CLINICAL MICROBIOLOGY REVIEWS, Jan. 2011, p. 110–140 Vol. 24, No. 1
0893-8512/11/$12.00 doi:10.1128/CMR.00033-10

Zoonotic Potential and Molecular Epidemiology of *Giardia* Species and Giardiasis†

Yaoyu Feng[1]* and Lihua Xiao[2]*

State Key Laboratory of Bioreactor Engineering, School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai, China,[1] and Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]

INTRODUCTION

Giardia sp. is one of the most common intestinal parasites of humans; about 200 million people in Asia, Africa, and Latin America have symptomatic infections (301). Once infected, *Giardia* causes a generally self-limited clinical illness (i.e., giardiasis) characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption. However, asymptomatic giardiasis occurs frequently, especially in developing countries (113, 256). *Giardia* is also a very common enteric parasite of domestic animals, including livestock, dogs, and cats (257, 260), and wildlife (19). One species within this genus, *Giardia duodenalis* (syn. *Giardia lamblia* and *Giardia intestinalis*), causes giardiasis in humans and most mammals. Thus, giardiasis is considered a zoonotic disease.

The life cycle of *Giardia* is direct, and the infective stage of the parasite, the cyst, is encysted when released into the feces and is immediately infectious (123). Cysts remain infectious for months in cool, damp areas and rapidly accumulate in the environment. In soil, cyst infectivity was reduced by only 11% after 49 days at 4°C and was noninfective after 7 days at 25°C (75). In tap water, *Giardia* cysts were infectious for 56 days at 0°C to 4°C and 14 days at 20°C to 28°C. Similar results were obtained in lake water, with 56 days of survival at 0°C to 4°C or 6°C to 7°C and 28 days at 17°C to 20°C. Longer survival was noticed in river water, with 84 days of survival at 0°C to 4°C and 28 days at 20°C to 28°C. In seawater,

* Corresponding author. Mailing address for Yaoyu Feng: State Key Laboratory of Bioreactor Engineering, School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai, China. Phone: (86-21) 6425-0664. Fax: (86-21) 6425-0664. E-mail: yyfeng@ecust.edu.cn. Mailing address for Lihua Xiao: Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30329. Phone: (404) 718-4161. Fax: (404) 718-4197. E-mail: lxiao@cdc.gov.

† Supplemental material for this article may be found at http://cmr.asm.org/.

TABLE 1. *Giardia duodenalis* infection rates and genotypes in humans in developed countries

Location(s)	Total no. of samples	Infection rate (%)	No. of samples genotyped	No. of samples with assemblage:			Reference
				A	B	Other(s)	
Europe			1,658	714	930	2 (C), 4 (D), 4 (E), 4 (F)	247
Belgium	373	4.0	72[a]	18	54		93
France			25	9	16		31
Germany	202	1.5	3	3			227
Italy	14	42.9	6	6			168
Italy	1,989	0.4	11[b]	5	5	1 (A + B)	58
Italy			30	24	6		39
Italy			37	17	15	5 (A + B)	148
Italy, Africa			61	28	33		37
Netherlands			98	34	64		282
Netherlands	892	2.0	18	9	9		117
Norway			21		21		223
Norway			63	3	60		218
Portugal	190	3.7	7	7			10
Portugal			25	25			245
Spain			108	43	61	4 (A + B)	228
United Kingdom			33	9	21	3 (A + B)	14
United Kingdom			199	48	145	6 (A + B)	35
Canada			6	6			283
Canada	52	28.9	15	3	9	3 (A + B)	106
United States			14	14			283
United States			2		2		249
Japan			3	2	1		2
South Korea			5	5			304
Australia			8	2	6		214
Australia			12		11	1 (A + B)	118
Australia	353	7.6	23	7	16		213
Australia			124	31	93		299
New Zealand			30	23	7		294
New Zealand	66	7.6	5	1	4		152
Total			2,722	1,096	1,589	37	

[a] Including positive samples from other sources.
[b] Multiple samples were collected from each patient.

Giardia cysts could survive for over 65 days at 4°C (75). When ingested by the host, cysts excyst in the duodenum, releasing the trophozoites. The latter undergo repeated mitotic division and form environmentally resistant cysts in response to the stimulation of bile salts and other conditions. Cysts pass through the intestine in feces and are spread by contaminated water, food, and fomites and by direct physical contact.

An important aspect of the epidemiology of giardiasis is to understand the host range of different *Giardia* species and strains/genotypes, the potential for cross-species transmission, and risk and environmental factors involved in the exposure of the pathogen. This is particularly important in determining the zoonotic potential of *Giardia* infections in domestic animals and in determining the human disease burden attributable to parasites of animal origin. It has been only recently, with the advent of molecular typing tools, that the epidemiology of *Giardia* is systematically addressed.

Public Health Importance of Giardiasis

Giardiasis exerts a significant public health impact because of the high prevalence and disease burden of the infection, its propensity in causing major outbreaks and emergency responses, and its effects on growth and cognitive functions of infected children. Giardiasis is also a common disease in livestock and companion animals; thus, it is of veterinary health importance.

Prevalence. *Giardia duodenalis* has a global distribution (Tables 1 and 2). Infection rates for giardiasis in humans are generally lower in developed countries, and data from some recent studies are listed in Table 1. Recent studies reported infection rates of 4.0% in Belgium (93), 1.5% in Germany (227), 0.4% to 6.2% in Italy (58, 97), 3.7% in Portugal (10), 5.4% in Spain (167), 1.3% in the United Kingdom (61), 1.4% in the United States (52), 1.1% to 6.6% in Saudi Arabia (8, 136), 2.5% in South Korea (126), 1.6% to 7.6% in Australia (113, 213), and 7.6% in New Zealand (152). Most of the surveys were conducted with asymptomatic children. The occurrence of giardiasis is probably higher in children with diarrhea. Thus, in the Nordic countries of Denmark, Finland, Norway, and Sweden, the infection rate of giardiasis was estimated to be 2.9% and 5.8% for asymptomatic and symptomatic persons, respectively (121). A similar trend was seen in the Netherlands, with infection rates of 14.0% in patients with persistent diarrhea and 2.0% in asymptomatic subjects (117). Although *Giardia* is not considered an opportunistic pathogen in immunocompromised patients, the infection rates of giardiasis in HIV-infected people ranged from 3.5% to 6.2% in Italy before the introduction of highly active antiretroviral treatment (97). The infection rates for giardiasis are 3.1% in HIV/AIDS patients in

TABLE 2. *Giardia duodenalis* infection rates and genotypes in humans in developing countries

Location	Total no. of samples	Infection rate (%)	No. of samples genotyped	No. of samples with assemblage:			Reference
				A	B	Others	
Albania	125	17.6	22	10	12		29
Argentina			43	40	3		174
Poland	232	1.3	3	2	1		243
Cuba			20	9	11		202
Mexico			19	19			206
Mexico			9	9			147
Mexico			12	12			70
Nicaragua			119	25	94		153
Brazil			37	29	8		246
Brazil	366	23.8	62	62			287
Peru	845	23.8	16	10	6		203
Peru			25	6	19		249
Peru	1,531	20.4	167	66	81	20 (A + B)	57a
Bangladesh	2,534	12.7	267	20	231	16 (A + B)	111
China			8	4	4		304
China			18	12	6		289
India			16	5	8	3 (A + B)	265
India			19	6	9	4 (A + B)	249
Nepal	1,096	4.1	35	7	26	2 (A + B)	238a
Laos			5		5		303
Malaysia	321	23.7	42	1	41		165
Thailand	204	20.3	35	25	1	1 (C), 2 (A + B), 6 (A/B + C/D)	264
Thailand	531	6.2	12	5	7		212
Thailand	6,967	0.9	61	5	31	25 (A + B)	274
Turkey			44	19	25		21
Egypt	52	34.6	18	1	14	2 (E), 1 (B + E)	83
Ethiopia			59	31	13	7 (A + F), 8 (A + B)	87
Ivory Coast			14		14		31
Sahrawi	120	34.2	32[a] (28[b])	12[a] (16[b])	18[a] (12[b])	2 (A + B)[a]	145
Saudi Arabia	1,500	6.5	40	23	15	2 (A + B)	12
Uganda	62	5.0	3	3			104
Uganda	108	40.7	5[a] (3[b])	4[a] (6[b])	5[a] (3[b])		137
Total			1,287	482	708	101	

[a] At the *tpi* locus.
[b] At the *gdh* locus.

Iran (60), 6.6% in immunocompromised patients in Saudi Arabia (8), and 13.9% in 252 patients with common immunodeficiency in France (193). Occasionally, high infection rates have also been reported for some poor regions in developed countries. For example, an infection rate of 42.9% was reported for a socially deprived small Rom community in Italy (168).

High infection rates for giardiasis have been reported for developing countries, and some recent data are listed in Table 2. In various Asian countries (Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Nepal, Philippines, Thailand, Turkey, Saudi Arabia, and Vietnam), North America (Cuba, Mexico, and Nicaragua), South America (Argentina, Brazil, Colombia, and Peru), and Africa (northern Africa, west Africa, and South Africa), most of the studies focused on children, and infection rates for *G. duodenalis* fell into the range of 8% to 30% in the majority of those studies (12, 29, 56, 62, 63, 65, 69, 144, 160, 166, 174, 184, 186, 202, 203, 229, 234, 237, 239, 252, 253, 281). In a few studies, the infection rate was lower than 4% or higher than 30% (65, 137, 145, 230, 243, 274, 280). High infection rates for giardiasis were also reported for adults in developing countries, with rates of 25.1% in pregnant women in Minatitlan, Mexico (224); 11.7% in adults in Settat, Morocco (72); and 5.0 to 14.0% in African refugees and new immigrants in the United States, the Netherlands, and Spain (167).

Disease burden. Giardiasis is highly underreported for various reasons (302). In the United States, the total number of reported cases remained about 20,000 annually from 2006 to 2008, and the incidence ranged from 7.4 to 7.6 cases per 100,000 population (302). Although the true burden of giardiasis in the United States is unknown, an estimated 2 million cases occur annually (302). In Britain, there are approximately 3,500 cases per year according to a report of Health Protection Scotland (http://www.hps.scot.nhs.uk/giz/giardia.aspx), with an incidence of giardiasis in the United Kingdom of 5.5 cases per 100,000 population in 2005. In Scotland, the annual number of reported *Giardia* infections remained constant at approximately 300 to 400 between 1988 and 1998 but decreased consistently thereafter; in 2003 only 192 infections were reported (205). In Germany, on average, 3,806 notified giardiasis cases (range, 3,101 to 4,626) were reported between 2001 and 2007, which corresponded to an average incidence of 4.6 cases/100,000 population (227). Much higher incidence rates were reported for some other countries. In the Netherlands, there were 11,600 cases in 2004, corresponding to 69.9 cases/100,000 population (286). In Canada, there were 5,295 cases in

2000, with an average annual incidence of 16.3 cases per 100,000 population (http://www.cureresearch.com/g/giardia/stats-country.htm). In New Zealand, 16,471 cases were reported in the 10 years of 1997 to 2006, with an average annual rate of 44.1 cases per 100,000 population (241). In China, there are approximately 28.5 million cases per year (290). The majority of giardiasis cases remain unreported. It was estimated that in the Nordic countries of Denmark, Finland, Norway, and Sweden, for every reported giardiasis case there were 254 to 867 cases of undetected and/or unreported cases and that the true incidence of symptomatic giardiasis was 4,670 cases annually per 100,000 general population (121). The disease burden in other countries is even more difficult to assess, as giardiasis reporting is not required in most countries, and data are available for only selected populations.

