with 3×10^4 pooled oocysts from the mice.

To compare the host range of the guinea pig isolate with that of *C. parvum*, the infectivity of four bovine isolates for 1-wk-old mice and weanling guinea pigs was determined. Isolates designated AUB, NT, and 9303 were received from B. Blagburn (Auburn University, Auburn, Alabama). AUB and NT were isolated from spontaneous infections in calves at Auburn University while 9303 was isolated from a calf infected with NT. All three isolates were passaged in calves housed at the APU in Beltsville, MD. Oocysts were isolated from feces by flotation on saturated sucrose and stored in 2.5% $K_2Cr_2O_7$. Oocysts from an additional bovine isolate (designated IOWA, originally isolated from a calf and received Dr. Harley Moon, National Animal Disease Center, Ames, Iowa) were passaged and isolated via discontinuous sucrose gradients [2] from a calf at Utah State University. Each mouse in each of two litters of 1-wk-old mice (9–11 mice/litter) was inoculated with 1×10^5 oocysts of each bovine isolate. In addition, two weanling guinea pigs were inoculated with 5×10^4 and 1×10^6 oocysts, respectively, of each bovine isolate.

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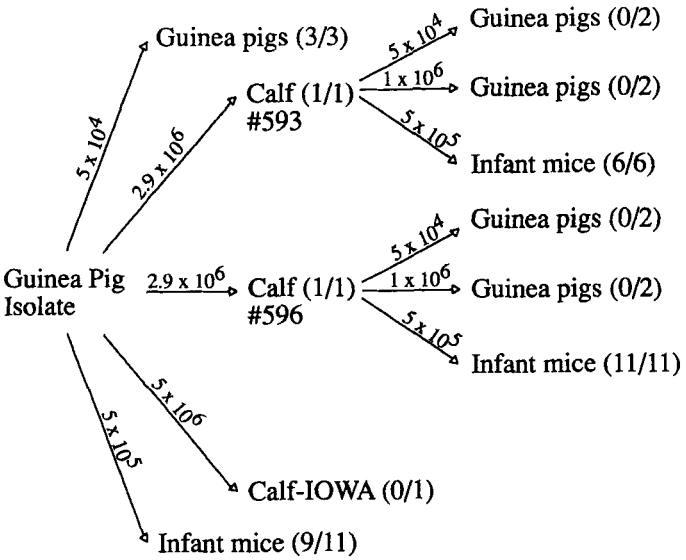

Fig. 1. Results of inoculation of guinea pig cryptosporidia. Numbers associated with arrows refer to numbers of oocysts inoculated. Numbers in parentheses refer to the number of animals positive for cryptosporidia (numerator) vs. the number of animals inoculated (denominator).

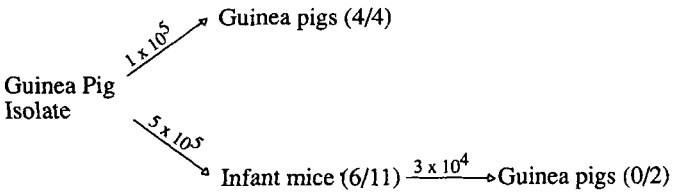

Fig. 2. Results of attempted passage of guinea pig cryptosporidia through mice and guinea pigs. Numbers associated with arrows refer to numbers of oocysts inoculated. Numbers in parentheses refer to the number of animals positive for cryptosporidia (numerator) vs. the number of animals inoculated (denominator).

To determine if *C. parvum* would infect very young guinea pigs, 1×10^5 oocysts from each of two bovine isolates, NT and 9303, were inoculated by gavage into 1-day-old guinea pigs. The two litters inoculated with NT contained two and three infants, whereas 9303 was inoculated into two litters with five and six animals. A litter of six 1-day-old guinea pigs were gavaged with saline as a negative control.

The reactivity of oocysts and sporozoites of the guinea pig isolate with monoclonal antibodies generated against oocyst and sporozoite antigens of *C. parvum* was determined using immunofluorescent assays [4, 12–14]. Parasite-coated microscope slides were prepared as follows. Oocysts were isolated from guinea pig feces using discontinuous sucrose gradients essentially as described for *C. parvum* oocysts [2]. Oocysts were excysted at 37° C in the presence of 0.25% trypsin and 0.75% sodium taurocholate, washed and finally resuspended in phosphate buffered saline. Poly-L-lysine (Sigma Chemical Co., St. Louis, MO) -coated microscope slides were prepared by immersing slides in a 0.05% solution at room temperature for 30 min, rinsing with distilled H_2O and wiping dry with lint-free paper towels. Aliquots of the excysted sporozoites, oocysts, and oocyst walls were immediately applied to the slides and allowed to air dry. Parasite-coated slides were stored at −20° C until used.

Immunofluorescent assays (direct and indirect) employed antibody incubation times of 30 min at room temperature [3]. The secondary antibody was goat anti-mouse IgG, conjugated with fluorescein isothyocyanate (Sigma). Mouse monoclonal antibodies examined for activity included those reactive with the oocyst surface (C1B3, OW3, OW50), oocyst suture (OW64), sporozoite (C3B4, C8C5, C4A1, and sporozoite cytoplasmic polar complex (C2A3). Monoclonal antibody specificities are described further in the results.