Effect on nutrition and growth. Deleterious effects of giardiasis on growth and development have been observed in many studies. Malnutrition is commonly seen in *Giardia*-positive children (237). In a case-control study including 30 children between 1 and 10 years old with giardiasis and 30 healthy children, the infected group had significantly lower body weight, serum iron levels, and zinc levels than controls (5). It is generally assumed that malnutrition results from malabsorption caused by giardiasis-associated chronic diarrhea. Nutrient malabsorption has been reported for at least 50% of patients with symptomatic giardiasis (27, 51, 238). In addition, a negative effect of giardiasis on growth and weight gain has been commonly reported (11, 186, 209). Results of cross-sectional studies suggested that symptomatic giardiasis delayed childhood growth, and diarrhea could cause growth delays up to 4 months after a diarrheal episode (51, 238). The duration of giardiasis episodes and their association with diarrhea appeared to be the most important factors associated with growth retardation (123). Even in asymptomatic giardiasis, reduction in growth (208), probably via malnutrition, was noticed (46, 186). This is in agreement with the observed association between an improvement in small intestinal mucosal function and better weight-for-age and weight-for-height *Z* scores (101) and the observation that giving antigiardial treatment three or four times a year improved growth in children in Brazil (208) and serum zinc levels in children in Mexico (209). Even more disturbing were the potential effects of stunting on cognition, intelligence, and psychosocial development, especially language-cognitive and fine-motor development (28, 238).

Outbreaks. Although most cases occur sporadically, outbreaks of giardiasis are well documented. A recent review (139) indicated that there have been at least 132 reported waterborne outbreaks of giardiasis worldwide since 1954. Among them, 104 were related to drinking water, 18 were related to recreational water, and 10 were related to foreign travel. The number of cases in each outbreak varied from several to 50,000. The majority of the outbreaks were reported in North America and Europe because of better surveillance and reporting systems. In addition, food-borne outbreaks of giardiasis linked to infected food handlers and food handlers who changed diapers of infected children prior to handling food have been reported (115). Food-borne outbreaks of giardiasis have also been associated with ice, vegetables, and chicken salad (302). Outbreaks resulting from person-to-person transmission in child care centers are common (15). Community-wide outbreaks might be waterborne initially but might spread subsequently by person-to-person transmission (142). Few direct animal-to-human outbreaks have been documented. Two food-borne outbreaks of giardiasis were linked to animals: the consumption of a Christmas pudding contaminated with rodent feces and tripe soup made from the offal of an infected sheep (240).

Veterinary health importance of giardiasis. *Giardia* infections are common in pigs (20, 108), cattle (296), sheep (298), goats (34, 50), elks and deer (201), and other ruminants (191).

For cattle, the infection rate varied markedly in different studies (Table 3), being 17.4% to 31.3% in Belgium (90, 91), 43.6% in Denmark (149, 163), up to 38.0% in Germany (135), 30.0% in Italy (30), 49.0% in Norway (107), 2.2 to 14.0% in Poland (22), 9.0% in Portugal (173), 26.6 to 30.1% in Spain (48, 49), 8.7 to 57.0% in Canada (17, 55, 103, 171, 192, 275), 19.1 to 52.0% in the United States (114, 231, 270–273), 3.7% in Taiwan (122), 10.2% in Vietnam (94), 58.0% in Australia (192), 4.5 to 40.6% in New Zealand (127, 152, 182, 295), and 8.0 to 10.0% in Uganda (104, 137). The age of the cattle and housing, feeding, and management practices probably contributed to the different infection rates observed. The infection rates were also different when different detection methods were used (285). Several longitudinal studies revealed cumulative infection rates of 73 to 100% in cattle (135, 210, 231, 275).

Giardia infection rates in sheep in some studies are listed in Table 3. The infection rates were 25.5% in Belgium (95), 1.5% in Italy (98), 26.8% in Norway (220), 1.3% in Poland (22), 19.2 to 42.0% in Spain (47, 48, 99), 55.6% in Mexico (66), 25.4% in the United States (232), and 11.1 to 44.0% in Australia (189, 226, 298). In goats, the infection rates were 35.8% in Belgium (95), 13.0 to 42.2% in Spain (48, 50, 225), and 12.3% in Uganda (137).

Giardia infections have been reported for pigs from Australia, Asia, Europe, and North America, with infection rates ranging from 0.1% to 20% in most studies (108). An infection rate of 31.1% was observed for preweaned piglets, postweaned piglets, and sows in Australia (20) (Table 3).

Giardiasis in dogs and cats was reviewed recently (23), and infection rates in some studies are listed in Table 4. In dogs, the infection rates were 24.8% in a large study in Europe (74), 22.7% in Belgium (53), 1.1% in the Czech Republic (68), 5.3% in Finland (215), 2.3% in Germany (73), 4.3% in Greece (198), 7.7 to 26.6% in Italy (30, 44, 197, 216, 235), 15.2% in the Netherlands (282), 8.2% in Norway (110), 2.0 to 36.0% in Poland (22, 242), 14.6% in Serbia (188), 1.0 to 7.0% in Spain (169, 175), 8.4 to 21.0% in the United Kingdom (25, 278), <0.1 to 12.9% in Canada (194, 236), 8.0% in Nicaragua (153), 3.3 to 15.6% in the United States (45, 85, 158), 1.3 to 8.9% in Argentina (82, 244), 0.8 to 36.8% in Brazil (43, 124, 141, 143, 172, 185, 287), 22.0% in Chile (161), 0.7% in Iran (235a), 0.9 to 37.4% in Japan (131, 133, 297), 11.2% in South Korea (158a), 7.9 to 56.8% in Thailand (129, 158a), and 9.4% in Australia (196). Among the various methods used, higher infection rates were detected by PCR or enzyme-linked immunosorbent assay (ELISA) than by conventional microcopy. For example, in two studies, the infection rates were 3.3% and 7.5% when micros-

TABLE 3. *Giardia duodenalis* infection rates and genotypes in farm animals

Animal(s)	Location(s)	Total no. of samples	Infection rate (%)	No. of samples genotyped	No. of samples with assemblage:				Reference(s)
					A	B	E	Other(s)	
Cattle	Europe			562	126	11	422		247
Cattle	Belgium	832	31.3	101	16		54	31 (A + E)	91
Cattle	Denmark	1,150	43.6	145	8		133	4 (unknown)	149, 163
Cattle	Italy			24	12	5	3	2 (A + B), 2 (A + E)	148
Cattle	Italy			4	4				37
Cattle	Italy	10	30	3			3		30
Cattle	Portugal	467	9.0	14	2	1	11		173
Cattle	Spain	379	26.6	4			4		48
Cattle	Sweden			17			17		154
Cattle	Canada	143	42.0	60		35	25		55
Cattle	Canada	495	33.9	42	1		41		17
Cattle	Canada	507[a]	49.0	14	6		14		275
Cattle	Canada, Australia	64	57.8	16	3		13		192
Cattle	United States			5	4		1		176
Cattle	United States			7			7		249
Cattle	United States	407	40.3	164	25		139		272
Cattle	United States	456	52.0	237	31		206		271
Cattle	United States			58	8		48	2 (A + E)	81
Cattle	United States	990[a]	31.5	312	44		266	2 (A + E)	231
Cattle	United States	571	35.7	204	18		186		273
Cattle	United States	541	26.6	144	9		135		270
Cattle	Brazil			5	1		4		246
Cattle	Japan			5	1		4		130
Cattle	Taiwan	107	3.7	4	2		2		122
Cattle	Vietnam	334	10.2	17	1		16		94
Cattle	Australia			31			31		26
Cattle	Australia			16			16		214
Cattle	New Zealand	715	40.6	15	11	4			127
Cattle	New Zealand	1,190	31.0	40	35	5			294
Cattle	New Zealand	724	6.6	48	26	22			152
Cattle	Uganda	25	8	1		1[c]	1[b]		137
Cattle	Uganda	50	10	5	5				104
Yak	Sweden			1			1		154
Water buffalo	Italy	57	26.3	8	2		6		40
Water buffalo	Italy			2	2				37
Sheep	Belgium	137	25.5	8	2		4	2 (A + E)	95
Sheep	Italy	325	1.5	5	5				98
Sheep	Italy			2		2			13
Sheep	Netherlands			2			2		282
Sheep	Norway	1,095	26.8	42		1	41		220
Sheep	Spain	386	42.0	75	1		74		99
Sheep	Spain	446	19.2	12		1	11		48
Sheep	Sweden			26	7	16		3 (A + E)	154
Sheep	Mexico	18	55.6	14			14		66
Sheep	United States			1			1		176
Sheep	United States	63	25.4	14	1		13		232
Sheep	Australia	477	11.1	52	5		36	11 (A + E)	298
Sheep	Australia	1,647[d] (500[e])	8.7[d] (44.0[e])	46	11		33	2 (unknown)	226
Sheep	Australia	284	15.1	43	30		13		189
Sheep and goat	Europe			207	35	2	170		247
Goat	Belgium	148	35.8	28	6		12	5 (A + E), 5 (unknown)	95
Goat	Netherlands			1			1		282
Goat	Spain	315	42.2	39			39		225
Goat	Spain	116	19.8	1			1		48
Goat	Uganda	57	12.3	3			2[b] (3[c])		137
Pig	Europe			140	29	1	109	1 (D)	247
Pig	Denmark	1,237	17.4	82	10		52	1 (D)	149, 163
Pig	Italy			1	1				37
Pig	Australia	289	31.1	55	17		35	1 (F), 2 (A + E)	20
Alpaca	United States	61	4.9	3	3				267

[a] Multiple samples were collected from each animal during a longitudinal study.
[b] At the *gdh* locus.
[c] At the *tpi* locus.
[d] Determined by microscopy.
[e] Determined by PCR.

TABLE 4. *Giardia duodenalis* infection rates and genotypes in companion animals

Animal	Location(s)	Total no. of samples	Infection rate (%)	No. of samples genotyped	No. of samples with assemblage:						Reference(s)
					A	B	C	D	F	Other(s)	
Dog	Europe			600	137	53	191	215		5 (E)	247
Dog	Belgium	1,159	22.7	119	40	4	26	49			53
Dog	Finland	150	5.3	8			3	4		1 (E)	215
Dog	Germany			55	33		5	2		15 (A + C)	155
Dog	Germany			150	4		54	83		8 (C + D), 1 (A + D)	24
Dog	Hungary	187	7.5[a] (58.8[b])	15			5	9		1 (C + D)	251
Dog	Italy	14	64.3	9	9						168
Dog	Italy	113	15.0	17	2		11	1		2 (A + C), 1 (C + D)	30
Dog	Italy			21	6		1	12		1 (A + D)	148
Dog	Italy	127	11.0[a] (20.5[c])	30	8		14	4			235
Dog	Italy	240	26.6	30	2		3	25			197
Dog	Netherlands			2				2			282
Dog	Netherlands	152	15.2	13	1		7	3		1 (C/D), 1 (unknown)	195
Dog	Poland	148	2.0	2			1	1			242
Dog	Sweden			28	1		8	14		5 (C + D)	154
Dog	United Kingdom	878	21.0	41	1		10	29		1 (C + D)	278
Dog	Mexico			5	4					1 (A + B)	147
Dog	Mexico			11	7					4 (A4 [probably C or D])	71
Dog	Mexico			19	19						70
Dog	Mexico			2	2						206
Dog	United States			15			15				249
Dog	United States			3			1	2			176
Dog	Canada	155	61.3	13	13						113a
Dog	Peru	605	14.5	67			9	32		26 (C + D)	57a
Dog	Argentina			1		1					174
Dog	Brazil			27			7	20			246
Dog	Brazil	19	36.8	7	7						287
Dog	Nicaragua	100	8.0	8			2	5		1 (C + D)	153
Dog	Japan			4				4			1
Dog	Japan			24	14		1	6		3 (A + D)	130
Dog	Japan	1,794	23.4	29			9	20			130a
Dog	India	101	3.0[a] (20.0[c])	7	5	2					265
Dog	Thailand	229	7.9	13	5		1	3		3 (A + B), 1 (A + D)	129
Dog	Thailand	229	56.8	60	33	9	5	13			264
Dog	Australia	1,400	9.4	88	1		41	44		2 (C + D)	196
Dog	Australia			11			10	1			178, 179
Dog	Unknown			9	1	2	4	2			214
Cat	Europe			158	68	3	5	3	77	2 (E)	247
Cat	Italy	1	100	1	1						30
Cat	Italy	27	37.0	10	10						199
Cat	Italy			1					1		148
Cat	Italy	181	4.4	11	3				8		197a
Cat	Italy, Croatia			3	3						37
Cat	Netherlands	60	13.6	2	1				1		195
Cat	Sweden			18	5				12	1 (E)	154
Cat	Mexico			1	1						206
Cat	United States	250	13.6	17	6				11		284
Cat	United States	18	44.4	8					8		79
Cat	United States			1					1		176
Cat	Brazil	1	100.0	1	1						287
Cat	Brazil			19	8				11		246
Cat	Colombia	46	6.5	3					3		233
Cat	Japan			3					3		130
Cat	Japan	321	8.1	26	6				20		250a
Cat	Australia			18	6	2	2	7		1 (E)	214
Cat	Australia	1,063	2.0	8				1	7		196
Horse	Italy	450	2.0[a] (13.3[d])	20						20 (E)	285
Horse	Australia, United States			10	4	6					263
Rabbit	Sweden			1		1					154
Rabbit	China			1		1					249
Ferret	Japan			3	3						3, 4

[a] Determined by microscopy.
[b] Determined by ELISA.
[c] Determined by PCR.
[d] Determined by direct fluorescent antibody assay.

copy was used, whereas the rates were 12.9% and 58.8% when ELISA was used (194, 251). In another study, the infection rates were 3.0% by microscopy and 20.0% by PCR (265). Similarly, the infection rate determined by PCR was almost twice as high as that determined by microscopy in another study (235).

The prevalence of *Giardia* has been studied less in cats than in dogs. The infection rates were 20.3% in a multicountry study in Europe (74), 15.8 to 37.0% in Italy (199, 200), 13.6% in the Netherlands (195), 5.3% in the United Kingdom (102), 10.8 to 44.4% in the United States (45, 79, 284), 4.1% in Canada (194), 5.9% in Brazil (54), 19.0% in Chile (161), 6.5% in Colombia (233), 40.0% in Japan (132), and 2.0% in Australia (196). However, low infection rates were reported in some studies, such as 0.2% in 8,160 feline specimens examined in Canada (236), 0.6% in 211,105 cats in 40 U.S. states during 2003 to 2004 (64), 2.3% in 1,566 cats in another U.S. study (85), 1.1% of 441 cats in Germany (73), none of 1,079 cats in Japan (297), and 0.9% of 113 cats in Iran (177). The health status of the cats (normal or diarrheic), age variations, and diagnostic techniques used probably contributed to the variations in infection rates in different studies. In one case, the infection rate obtained by ELISA (4.1%) was higher than that obtained by microscopy (1.0%) (194).

Giardiasis in animals is often asymptomatic but has been associated with the occurrence of diarrhea and ill thrift in calves, puppies, and kittens (257). Although it is commonly believed that infection with *Giardia* is associated with economic losses through the occurrence of diarrhea, poor growth, and even death in farm animals (48, 89), only a few studies have been conducted to assess the effect of giardiasis on the production or growth rates in livestock. In bottle-fed specific-pathogen-free lambs experimentally infected with *Giardia* cysts, the infection was associated with delays for lambs to reach slaughter weight and decreased carcass weight (191). In calves experimentally infected with *G. duodenalis* and treated with fenbendazole, a significant difference in weight gain was noticed between fenbendazole-treated and untreated calves. Animals in the treatment group gained on average 2.86 kg (equal to 102 g per day) more than the animals in the control group ($P < 0.031$) (96). In an outbreak of giardiasis on a sheep farm, *Giardia*-infected lambs (30 to 90 days of age) had malabsorption, decreased weight gain, and reduced feed efficiency. After treatment with fenbendazole, giardiasis-infected animals recovered rapidly from the symptoms and poor weight gain (13). However, in some other treatment studies with fenbendazole or paromomycin sulfate, differences in mean body weight, average daily weight gain, or feed intake between the control and treated groups were not significant, although there was a slightly higher weight gain and lower occurrence of diarrhea in the treated groups (88, 190). Rapid reinfection after antigiardial treatment was one possible reason for the failure to detect some benefits of the treatment.

GIARDIA TAXONOMY

Giardia Species

Species of the genus *Giardia* infect numerous hosts, ranging from mammals to amphibians and birds. The taxonomy of *Giardia* was reviewed previously (42, 180, 260). Currently, six *Giardia* species are accepted by most researchers. Among them, *G. agilis*, *G. ardeae*, *G. muris*, *G. microti*, and *G. psittaci* infect various animals, whereas *G. duodenalis* infects humans and many mammals (Table 5). A unique species is probably also present in reptiles, as a *G. duodenalis*-like parasite was found in lizards. This parasite, however, mostly lacked median bodies and had binucleated cysts, and it was considered *G. varani* (279). Although it is expected that fish have unique *Giardia* spp., a recent study of cultured and wild freshwater and marine water fish in Australia showed the occurrence of zoonotic (assemblages A and B) and artiodactyl-specific (assemblage E) genotypes of *G. duodenalis* and *G. microti* (300). It was not clear whether the fish were infected with these *Giardia* spp. or merely served as mechanical vectors for the dissemination of waterborne *Giardia* cysts. Thus, *Giardia* species differ significantly in host range, with *G. duodenalis* having the broadest host range and greatest public health significance.

TABLE 5. Established *Giardia* species and *G. duodenalis* assemblages

Species	Major host(s)
G. agilis Kunstler, 1882	Amphibians
G. ardeae Noller, 1920	Birds
G. microti Benson, 1908	Muskrats and voles
G. muris Benson, 1908	Rodents
G. psittaci Erlandsen and Bemrick, 1987	Birds
G. varani Lavier, 1923[a]	Lizards
G. duodenalis Davaine, 1875	Mammals
Assemblage A (=*G. duodenalis* sensu stricto?[b])	Humans, nonhuman primates, domestic and wild ruminants, alpacas, pigs, horses, domestic and wild canines, cats, ferrets, rodents, marsupials, other mammals
Assemblage B (=*G. enterica*?[b])	Humans, nonhuman primates, cattle, dogs, horses, rabbits, beavers, muskrats
Assemblage C (=*G. canis*?[b])	Domestic and wild canines
Assemblage D (=*G. canis*?[b])	Domestic and wild canines
Assemblage E (=*G. bovis*?[b])	Domestic ruminants, pigs
Assemblage F (=*G. cati*?[b])	Cats
Assemblage G (=*G. simondi*?[b])	Mice, rats
Assemblage H	Seals

[a] To be supported by molecular biological characterizations.
[b] Species names recently proposed (180, 260, 261).

The species names *Giardia duodenalis*, *Giardia intestinalis*, and *Giardia lamblia* are used interchangeably in current literature referring to the same organism (296). Both *G. duodenalis* and *G. intestinalis* are used in equal frequency in referring to the *Giardia* species infecting most mammals, including humans, their companion animals, and livestock, and opinions differ regarding the legality of the name *G. intestinalis*, largely because of different interpretations of the complex rules of the International Code of Zoological Nomenclature (180, 259). For purposes of consistency, *G. duodenalis* is used in this review. In the medical field, *G. lamblia* is still commonly used to discuss *Giardia* species infecting humans (180, 259). Because numerous recent bio-

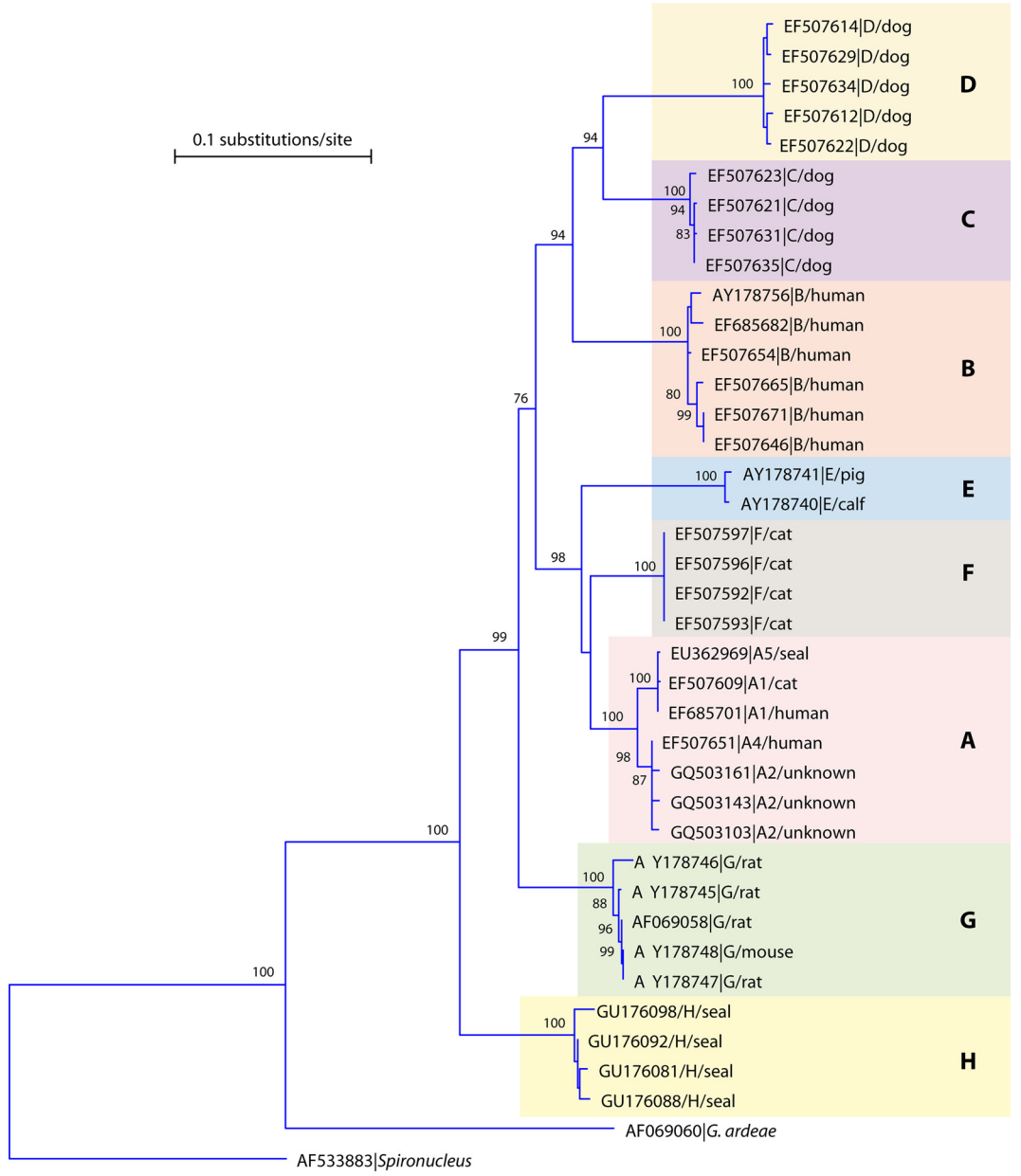


FIG. 1. Phylogenetic relationships among assemblages of *G. duodenalis* at the *gdh* locus as assessed by a neighbor-joining analysis of the nucleotide sequence covering a 709-bp region (positions 256 to 964 of GenBank accession number AY178740) of the gene, using distance calculated by the Kimura two-parameter model.

logical and genetic analyses have shown that the same *Giardia* species present in humans are also found in a range of other mammalian species (296), there is no taxonomic basis for the use of the name *G. lamblia*, which was preempted by both *G. duodenalis* and *G. intestinalis*.