Those hybridomas secreting monoclonal antibody C2A3 were prepared as previously described [13], with the following modifications: the immunizing antigen was composed of Percoll-purified *C. parvum* oocyst walls [2] and the myeloma SP2/0 was utilized in the fusion procedure in place of the myeloma P3/X63/Ag8.653. Isotypic analyses of monoclonal antibodies OW50, OW64 and C2A3 were performed by indirect immunofluorescent assay using goat anti-mouse subclass-specific reagents (Zymed Laboratories Inc., San Francisco, CA).

RESULTS

Two of three calves inoculated with the guinea pig isolate shed oocysts (Fig. 1). Calf #593 shed oocysts on days 4, 6 and 7 PI, whereas calf #596 shed oocysts on days 4–7 PI. Calves #596 and #593 had foul smelling diarrhea starting on days 4 and 5 PI, respectively, and continuing through day 7. Guinea pigs were always infected by the guinea pig isolate throughout all experiments. None of five 11-wk-old mice were infected. None of the animals inoculated with saline were infected. Nine of 11 1-wk-old mice were infected by the guinea pig isolate and all 1-wk-old mice were infected by oocysts isolated from both calves after infection by the guinea pig isolate (Fig. 1). However, weanling guinea pigs could not be infected with oocysts isolated from either of two calves. Similarly, guinea pigs could not be infected with cryptosporidia after passage in mice (Fig. 2). Relatively low numbers of oocysts (6×10^4) could be isolated from the pooled intestines of infected mice.

Results of transmission experiments of bovine isolates of *C. parvum* to 1-wk-old mice, 1-day-old guinea pigs and weanling guinea pigs are shown in Table 1. Three isolates infected all the mice and the fourth infected 9 of 11 mice as determined by histopathology. In contrast, none of the bovine isolates infected weanling guinea pigs. No evidence of infection was found in the five 1-day-old guinea pigs from two litters inoculated with the NT bovine isolate. All five 1-day-old animals in one litter inoculated with bovine isolate 9303 were infected, but six guinea pigs in another litter had no morphologic evidence of infection seven days PI. Evidence of infection in the five infected guinea pigs was limited to occasional small clusters of cryptosporidia

Table 1. Results of inoculation of bovine isolates of *C. parvum* into infant mice and two age groups of guinea pigs.

	Infant mice (7 days old)		Weanling guinea pigs (3–4 wk old)		Infant guinea pigs (1 day old)	
Isolate designation	No. of oocysts inoculated	No. infected/ no. inoculated	No. of oocysts inoculated	No. infected/ no. inoculated	No. of oocysts inoculated	No. infected/ no. inoculated
NT	1×10^5	9/9	5×10^4	0/2	1×10^5	0/5
			1×10^6	0/2		
9303	1×10^5	9/11	5×10^4	0/2	1×10^5	5/11
			1×10^6	0/2		
AUB	1×10^5	11/11	5×10^4	0/2	—	—
			1×10^6	0/2		
IOWA	1×10^5	11/11	5×10^4	0/2	—	—
			1×10^6	0/2		

Table 2. Monoclonal antibodies generated against *C. parvum* cross-reactive by immunofluorescence for antigens of the guinea pig *Cryptosporidium* isolate.

Monoclonal antibody	Subclass	*C. parvum* reactivity[a]	Western blot reactivity[b]
C1B3	IgG_1	Oocyst surface [4, 12, 14]	ca. 40 kDa to ≥200 kDa [3]
OW3	IgM	Oocyst surface [4, 12]	ca. 200 kDa [3]
OW50	IgG_1	Oocyst surface	ND[c]
OW64	IgG_1	Oocyst suture	ND
C3B4	IgG_1	Sporozoite surface [12]	ca. 23 kDa [12]
C8C5	IgG_3	Sporozoite surface [3, 12]	ca. 23 kDa [4, 12]
C4A1	IgM	Sporozoite polar surface [3]	ca. 25 kDa to ≥200 kDa [4]
C2A3	IgG_{2a}	Sporozoite cytoplasmic polar complex	NR[d]

[a] Immunofluorescent appearance (reference number).
[b] Reactivity to detergent-solubilized sporozoite or oocyst antigens of *C. parvum* following SDS-PAGE (reference number)
[c] ND, not determined.
[d] NR, no reactivity in blots utilizing detergent-solubilized sporozoite antigens.

deep in the crypts of the cecum and colon. Unexpectedly, when tissues from the dam housed with the infected litter were examined, small numbers of cryptosporidia were found in the same locations as in the pups. None were found in the two dams whose pups were inoculated with the NT isolate and none were seen in the other dam whose pups were inoculated with the 9303 isolate.

Monoclonal antibodies generated against *C. parvum* sporozoite and oocyst antigens (Table 2) all reacted with antigens on the guinea pig isolate with equal intensity in indirect immunofluorescent assays. Immunofluorescent patterns on guinea pig oocysts and sporozoites were not distinguishable from those observed on *C. parvum*.