Although *G. duodenalis* is the only species found in humans and many other mammals, including pets and livestock (261) (Table 5), it is now considered a multispecies complex. Historically, allozyme analyses placed all isolates from humans into two genetic assemblages (assemblages A and B) encompassing at least four genetic clusters (groups I to IV) (reviewed in reference 181). Phylogenetic analyses of a large set of nucleotide sequence data from the small-subunit (SSU) rRNA gene and several housekeeping genes coding for glutamate dehydrogenase (*gdh*), β-giardin (*bg*), elongation factor 1 alpha (*ef1*α), and triosephosphate isomerase (*tpi*) confirmed the genetic uniqueness of assemblages A and B. Additional lineages of *G. duodenalis* from animals were identified: assemblages C and D from dogs, assemblage E from artiodactyls, assemblage F from cats, and assemblage G from rodents (42, 261) (Fig. 1 and see Fig. S1 and S2 in the supplemental material).

In view of the observed host specificity and genetic characteristics of the *G. duodenalis* assemblages, attempts have been made recently to resurrect some of the previously used species names based on host occurrence (180). Thus, it has been proposed to adopt the names of *G. duodenalis* for assemblage A, *G. enterica* for assemblage B, *G. canis* for assemblages C and D, *G. bovis* for assemblage E, *G. cati* for assemblage F, and *G. simondi* for assemblage G (180, 260, 261). This suggestion seemingly makes logical sense and could lead to reduced confusion in *Giardia* taxonomy and a better understanding of giardiasis epidemiology. Because of the uncertainty related to the identity of the parasites at the initial description of these species, in compliance with the International Code of Zoological Nomenclature, redescriptions of these *Giardia* species that incorporate modern biological and genetic data may be needed before the proposed new *Giardia* species nomenclature can be accepted by the research community. This probably should include morphological descriptions and measurements of the cysts and trophozoites, data on natural host spectrum and, if any, infectivity in animal models, other biological characteristics (prepatent period, patent period, and infection site in the gastrointestinal tract, etc.), and a summary of the nucleotide sequence uniqueness of the assemblage. Such data are already available for most of the proposed *Giardia* species, although they are scattered in the literature. The only difficulty is probably in the naming of the species that infects canines, as lumping assemblages C and D into one *Giardia* species may be problematic; the sequence divergence between the two assemblages is of an order of magnitude similar to that separating the other assemblages, and at some genetic loci, such as *tpi*, they do not form a monophyletic group (see Fig. S2 in the supplemental material).

Giardia duodenalis Assemblages

Among assemblages of *G. duodenalis* (Fig. 1 and see Fig. S1 and S2 in the supplemental material), assemblages A and B have the broadest host specificity, having been found to infect humans and various other mammals (42, 261, 296). Assemblage A is frequently found in livestock (cattle, water buffalo, sheep, goats, alpacas, and pigs) and companion animals (dogs, cats, and horses) (Tables 3 and 4). In comparison, assemblage B is less frequently reported for livestock and companion animals, with only a few reports of infection of cattle, sheep, horses, dogs, cats, and rabbits (Tables 3 and 4). Assemblage A and, to a lesser extent, assemblage B are commonly found in wild animals, with the exception of beavers and muskrats, which seemingly have a high occurrence of assemblage B (Table 6). Both assemblages A and B are commonly reported to infect humans (Tables 1 and 2). Because they are found in humans and numerous species of mammals, both assemblages A and B are considered to have broad host specificity and can be transmitted zoonotically (41, 261, 296). The host range of assemblages A and B is shown in Table 5, and their distributions in humans and various animals are shown in Tables 1 to 4 and 6.

There is substructuring within assemblage A, which consists of mostly two subgroups, subgroups or subassemblages AI and AII. The separation of subgroups AI and AII was initially made by allozyme analysis and is supported by phylogenetic analyses of assemblage A sequences at the *gdh* locus (Fig. 2). The existence of numerous subtypes related to subgroups AI and AII, however, made the separation of subgroups AI and AII less obvious at some other loci, such as *tpi* and *bg* (see Fig. S3 and S4 in the supplemental material). Recently, a third subgroup within assemblage A, subgroup AIII, was identified and appears to be associated mostly with wild hoofed animals (37, 219, 282). It has significant sequence differences from subgroups AI and AII at all loci examined thus far (Fig. 2 and see Fig. S3 and S4 in the supplemental material). More recently, based on an analysis of the *tpi*, *gdh*, and *bg* loci, subgroup AIII was found in three cats, four cattle, and 45 wildlife but has not been found in dogs, goats, sheep, pigs, and humans thus far (247). In comparisons with subgroup AIII, subgroups AI and AII always form one cluster with high bootstrap values in phylogenetic analyses of nucleotide sequences of all loci (Fig. 2 and see Fig. S3 and S4 in the supplemental material). In contrast, there is no clear subgrouping within assemblage B (293) (Fig. S5 to S7).

Assemblages C, D, E, F, and G have strong host specificities and narrow host ranges. Assemblages C and D have been found mostly in dogs and other canines (foxes and coyotes) and canine-related animals (seals) (Table 4). Assemblage E has been found largely in cloven-hoofed domestic mammals (cattle, water buffaloes, sheep, goats, and pigs) (Table 3). Assemblages F and G have been found mostly in cats and rodents, respectively (Tables 4 and 6). However, there are occasional exceptions to the host specificity. Thus, assemblages C and D were reported in a few cats (196, 214) and humans (264), assemblage D was also reported in two pigs (149, 247), assemblage E was reported in cats (154, 214, 247) and humans (83), and assemblage F was reported in one pig (20) and seven humans (87). It is commonly believed that humans are infected only with assemblages A and B (Tables 1 and 2), and the recent identification of unusual *G. duodenalis* genotypes, such as assemblages C, D, E, and F in humans (83, 87, 264), requires thorough genetic characterizations of the parasites.

In addition to the above-described accepted assemblages, several novel genotypes have been reported. *Giardia* cysts isolated from U.S. seals were reported to belong to a new genotype based on a sequence analysis of the *gdh* gene (86), and the same genotype was found in gray seals, harbor seals, and a gull in a later study and was recently named assemblage H (151) (Fig. 1). However, the existence of this new assemblage was supported by the sequence analysis of only *gdh* but not *tpi* (151), and the *gdh* sequence of the new genotype is placed outside the *G. duodenalis* cluster in phylogenetic analyses (Fig. 1). One *Giardia* isolate from a southern brown bandicoot (*Isoodon obesulus*, commonly known as quenda) in Western Australia was reported to be a novel genotype based on a phylogenetic analysis of the SSU rRNA (GenBank accession number AY309064) and *ef1*α (accession number AY309065) gene sequences. Those authors believed that this isolate constituted a distinct species, because phylogenetic analysis shows it to be distinct from other recognized species such as *G. microti*, *G. psittaci*, *G. ardeae*, and *G. muris* (7). This new genotype was identified in another six quenda specimens in a recent study (262). Two novel genotypes of *G. duodenalis* were found in house mice on Boullanger Island, Australia, based on

TABLE 6. *Giardia duodenalis* infection rates and genotypes in wild animals

Animal	Location	Total no. of samples	Infection rate (%)	No. of sample genotyped	No. of samples with assemblage:			Reference
					A	B	Other(s)	
Gorilla	Uganda	100	2.0	2	2			104
Chimpanzee	Italy			2		2		37
Mandrill	Italy			1		1		37
Macaque	Italy			7		7		37
Japanese macaque	Japan			3		3		130
Pygmy marmoset	Sweden			1		1		154
Cotton-top mandarin	Sweden			1		1		154
Vervet monkey	Sweden			1		1		154
Southern brown howler monkey	Brazil	28	100	16	16			288
Red colobus monkey	Uganda	30	23.3	4		3[a]	1 (E)[a,b]	137
Moose	Norway			13	13			219
Moose	Sweden			1	1			154
Fallow deer	Italy	139	11.5	8	8			146
Fallow deer	Sweden			2	1		1 (E)	154
Reindeer	Norway			6	6			219
Reindeer	United States			1	1			176
Roe deer	Netherlands			1	1			282
White-tailed deer	United States	26	3.9	1	1			269
Thresher shark	United States			1		1		150
Mako shark	United States			1			1 (A + B)	151
Common dolphin	United States			4		2	2 (A + B)	150
Common dolphin	United States			1	1			151
Atlantic white-sided dolphin	United States			3		3		150
Risso's dolphin	United States			1		1		150
Harbor porpoise	United States			3		2	1 (A + B)	150
Red fox	Norway	269	4.8	7	5	2		109
Coyote	Canada	70	18.6	8[c] (9[a])	3[c] (5[a])		5[c] (2[a]) (D), 1[a] (A + C), 1[a] (A + D)	258
Coyote	United States	22	31.8	7		1	3 (C), 3 (D)	268
African painted dog	Namibia, Zambia, and Australia	87	33.3	30	2	12	10 (A + B), 2 (A + B + C), 1 (B + C), 1 (A + D), 1 (B + D), 1 (A + C + D)	20a
Wild boar	Croatia			1	1			37
Ringed seal	Canada	55	80.0	2		2		67
Gray seal	United States			21	6	5	10 (H[d])	151
Harp seal	Canada	58	27.6	16	16			18
Harp seal	United States			1			1 (A + B)	150
Harbor seal	United States	97	42.3	14			3 (D), 11 (H[d])	86
Harbor seal	United States			1		1		150
Harbor seal	United States			8	2	5	1 (A + B)	151
Hooded seal	Canada	10	80	8	8			18
Muskrat	United States			5		5		249
Beaver	Canada			12	12			16
Beaver	United States	62	6.5	4		4		78
Beaver	United States			7		7		249
Guinea pig	Sweden			1		1		154
Bush rat	Australia	12	8.3	1			1 (F + C)	262
Rat	Sweden			8			8 (G)	154
Rat	Australia			2			2 (G)	178
Ash-gray mouse	Australia	2	50.0	1			1 (E)	262
Chinchilla	Germany			1	1			138
Marsupials	Australia	421	13.6	49	41	8		255
Common planigale	Australia	5	20.0	1	1			262
Quenda	Australia	72	1.4	1			1 (quenda genotype)	7
Quenda	Australia	18	78.6	10	1		6 (quenda genotype), 3 (C), 1 (E)	262
Herring gull	United States			6			6 (A + B)	150
Herring gull	United States			1				151
Gull	United States			3		1	1 (A + B), 1 (B + H[b])	151
Common eider	United States			3	1		2 (A + B)	150
Wildlife	Europe			172	93	34	3 (C), 3 (D), 10 (E), 28 (G)	247

[a] At the *gdh* locus.
[b] At the *tpi* locus.
[c] At the SSU rRNA gene locus.
[d] Defined in reference 151.

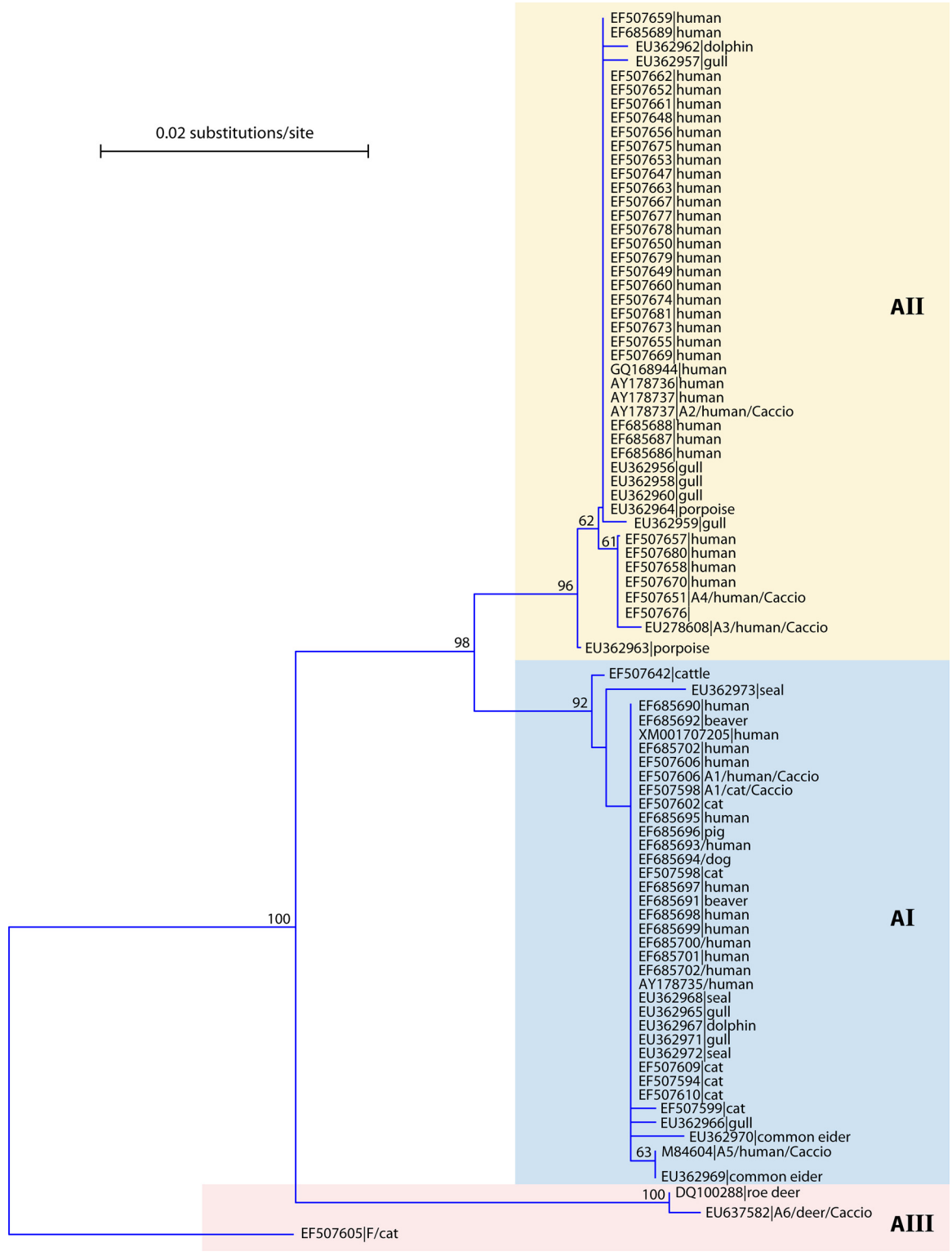


FIG. 2. Phylogenetic relationships among subtypes within assemblage A of *G. duodenalis* at the *gdh* locus as assessed by a neighbor-joining analysis of the nucleotide sequence covering a 530-bp region (positions 267 to 796 of GenBank accession number AY178735) of the gene, using distance calculated by the Kimura two-parameter model. Sequence names with Caccio are subtypes described previously by Caccio et al. (37).

TABLE 7. Target, primer, assay type, and main use of some commonly used *G. duodenalis* genotyping tools

Gene	Primer (sequence [5′–3′])	Size (bp)	Specificity	Assay type	Usage(s)	References
tpi	AL3543 (AAATIATGCCTGCTCGTCG) AL3546 (CAAACCTTITCCGCAAACC) AL3544 (CCCTTCATCGGIGGTAACTT) AL3545 (GTGGCCACCACICCCGTGCC)	605 532	Genus specific[a]	Nested PCR, sequencing	Genotyping and subtyping	4, 13, 32, 37, 59, 78, 83, 87, 93, 145, 154, 225, 249, 263, 300
gdh	Ghd1 (TTCCGTRTYCAGTACAACTC) Gdh2 (ACCTCGTTCTGRGTGGCGCA) Gdh3 (ATGACYGAGCTYCAGAGGCACGT) Gdh4 (GTGGCGCARGGCATGATGCA)	754 530	Genus specific	Nested PCR, sequencing	Genotyping and subtyping	37, 145, 154, 156
gdh	GDH1 (ATCTTCGAGAGGATGCTTGAG) GDH4 (AGTACGCGACGCTGGGATACT)	778	Genus specific	PCR, RFLP sequencing	Genotyping and subtyping	3, 4, 93, 116, 130, 170, 282
gdh	GDHeF (TCAACGTYAAYCGYGGYTTCCGT) GDHiF (CAGTACAACTCYGCTCTCGG) GDHiR (GTTRTCCTTGCACATCTCC)	432	Genus specific	Seminested PCR, RFLP	Genotyping and subtyping	31, 48, 86, 109, 128, 154, 202, 214, 218, 223, 300
SSU rRNA gene	RH11 (CATCCGGTCGATCCTGCC) RH4 (AGTCGAACCCTGATTCTCCGCCC AGG) GiarF (GACGCTCTCCCCAAGGAC) GiarR (CTGCGTCACGCTGCTCG)	292 130	Genus specific	PCR, sequencing	Genotyping	3, 4, 37, 77, 118, 148, 164, 196, 213, 231, 255, 264, 270, 300
bg	G7 (AAGCCCGACGACCTCACCCGCAGTGC) G759 (GAGGCCGCCCTGGATCTTCGAGAC GAC) GiarF (GAACGAACGAGATCGAGGTCCG) GiarR (CTCGACGAGCTTCGTGTT)	753 511	Unknown	Nested PCR, sequencing	Genotyping and subtyping	4, 37, 39, 87, 92, 98, 146, 148, 153, 154, 156, 170, 196, 202, 217, 287, 288

[a] Does not amplify assemblage D (154).

a sequence analysis of a 130-bp region of the SSU rRNA gene (183), although the sequence data were not presented. All these new genotypes need to be characterized extensively, at least at the common loci used for genotyping *G. duodenalis*.

MOLECULAR DIAGNOSTIC TOOLS

Molecular biology has provided powerful new tools for characterizing *Giardia*, and the analysis of previously unrecognized genetic differences within this genus has revolutionized our understanding of the taxonomy, population genetics, and epidemiology of giardiasis in humans and domesticated animals. Although some simple PCR assays have been used for the detection of *Giardia* in clinical and environmental samples, most recent molecular tools are used for the differentiation of *Giardia* at the species/assemblage and genotype levels. These tools are widely used for the identification of *G. duodenalis* genotypes in clinical specimens (293).

The utility of molecular diagnostic tools is determined by the gene targeted (such as the SSU rRNA, *gdh*, *tpi*, *ef1*α, *bg*, and variant surface protein [*vsp*] genes), the number of loci used in the analysis, the specificity of the assay (*Giardia* specific, *G. duodenalis* specific, or assemblage specific), and downstream procedures (restriction fragment length polymorphism [RFLP] analysis or DNA sequencing of PCR products). The usage of these loci for the genotyping and subtyping of *G. duodenalis* and their sequence characteristics were reviewed recently (293). Some commonly used primers for the species/genotype and subtype differentiation of *Giardia* isolates in animal and human specimens and water samples in recent studies are listed in Table 7. Among them, the SSU rRNA, *gdh*, *tpi*, and *bg* genes are frequently targeted.

Since the genetic loci of *Giardia* differ in substitution rates, the resolution for parasite typing is different among loci. For example, substitution rates for the partial SSU rRNA, *bg*, *gdh*, and *tpi* genes were reported to be 0.01, 0.03, 0.06, and 0.12 substitutions per nucleotide, respectively (293). Thus, the conserved SSU rRNA gene is traditionally used for species and assemblage differentiation (mostly genotyping), whereas the most variable locus, *tpi*, is frequently used for subtyping. The *bg* and *gdh* loci, with substitution rates between those of the SSU rRNA and *tpi* genes, have a broad application spectrum (293).

Genotyping Tools

The SSU rRNA gene is a commonly used marker for the species and assemblage differentiation of *Giardia*, and different regions have been targeted for different applications. The variable 5′ and 3′ ends of the SSU rRNA gene locus can be used for identifying the relatively closely related assemblages, whereas the more conserved regions would provide sufficient information only for the differentiation of *Giardia* species. Thus, when the SSU rRNA gene locus is used for assemblage differentiation, primer selection should be careful because the products by some primer sets are too small to differentiate all *G. duodenalis* assemblages. For example, some primers (118, 283, 291) amplify only the first 1/5 or the last 1/10 of the gene (~1,400 bp in total). Using one such set of primers (118), cat isolates were genotyped as assemblage A in one study (30) and assemblage F in another study (79) due to the fact that assem-

blage F is mostly identical to assemblage A in the fragment under analysis (293). The identification of unusual assemblages (assemblages C, D, E, and F) in humans involved mostly the use of SSU rRNA gene-based tools. One reason for targeting small fragments of the SSU rRNA gene is the difficulty in the PCR amplification of the locus; the use of dimethyl sulfoxide or special PCR buffers designed for GC-rich targets is frequently needed for efficient PCR amplification of the target.

The *tpi*, *gdh*, and *bg* loci are also common genotyping markers. Because most primers amplify 40% to 60% of the *gdh* gene and 60% of the *tpi* and *bg* genes, these loci have been used for both genotyping and subtyping. However, inconsistent genotyping results are sometimes generated among different loci. In a study of human and dog specimens in a tea-growing community in India, SSU rRNA gene sequencing suggested that assemblages C and D were the dominant genotypes in humans but were absent in dogs. The finding of assemblages C and D in humans was not supported by a sequence analysis of the specimens at the *ef1*α and *tpi* loci (265).

The occurrence of infections with mixed assemblages in humans and animals is common. For 908 isolates from human and various animals characterized at two or more loci, mixed infection of *Giardia* assemblages was found for 121 (13.3%) isolates. Among them, mixed infection was found for 46 of 392 (12%) human specimens and 45 of 134 (34%) dog specimens analyzed. Mixed infection was also found in cat, cattle, goat, sheep, pig, and wildlife specimens. Mixed infection involving assemblages A to E is especially common. In some dogs, the concurrent occurrence of three assemblages (assemblages A, B, and C or B, C, and D) was seen (247). Therefore, assemblage-specific PCR assays are now used to assess more accurately the occurrence of mixed infections. The usage of such a system targeting the *tpi* gene in analyses of specimens from dairy and beef calves led to the identification of mixed assemblage A and E infections in 31% of positive calves and a more frequent occurrence of assemblage A in dairy calves (59% of all positive samples) (91). Primers specific for assemblage A or B based on the *tpi* gene were reported and should be useful for an accurate assessment of the occurrence of mixed infections in clinical specimens (14, 31, 223). Such data will improve our understanding of the clinical spectrum of giardiasis, intensity of giardiasis endemicity in study areas, tracking of infection sources, and identification and differentiations of outbreaks of giardiasis.

Ideally, genotyping should be performed at the single-cyst level, as this will allow the differentiation between mixed infections and the occurrence of recombinants, because there is a possibility of genetic exchanges between isolates of assemblage A (57) or even between isolates of assemblages A and B (254). The use of real-time PCR appears to be promising in reaching this technically demanding level of sensitivity and specificity. A real-time PCR assay targeting the *bg* gene was developed. It could differentiate assemblages A and B of *G. duodenalis* with a sensitivity of detecting an equivalent of one cyst of *G. duodenalis* (105). More recently, three real-time PCR assays targeting the *tpi*, *gdh*, and open reading frame C4 sequences were developed to differentiate assemblages A and B. The assays had high specificity and detected DNA from a single trophozoite or cyst (9). When these assays and a TaqMan assay targeting the *bg* gene were used to analyze 30 human stool samples, a simultaneous detection of both assemblages was seen for 37 to 83% of specimens, depending on the genes targeted. PCR analyses of DNA extracted from single cysts purified by immunomagnetic separation from the same specimens showed that the simultaneous amplification of DNA of both assemblages was attributable to mixed infections. However, potential genetic exchanges between assemblages A and B were suggested by the detection of both assemblages in individual cysts (9). There is also a single-tube multiplex real-time PCR assay based on the SSU rRNA gene for the differentiation of assemblages A and B in fecal specimens but with a low sensitivity of an equivalent of 20 trophozoites per PCR (187).