DISCUSSION

We previously showed that guinea pigs could be infected with small numbers of guinea pig-derived cryptosporidial oocysts [5]. For example, as few as 325 oocysts infected five of five weanling guinea pigs [5]. Thus, it was not surprising that positive control guinea pigs in the present experiments were all infected. As shown previously for *C. parvum* [8], adult mice could not be infected with the guinea pig isolate. Although 1-wk-old mice could be infected with the guinea pig isolate, the numbers of oocysts produced was low. This confirmed previous results that showed that mice infected with guinea pig cryptosporidia shed fewer oocysts than those infected with *C. parvum* [16]. Two of three calves shed oocysts following oral inoculation with the guinea pig isolate; however, oocysts subsequently isolated from both mice and calves failed to infect other guinea pigs. Although *C. parvum* contamination of the calves could have taken place, it is unlikely in the mice. Therefore, the results might be explained by biological alteration during passage through unnatural hosts.

As expected, *C. parvum* isolates from calves readily infected 1-wk-old mice [8]; however, in contrast to the guinea pig isolate, *C. parvum* did not infect weanling guinea pigs. In addition, only one of two bovine isolates infected 1-d-old guinea pigs and only very light infections of the large intestine resulted. In contrast, Tzipori et al. were able to infect seven of seven newborn guinea pigs with a bovine strain of cryptosporidia [18]. These variable results might be explained by differences in virulence between isolates. For example, Fayer and Ungar [8] reported marked differences between isolates of *C. parvum* in the number of oocysts required to infect calves. Although the host range of the guinea pig isolate and *C. parvum* appears to overlap somewhat, the restricted infectivity of *C. parvum* versus the guinea pig isolate for other than neonatal guinea pigs suggests a distinct difference.

Guinea pig cryptosporidia have essentially the same oocyst shape and size and the same stages in mouse ilea as does *C. parvum* [16]. At first glance, there appear to be many antigenic similarities between the guinea pig isolate and *C. parvum*. Eight monoclonal antibodies generated against either sporozoites or oocysts of *C. parvum* reacted with oocysts and sporozoites of the guinea pig isolate. It was shown previously that monoclonal antibody OW3 reacted with the surface of oocysts of *C. muris* but not with those of *C. baileyi* from chickens [12]. In addition, a monoclonal antibody to a 15-kDa protein from sporozoites and merozoites of *C. parvum* that partially protected mice from infection with *C. parvum* also reacted with guinea pig sporozoites [15]. This antigen was not shared by *C. muris, C. baileyi,* or *C. serpentis*. On the other hand, striking differences were found between the ^{125}I-labeled oocyst surface proteins of *C. parvum* and the guinea pig isolate [16]. Only six oocyst surface proteins were shared but four others had similar molecular weights.

Cryptosporidia have been described previously in guinea pigs [1, 5, 9–11, 19, 20], but there have been few attempts to transmit the infection to other species [1, 5, 19]. A separate species of *Cryptosporidium* has been previously described in guinea pigs and named *C. wrairi* [19, 20]. This isolate could be transmitted by inoculation with intestinal scrapings [19]. Although oocysts were not found to be associated with that isolate, methods for isolating oocysts were not known at that time. Therefore, that isolate may have been the same as the one we have described previously [5] and in this study.

Another guinea pig cryptosporidial isolate has been described [1]. In that study intestinal contents were used to transmit cryptosporidia rather than isolated oocysts. Guinea pigs were readily infected and the infection was successfully transmitted to one of two lambs. No attempt was made to transmit the organism from lambs to guinea pigs. In contrast to our results, infection could be transmitted from guinea pigs to mice and back to guinea pigs. Because intestinal contents rather than isolated oocysts were used in that study, the dose and the infective form of the cryptosporidia is unknown. For example, thin-walled oocysts that are thought to be autoinfective have been described in the intestinal contents but not in the feces of animals infected with *C. parvum* [6]. Because their study did not include observations of oocyst or sporozoite surface proteins, or other possible differentiating characteristics, it is impossible to know whether that

organism was the same as the isolate described in the present report.

The differences between *C. parvum* and *Cryptosporidium* from guinea pigs merits separation of the guinea pig isolate into a distinct species. We propose that the name *C. wrairi* be retained according to precedent. The differences are summarized as follows: 1) *C. wrairi* infects immunocompetent adult guinea pigs [5], whereas *C. parvum* infects immunocompetent adult humans [8]; 2) although both *C. wrairi* and *C. parvum* can be transmitted to infant mice and newborn calves, *C. wrairi* isolated from those animals is not capable of reinfecting guinea pigs; 3) *C. wrairi* infects only the small intestine of guinea pigs, whereas *C. parvum* is not readily transmitted to guinea pigs and infects only the large intestine of those animals; and 4) *C. wrairi* has a distinctly different surface protein pattern from that of *C. parvum* [16].

The antigenic similarities between these two species, like those between *Hammondia* and *Toxoplasma* (which are otherwise morphologically identical organisms with similar life cycles and hosts [7]), indicate they may have been derived from a common ancestor.

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