Subtyping Tools

Assemblages A and B are zoonotic; therefore, genetic differences within them may provide information on the relationship of subtypes and hosts, the zoonotic potential of each subtype, contamination source tracking, outbreak investigation, as well as characterizations of transmission dynamics. Based on sequence analysis of the *tpi*, *gdh*, and *bg* genes, multiple subtypes of assemblage A were noticed. At the *gdh* locus, these subtypes can be grouped easily into three subassemblages, subgroups AI, AII, and AIII, and there are multiple subgroup- or subassemblage-specific polymorphisms (Fig. 2). At the *tpi* and *bg* loci, subassemblage AIII also has many distinct nucleotide substitutions, but there is no formation of robust subgroup AI and AII clusters in phylogenetic analyses of the sequences (see Fig. S3 and S4 in the supplemental material). The initially identified two subassemblage-specific substitutions in the *tpi* gene apply only to the common A1 and A2 subtypes, which belong to subassemblages I and II, respectively. Thus, the A5 subtype (belonging to the AI subassemblage) (37) has a *tpi* sequence more similar (two nucleotide substitutions versus four) to A2 (an AII subtype) than to A1 (an AI subtype).

To systematically characterize intra-assemblage genetic diversity and provide more-robust typing of *G. duodenalis*, Caccio et al. proposed a subtype nomenclature system (Table 8) for assemblage A based on multilocus genotype (MLG) analysis of the *bg*, *gdh*, and *tpi* genes (37). This would reduce confusions in subtype terminology and improve understandings of the host segregation of subtypes. Nevertheless, the subgroup AI, AII, and AIII classification system proposed was determined largely by the extent of nucleotide substitutions at the *gdh* locus, although concatenated sequences of the *gdh*, *tpi*, and *bg* genes were used in the phylogenetic assignment of the subassemblages. The designation of rare subtypes within the AI and AII subassemblages may need support from genetic characterizations of other loci.

The BIII and BIV subassemblages originally described by allozyme electrophoretic studies are not supported by DNA sequence analyses. The sequences from known subgroup BIII or BIV isolates are insufficient to assess the existence of subgroup BIII/BIV-specific sequence polymorphisms, and subtyping analyses of field isolates produced inconsistent subassemblages among different loci, most of which were not supported by bootstrap analyses (293) (see Fig. S5 to S7 in the supplemental material). Assemblage B has many more subtypes than does assemblage A.

TABLE 8. Definition of different subtypes in assemblage A[a]

Subassemblage	MLG type	Subtype			GenBank accession no.			Host(s) (no. of positive samples)
		gdh	*bg*	*tpi*	*gdh*	*bg*	*tpi*	
AI	AI-1	A1	A1	A1	AY178735, EF507606, EF685701, EF507610	X14185, AY258617, EU769204, X85958, GQ919292, GQ919293, EU769204	L02120, AY655704, AF069556, EF688040, AB509384	Human (2), cattle (4), water buffalo (2), cat (1), pig (1), sheep (1)
	AI-2	A5	A5	A5	M84604, EU362969, EF507598	AB469365, DQ649780, DQ984131, AB218605	AB509383, EU781000	Cat (1)
AII	AII-1	A2	A2	A2	AY178737, EF507674, EU362964, EF507675, L40510	AY072723, FJ971422, EU594669, FJ560582	U57897	Human (9), cat (1)
	AII-2	A3	A3	A2	EU278608	AY072724, FJ971415, EU188635, FJ471821	U57897	Human (12)
	AII-3	A3	A2	A2	EU278608	AY072723, FJ971422, EU594669, FJ560582	U57897	Human (4)
	AII-4	A4	A3	A2	EF507657, EF507680, EF507651, EF507676	AY072724, FJ971415, EU188635, FJ471821	U57897	Human (5)
	AII-5	A3	A3	A1	EU278608	AY072724, FJ971415, EU188635, FJ471821	L02120, AY655704, AF069556, EF688040, AB509384	Human (1)
	AII-6	A3	A3	A3	EU278608	AY072724, FJ971415, EU188635, FJ471821	EU041754	Human (1)
	AII-7	A3	A3	A4	EU278608	AY072724, FJ971415, EU188635, FJ471821	GQ329677, AB509382, EU781027, EU637593	Human (1)
AIII	AIII-1	A6	A6	A6	EU637582, DQ100288	DQ650649, EU621373	DQ650648, EU781002	Fallow deer (9), wild boar (1), cat (1)

[a] The definitions for subtypes A1 and A5 based on the *gdh* gene are switched. In the original definitions, only two sequences belonged to subtype A1, whereas the majority of assemblage AI sequences belonged to subtype A5. (Adapted from reference 37 with permission from Elsevier.)

Many subtypes are also seen in assemblage E. Although assemblage E also infects a range of hoofed livestock, there are no host-specific subgroups in nucleotide sequences of various genetic loci. There are insufficient data to assess the substructure of other assemblages (assemblages C, D, F, and G). Host-specific sequence polymorphism was not seen at the *gdh* locus when assemblage G isolates from rats and mice were examined (293).

Multilocus Genotyping Tools

The low resolution of current genotyping tools has limited their potential for the characterization of the transmission of human giardiasis (42). Thus far, the vast majority of studies have relied on the sequence characterization of human- and animal-derived cysts at one or two genetic loci (41). In early studies, there was a bias toward the use of the SSU rRNA gene target because of its multicopy nature and high degree of sequence conservation. This has led to some problematic interpretations of the data acquired. In addition, recent data have shown that inconsistent genotyping results may be obtained when different genetic loci are targeted. Thus, MLG analysis is increasingly used for the characterizations of *G. duodenalis* from humans and animals (37).

Several recent studies using the MLG approach showed that some isolates of both human and animal origins could not be unequivocally assigned at the assemblage level (37, 265). In one study where a sequence analysis of four genes (the SSU rRNA, *bg*, *gdh*, and *tpi* genes) of human and animal isolates was performed, the data generated showed that although congruent genotyping results were obtained at the four genetic loci for isolates of assemblage A, it was difficult to assign some assemblage B isolates from humans and nonhuman primates to an assemblage, because the result obtained at one locus was not consistent with data obtained at the other three loci. In addition, there were variable levels of intraisolate sequence heterogeneity in assemblage B isolates, which prevented the unambiguous identification of MLGs (37). Similar results were reported by other studies of human and animal isolates using some of the same targets (93, 265). This inconsistency in genotyping results was more frequently reported for isolates from dogs, where, depending on genetic loci, isolates were typed as either host-adapted assemblages C and D or zoonotic assemblage B (265). This has important repercussions, as different

conclusions may be reached. These findings also raise concerns about the interpretation of genotyping data based on single markers. It was reported that 15% of isolates genotyped (including 2,400 *Giardia* sequences from the GenBank database) in the ZOOnotic Protozoa NETwork (ZOOPNET) had inconsistent typing between two markers among the SSU rRNA, *bg*, *gdh*, and *tpi* genes, and this inconsistency was observed predominantly for specimens from humans and dogs (247).

One possible factor contributing to the inconsistent genotyping among markers is the high level of occurrence of mixed infections as a result of the high prevalence of giardiasis in humans and animals. This was supported by the frequent detection of concurrent infections of both assemblages A and B in humans and the high level of occurrence of assemblage A infection in animals when assemblage-specific PCR was used for genotyping. The grouping of the parasites into individual MLGs was further complicated by the fact that many isolates exhibited double peaks at specific positions in electropherograms of otherwise clean sequences. In one recent study, double peaks in electropherograms were seen in assemblage B, C, D, and E isolates but never in assemblage A, F, and G isolates, suggesting that MLG analysis of *G. duodenalis* might be more useful for the typing of assemblage A (154).

POPULATION GENETICS OF *GIARDIA DUODENALIS*

Giardia species have long been assumed to have exclusively asexual reproduction. Results of recent MLG studies suggest the occurrence of genetic recombination in *Giardia* (57, 151a, 207, 211). Possible meiotic recombination was seen within some loci under analysis when four regions of three chromosomes of the subgroup AII reference strain JH and five subgroup AII isolates from an area in Peru where the disease is highly endemic were sequenced (57). In another study of 978 human and 1,440 animal isolates at four loci (the SSU rRNA, *bg*, *tpi*, and *gdh* genes), the allelic sequence heterogeneity and genetic recombination within assemblage B were inferred. Mixed genotypes were repeatedly seen for individual isolates, particularly those of assemblage B (247). The high level of allelic sequence heterozygosity in assemblage B in contrast to assemblage A was confirmed by sequence analysis of 30 Thai isolates at the *bg* locus (144a) and by the whole-genome sequencing of isolate GS, which showed that only a few regions of the genome appeared to be free of heterozygosity (84). The possibility of recombination between different assemblages, especially between assemblage AI and B isolates, was inferred in another study. When PCR products of nine isolates of assemblages AI, AII, and B were clone sequenced at 10 loci, there were rare assemblage B haplotypes that grouped with assemblage AI at several loci. In contrast, all assemblage AII isolates formed a single clade with no evidence of recombination (254).

Current population genetic data do not allow us to distinguish between meiotic sex and parasexual reproduction (33). Further investigations are required to better understand the population structure and reproduction of *Giardia* spp. Emphasis should be placed on intra-assemblage comparisons, as available data indicate that the genetic assemblages of *Giardia* are conserved in terms of geographic location and host occurrence, suggesting that any recombination is probably not reflected at the assemblage and species levels (180). This was supported by results of a recent MLG study of 114 *Giardia* isolates from various animals (pets, livestock, wildlife, and captive nonhuman primates) in Sweden at three loci (*bg*, *tpi*, and *gdh*), which showed no evidence of recombination between assemblages, although allelic sequence divergence was commonly noticed (154). However, in a recent study, both intra- and interassemblage recombination and meiotic sex were seen in assemblages A to G, which challenges the rationale for naming *G. duodenalis* assemblages different species (151a).

The evolutionary advantage of recombination is the capacity for the organism to respond to adverse conditions such as selection pressures imposed by immunity and antigiardial treatment (144a, 180). Thus, it was observed many years ago that metronidazole- or furazolidone-resistant *G. duodenalis* clones underwent chromosomal rearrangement (277). More recently, a whole-genome sequence analysis suggested that the large *vsp* gene repertoire (270 to 303 copies) in isolate WB of assemblage A is probably the result of gene duplication and recombination (6). The occurrence of allelic heterogeneity and genetic recombination makes the development of high-resolution subtyping tools more difficult and the analyses of sequence data intricate. In contrast, if *G. duodenalis* has a clonal population structure, the use of only a few highly polymorphic markers in epidemiological investigations would be justified (249). As the clonality in *G. duodenalis* is currently challenged by data indicative of both heterozygosity and genetic recombination, the single-genetic-locus approach used by many previous studies should be reevaluated. Thus, investigations of the distribution of *G. duodenalis* genotypes and subtypes in humans and the identification of infection sources and risk factors now require more discriminatory typing techniques that allow the identification of individual lineages. These techniques will undoubtedly improve the understanding of the molecular epidemiology of giardiasis.

ZOONOTIC POTENTIAL OF GIARDIASIS

Epidemiologic Evidence for Zoonotic Transmissions

Few epidemiological studies have assessed the importance of zoonotic transmission in the occurrence of human giardiasis. In New Zealand, case-control studies of giardiasis did not identify contact with pets as a risk factor for children or adults, although contact with farm animals was associated with an increased risk of infection for adults (119, 120). In agreement with this finding, the infection rate of human giardiasis in New Zealand was 23% higher in rural areas than in urban areas (241). In the United Kingdom, farm visitation was frequent among case patients, but specified exposures to dogs, cats, horses, cattle, and sheep was not a significant risk factor (248). One case-control study in eastern England found an association of giardiasis with exposure to farm animals and pets, particularly pigs, dogs, and cats (296). Other studies in the United States, Canada, and the United Kingdom did not show such an association (296).

Beavers are probably the animals most commonly implicated in the zoonotic transmission of giardiasis. This originated largely from investigations of waterborne outbreaks and reports of frequent giardiasis in hikers and campers, who recalled

drinking water from streams or lakes. The first observation was made in 1976 with an outbreak of giardiasis in Camas, WA, when 128 townspeople had laboratory-confirmed giardiasis. A questionnaire survey revealed that 3.8% of residents had clinical giardiasis, whereas none of 318 residents in a control town were ill. *Giardia* cysts were recovered from water entering the city water treatment system and two storage reservoirs containing chlorinated and filtered stream water. Trapping in the watershed yielded three beavers infected with *Giardia* that was infective for beagle pups. Similar observations were made by subsequent investigations of waterborne giardiasis outbreaks in New Hampshire and Nevada, based largely on the finding of *Giardia* cysts in beavers caught in source watersheds (reviewed in reference 296). These reports did not provide concrete evidence of an involvement of beavers in human infections and reported merely circumstantial evidence based on beavers found at contaminated sites. The possible role of humans and other animals as a source of infection for beavers was not addressed in these reports. In a review of data from case-control studies, the association between the drinking of wilderness water and acquisition of giardiasis was deemed minimal (292).

The consumption of raw surface water clearly represents a significant risk for giardiasis (119). However, the contamination of such water supplies may result from humans, farm animals, and wildlife (112). A 2-year study assessed the significance of each source in the environmental contamination of surface water with *Giardia* cysts (112). Sewage effluent was shown to have the highest prevalence of *Giardia*, although the concentration of cysts was minimal compared with that detected in cattle feces. Although the overall prevalence of *Giardia* was lower in wildlife, giardiasis was prevalent in aquatic mammals such as beavers and muskrats. Nevertheless, an interpretation of these results in the context of the source of human *Giardia* infections can be made only in conjunction with data on the distribution of *Giardia* species and genotypes in these potential contamination sources (257).

Genetic Evidence for Zoonotic Transmissions

Numerous isolates of *Giardia* collected from different host species in various geographical locations have been genotyped, and the occurrence of the same species/genotype in humans and other animals has been well demonstrated (181). Such data are indicative of zoonotic potential, and most experts would agree that *G. duodenalis* strains are potentially zoonotic, especially assemblages A and B of *G. duodenalis*. The use of genotyping and subtyping tools, especially the more recent MLG tools, in well-designed epidemiological studies, however, is needed before we can fully assess the human disease burden caused by zoonotic giardiasis (37).

The occurrence of *Giardia* in aquatic wildlife in investigations of waterborne outbreaks of giardiasis, particularly of isolates that are morphologically identical to *G. duodenalis*, has been the single most important evidence implicating *Giardia* as a zoonotic agent. Indeed, recent studies have shown the common occurrence of *G. duodenalis* assemblage B in beavers and muskrats (78, 249). However, there is little evidence to implicate these animals as the original contaminating source in waterborne outbreaks. It has been suggested that these animals are more likely to become infected from water contaminated with fecal material of human or even domestic animal origin, thus serving to amplify the numbers of the original contaminating isolate (257).

In view of the frequency of human-pathogenic *Giardia* genotypes, the public health risk of giardiasis from domestic animals appears to be small. This is the case at least for cattle in North America, Australia, and Europe, where *G. duodenalis* assemblage E predominates (149, 231, 249, 271, 272). The human-pathogenic assemblages (assemblages A and, occasionally, B) in cattle may have to compete with the more common assemblage E. Similar conclusions can probably be drawn for other livestock such as sheep, goats, and pigs, where assemblage E is also the predominant genotype (Table 3), or domestic pets such as dogs and cats, which have other host-adapted *G. duodenalis* genotypes (assemblages C and D in dogs and assemblage F in cats) as the dominant parasites (Table 4).

Most interests in the zoonotic transmission of *G. duodenalis* focus on assemblage A, as it is the most common non-host-specific assemblage in animals (Tables 3, 4, and 6). Subtyping data accumulated so far do not support a widespread occurrence of zoonotic transmission. The two most common subassemblages of assemblage A, subgroups AI and AII, appear to differ in host preference (247, 296). Humans are more commonly infected with subgroup AII, although subgroup AI has also been seen in some areas or studies (Table 9). In contrast, animals are commonly infected with subgroup AI, although subgroup AII is sometimes seen (Table 10). More systematic characterizations of the less common subtypes within subgroups AI and AII, however, are needed to improve our understanding of the host specificity of subgroups AI and AII. The other subgroup, subgroup AIII, has thus far been found only in animals, mostly in wildlife (Table 10). There are no clear geographic differences in the distribution of the two common assemblage A subgroups in humans; although subgroup AI was detected in humans at a high frequency in two studies in South America, two other studies conducted in the same countries found only subgroup AII in humans (Table 9). Increasing the typing resolution and detailed epidemiological data are needed to determine what portion of human subgroup AI infections is the result of zoonotic transmission.

MLG tools have recently been used in assessments of the zoonotic transmission of giardiasis because of their highly discriminatory power. When 978 human and 1,440 animal isolates were characterized using sequence analysis of four loci (the SSU rRNA, *bg*, *tpi*, and *gdh* genes), the zoonotic potential of both assemblages A and B was apparent at the levels of assemblages and subassemblages at each locus. However, when isolates were defined using an MLG scheme, only two MLG types of assemblage A and none of assemblage B appeared to have occurred in both humans and animals (247). In contrast, three assemblage A MLG types that were previously detected in humans were present among 114 *Giardia* isolates from various animals (pets, livestock, wildlife, and captive nonhuman primates) in Sweden when they were analyzed by an MLG technique targeting three genes (*bg*, *gdh*, and *tpi*) (154).

The finding of the same genotypes or MLG types in humans and animals is not by itself conclusive evidence that zoonotic transmission has taken place. A better assessment of zoonotic transmission can only come from studies that examine the dynamics of *Giardia* transmission between humans and animals living in the same household or localized focus of ende-

TABLE 9. Subtypes of *Giardia duodenalis* assemblage A in humans

Location(s)	Locus	Total no. of samples	No. of samples with subtype:			Reference
			A-I	A-II	Other(s)	
Europe	*gdh*, *bg*, or *tpi*	594	148	446		247
Belgium	MLG[a]	18		18		93
France	*gdh* and *tpi*	8		8 (A2)		31
Germany	*bg*	3		3 (A3)		227
Italy	*bg*	6	6 (A1)			168
Italy	*bg*	17	1 (A1)	7 (A2), 3 (A3), 2 (A4), 3 (new)	1 (A1 + A2)	148
Italy	*bg*	13		10 (A2), 2 (A3), 1 (A2 + A3)		39
Italy, Africa	MLG	32	2	30		37
Portugal	*bg*	2		1 (A2), 1 (A3)		10
Portugal	*bg*	25	25 (A1)			245
United Kingdom	*tpi*[b]	12		12		14
United Kingdom	*tpi*	9		9		35
Mexico	*vsp*[b]	19		19		206
Mexico	*bg*	17	15 (A1)	2 (A3)		147
Mexico	*bg*[b]	18	11	7		70
Argentina	*tpi*	3		3 (A2)		174
Brazil	*bg*	62	60 (A1)	2 (A2)		287
Brazil	*gdh*	29		23 (A2), 6 (A4)		246
Nicaragua	*bg*	16		3 (A2), 13 (A3)		153
Peru	*gdh*[b]	10	9	1		203
Peru	*tpi*	6		6 (A2)		249
Peru	*tpi*	86		86		57a
Bangladesh	*tpi*	29	8 (A1)	20 (A2)	1 (A1 + A2)	111
China	*tpi*	12	8 (A1)	4 (A2)		289
Japan	*gdh*	2		1 (A2), 1 (A4)		2
India	*tpi*	8	3 (A1), 2 (AI, unknown)	3 (A2)		265
Philippines	*tpi*[b]	50	3	47		301
Saudi Arabia	*igs*[c]	25	12	11	2 (AI + AII)	12
Thailand	*gdh*[b]	5		5		212
Thailand	*bg*[b]	18	3	15		274
Australia	*gdh*	4	1 (A1)	3 (A2)		214
Australia	*gdh*	27		27 (A2)		299
Australia, China, Cambodia	*tpi*	5	3 (A1)	2 (A2)		162
Ethiopia	*bg*	23	1 (A1)	5 (A2), 16 (A3), 1 (new)		87
Sahrawi	*gdh* and *tpi*	16	1 for *tpi* (A1)	2 (A2) and 14 (A3) for *gdh*; 6 (A2), 1 (A3), 2 (A4), and 2 (new) for *tpi*		145
Total		1,229	322	903	4	

[a] Multilocus genotype analyses of the *gdh* and *bg* genes and the *tpi gdh*, *bg*, and *tpi* genes.
[b] By PCR-RFLP analysis instead of sequencing.
[c] Intergenic spacer of the rRNA gene.

micity. In Aboriginal communities in Australia, SSU rRNA gene sequences from 13 humans and nine dogs identified assemblages A and B in humans and assemblages C and D in dogs. Only one dog had an assemblage B isolate, suggesting that zoonotic transmission between humans and dogs, if present, was infrequent, and the dog could have acquired the assemblage B infection from a human source (265). Similarly, in a community in which giardiasis is highly endemic in Peru, multiple examinations of dogs and humans in 22 households showed the presence of assemblages A and B in 167 human specimens genotyped and assemblages C and D in 67 canine specimens genotyped (57a). In contrast, in a study conducted in 20 temples and surrounding communities in Bangkok, Thailand, of 13 *Giardia* isolates from dogs and 3 from humans, 1 dog and 2 monks in the same temple had assemblage A isolates (129). In another similar study in the same area, 42 and 35 *Giardia*-positive specimens from dogs and humans, respectively, were chosen for genotyping. Assemblage A was shown to be the most common genotype in dogs (79%), followed by assemblages D (31%), B (21%), and C (12%). Likewise, 73% of humans were infected with assemblage A, followed by assemblage B and assemblage C. Based on the high level of occurrence of assemblage A in both dogs and humans, dogs were considered reservoirs for human giardiasis in temple communities in Bangkok (264).

More recently, subtyping studies of isolates from humans and animals living in the same community or area were conducted to further assess the likelihood of the zoonotic transmission of giardiasis. In a socially deprived small Rom community in Italy where dogs roamed freely, *G. duodenalis* specimens from children and dogs both had only subassemblage AI (168). Likewise, one child and a dog living in the same household were found to be infected with a subassemblage AI strain in a Brazilian study (287). In contrast, in Assam, India, subassemblage AII was found to be the dominant *G. duodenalis* assemblage in both humans and a dog living on a tea estate (265, 266), and in one household, two isolates from humans and one isolate from a dog all belonged to subassem-

TABLE 10. Subtypes of *Giardia duodenalis* assemblage A in animals

Animal(s)	Location(s)	Gene(s)	Total no. of samples	No. of samples with subtype:				Reference
				A-I	A-II	A-III	Other	
Cattle	Europe	*gdh*, *bg*, or *tpi*	113	70	39	4		247
Cattle	Denmark	*gdh*	8	8 (new)				149
Cattle	Italy	MLG[a]	4	4 (A1)				37
Cattle	Portugal	*bg*, *gdh*	2		2 (A2)			173
Cattle	United States	*tpi*	10	3 (A1), 1 (new)	6 (A2)			81
Cattle	Brazil	*gdh*	1	1 (A1)				246
Water buffalo	Italy	MLG[a]	2	2 (A1)				40
Sheep	Belgium	*bg*	2		2 (A2)			95
Sheep	Italy	*gdh*, *bg*	5	5 (A1 at *bg*, A5 at *gdh*)				98
Sheep	Spain	*bg*	1	1 (A5)				99
Sheep	Sweden	MLG[a]	7	7				154
Sheep	Australia	*tpi*	30	26 (A1), 3 (new)	1 (new)			189
Sheep and goat	Europe	*gdh*, *bg*, or *tpi*	36	28	8			247
Goat	Belgium	*bg*	6	6 (A1)				95
Pig	Europe	*bg*, *tpi*, or *gdh*	14	12	2			247
Pig	Denmark	*gdh*	10	10 (A1)				149
Pig	Italy	MLG[a]	1	1 (A1)				37
Horse	United States and Australia	*tpi*	4	3 (A1)	1 (A2)			263
Dog	Europe	*gdh*, *bg*, or *tpi*	120	88	32			247
Dog	Belgium	*bg*	40		2 (A2), 36 (A3)		2 (unknown)	53
Dog	Germany	*gdh*	14	14 (A1)				155
Dog	Italy	*bg*	2	2 (A1)				197
Dog	Italy	*bg*	6	5 (A1)	1 (new)			148
Dog	Italy	*bg*	9	9 (A1)				168
Dog	Mexico	*bg*	6	5 (A1)	1 (new)			148
Dog	Mexico	*vsp*[b]	2		2			206
Dog	Brazil	*bg*	7	7 (new)				287
Dog	India	*tpi*	5	2 (A1)	3 (A2)			265
Cat	Europe	*gdh*, *bg*, or *tpi*	59	41	15	3		247
Cat	Italy and Croatia	MLG[a]	3	2	1 (A2)			37
Cat	Sweden	MLG[a]	5	3		1	1	154
Cat	Mexico	*vsp*[b]	1		1			206
Cat	United States	*gdh*	6	6 (new)				284
Cat	Brazil	*gdh*	8	8 (A1)				246
Cat	Brazil	*bg*	1	1 (new)				287
Cat	Australia	*gdh*	5	5 (A1)				214
Cat	Japan	*gdh*, *tpi*	6	6				250a
Moose	Norway	*gdh* and *bg*	13	6		5	2 (AI + AIII)	219
Moose	Sweden	MLG[a]	1	1 (A5 at *gdh* and *bg*)	1 (A4 at *tpi*)			154
Reindeer	Norway	*gdh* and *bg*	6	6 (A5 at *bg*, A1 at *gdh*)				219
Fallow deer	Sweden	MLG[a]	1	1 (A5 at *gdh* and *bg*)	1 (A4 at *tpi*)			154
Fallow deer	Italy	MLG[a]	8			8		37
Ferret	Japan	*gdh* and *bg*	1	1 (A5 at *bg*, new at *gdh*)				3
Ferret	Japan	MLG[a]	2	2				4
Common dolphin	United States	*gdh* and *tpi*	1				1 (A1 + A2)	150
Harbor porpoise	United States	*gdh*	1				1 (A2 + new)	150
Wild boar	Croatia	MLG[a]	1			1		37
Harp seal	United States	*gdh*	1				1 (A1 + new)	150
Herring gull	United States	*gdh* and *tpi*	6	1 (A1)			5	150
Common eider	United States	*gdh*	2	1 (A5)			1 (new)	150
Wildlife	Europe	*bg*, *tpi*, or *gdh*	86	38	3	45		247
Total			691	452	160	67	14	

[a] Multilocus genotype analyses of the *gdh*, *bg*, and *tpi* genes.
[b] By PCR-RFLP analysis instead of sequencing.

blage AII. Evidence for zoonotic transmission was further supported by epidemiological data showing a highly significant association between *Giardia* infection of humans and the presence of a *Giardia*-positive dog in the same household. Unfortunately, it was not clear how many subassemblage AII cases in dogs in the community represented active infection. It was noticed that over 30% of dogs in the community had *Ascaris lumbricoides* eggs in high intensities in their feces (265, 266). As *A. lumbricoides* is a human-specific pathogen and dogs were shedding *A. lumbricoides* eggs after coprophage of human fe-

ces, some dogs might have acted as mechanical disseminators of *Giardia* subtype AII rather than being actively infected with this more human-adapted parasite.

MOLECULAR EPIDEMIOLOGY OF GIARDIASIS

Molecular Epidemiology of Giardiasis in Animals

Farm animals. Most cattle, sheep, and pigs are infected with *G. duodenalis* assemblage E (Table 3). Among 562 studied samples from cattle in Europe, 422 (75%) were found to be positive for assemblage E (247). A longitudinal study of dairy herds in Australia over several months found that 100% of calves became infected during the first 12 weeks of life, and all the animals sampled had assemblage E isolates (26). This livestock genotype was found to predominate in cattle in North America, Europe, and Australia (26, 149, 231, 271, 272). Although assemblage E appears to occur most frequently in cattle, studies in Belgium, the United States, and Europe have shown that a small proportion (<20%) of cattle in a herd may harbor assemblage A, the most common zoonotic genotype (231, 247, 271, 272) (Table 3). In contrast, assemblage B was found in only a small number of cattle in a few studies, and other assemblages have never been convincingly found in cattle (Table 3). The only exception is New Zealand, where limited studies indicated that assemblages A and B appeared to be common in cattle and that assemblage E was largely absent (127, 152, 294).

The actual infection rate of assemblage A in cattle may be higher than previously believed. A longitudinal study of 30 calves from birth to 24 months of age on a dairy farm in Maryland showed that the cumulative infection rate of assemblage E reached 100% by 7 weeks of age and that of assemblage A reached 70% by 15 months of age (231). Another longitudinal study of adult cattle also reported that 43% of positive isolates were assemblage A and 57% were assemblage E isolates (275). The findings of the two recent longitudinal studies were somewhat unexpected considering the low levels of assemblage A previously reported for cattle (17, 125, 192). In addition, a previous longitudinal study of dairy herds in Australia (26) indicated that zoonotic genotypes might be present only transiently in cattle when the frequency of transmission with assemblage E was high and competition between genotypes was likely. Additional longitudinal studies are necessary to determine if the data mentioned above are representative of dairy farms in general.

An age-associated difference in the distribution of assemblages A and E was reported for cattle. Four multistate pooled point-prevalence studies of *G. duodenalis* on 14 dairy farms in the eastern United States involving nearly 2,000 cattle from birth to adulthood reported that assemblage E was found in 34% of preweaned calves, 45% of postweaned calves, 33% of heifers, and 25% of cows, whereas assemblage A was detected in 6%, 7%, 3%, and 2% of the animals, respectively (270–273). However, in a longitudinal study of 30 calves from birth to 24 months of age on a dairy farm in Maryland conducted by that same group, assemblage E was detected in 61%, 25%, and 6% of preweaned calves, postweaned calves, and heifers, respectively, whereas assemblage A was detected in 0%, 7%, and 5% of the animals, respectively (231). Differences in sample sizes, housing conditions, and management practices were believed to be responsible for the differences in the distribution of *G. duodenalis* assemblages in dairy cattle among studies (231). Some studies reported that assemblage A was less commonly found in beef than in dairy cattle (17, 66a, 91).

There are only a few studies of subtypes of *G. duodenalis* in cattle, and subassemblage AI was found to be the major subassemblage (Table 10). In one study conducted in Europe, among 113 samples tested, 70 belonged to subassemblage AI, 39 belonged to subassemblage AII, and 4 belonged to subassemblage AIII (247). In one small-scale study conducted on three dairy farms, the intragenotypic diversity of *G. duodenalis* was studied by analyzing 58 bovine specimens harboring assemblages E (48) and A (8) and a mixture of them (2). Eleven distinct subtypes were identified in assemblage E; all farms had multiple subtypes of assemblage E, and concurrent infection with mixed subtypes occurred in 24% of animals. In addition, both subassemblages AI and AII were identified in assemblage A isolates. Therefore, the high intragenotypic diversity and occurrence of mixed infections reflect the high intensity of *G. duodenalis* transmission in cattle (81). More subtyping studies are needed to understand the transmission dynamics of *Giardia* infection in cattle.

Like in cattle, sheep and goats are infected predominantly with assemblage E, with assemblage A being identified infrequently (Table 3). In studies with reasonable sample sizes, assemblage E was the most common genotype identified in preweaned lambs (36/52 lambs in one study and 74/75 lambs in another), juvenile and adult sheep (33/46 sheep), goats (39/39 goats), or both animal species (170/207 animals) (99, 225, 226, 247, 298). Multiple *bg* subtypes of assemblage E were also found in lambs, with 1 to 6 subtypes on each farm (99, 225, 226, 298). Although assemblage A was also found at a high frequency in some studies, subtyping showed that the isolates belonged mostly to subassemblage AI instead of subassemblage AII (Table 10). In contrast, assemblage B is rarely found in sheep (Table 3). One outbreak of giardiasis in lambs with severe weight loss and some mortality was attributed to assemblage B, although the conclusion was based on a PCR analysis of only two specimens (13).

Although assemblage E is the predominant genotype, assemblage A has also been found frequently in pigs (Table 3). In Australia, assemblage E was the most common genotype and was detected in 64% and 67% of positive specimens from preweaned and postweaned pigs, respectively, with the balance as assemblage A (20). In Denmark, assemblage E was also the most common genotype, being identified in 62% of specimens from postweaned pigs, while assemblage A was detected in only 12% of specimens (149). In Europe, assemblage E was the most common genotype, being detected in 78% of 140 positive pigs studied, followed by assemblage A in 21% of pigs. Surprisingly, assemblages B and D were also found in pigs, although they accounted for less than 1.5% of samples from the positive pigs (247). The majority of the assemblage A strains in pigs belonged to subassemblage AI (Table 10).

Companion animals. Earlier studies reported that dogs were infected mostly (but not exclusively) with host-specific *G. duodenalis*, with assemblages C and D being the major parasites detected. Several recent studies, however, have shown modest to high infection rates of assemblage A (Table 4). In contrast,

assemblage B was found only occasionally in dogs (53, 174, 214, 247, 264, 265), some of which might have resulted from the ingestion of human feces (266). Among assemblage A isolates from dogs subtyped, the majority of them belonged to subassemblage AI, with subassemblage AII being occasionally noticed (Table 10). One study, however, reported that subgroup AII was the dominant subgroup (53).

Differences in social and environmental conditions might have contributed to the variations in the distribution of *G. duodenalis* assemblages in dogs. It was suggested that there are probably two transmission cycles in domestic urban environments, with the transmission of dog-specific genotypes among dogs and the possible transmission of assemblage A between pets and humans. The transmission of dog-specific genotypes may be favored by intensive contact among large numbers of dogs living together and may outcompete the transmission of other genotypes. In household dogs, the frequency of dog-to-dog transmission may be lower, and consequently, infections with assemblage A in dogs are likely to persist (260). This was supported by the finding of high percentages of assemblage A isolates in household dogs (147, 155) and high percentages of assemblages C and D in kennel dogs (30, 251). This was further confirmed by a study from northern Belgium, where 80.5% of 41 *Giardia*-positive household dogs excreted assemblage A, while dogs in breeding kennels and clinically affected dogs had mainly dog-specific assemblages C and D (93.9% of 33 dogs and 80.0% of 45 dogs, respectively) (53). In some other surveys, however, household dogs were shown to be infected mainly with assemblages C and D (130, 195), and kennel dogs were shown to be infected predominantly with assemblage A (130, 155). Because only one sample was collected from each dog in these studies, it was hard to know whether the genotype detected was from new infection or from stable transmission. The percentage of zoonotic and dog-specific assemblages may change over time, depending on the competition of the two transmission cycles. In a study of domestic dogs living in urban environments (155), mixed infections of assemblages A and C in both group dogs (38%) and individual dogs (21%) were common, indicating the existence of the two transmission cycles in domestic urban environments. In addition, although assemblage A was prevalent in both dogs kept individually (91%) and dogs kept in groups (81%), the higher infection rates of assemblages C and D in group dogs (57%) than in individual dogs (30%) suggests that under conditions of intensive dog-to-dog transmissions of giardiasis, the host-adapted genotype is likely to outcompete the zoonotic genotype and become the dominant genotype.

Cats are infected with assemblages A and F, with the cat-specific assemblage F being found more frequently (Table 4). Among the limited numbers of assemblage A isolates from cats subtyped, subgroup AI was the dominant one, although subgroups AII and even AIII were also reported (Table 10). Surprisingly, in a multicountry study, assemblages A to F were all found in cats, although assemblages A and F were the dominant ones (247). Similar results were found in another study where almost all known *G. duodenalis* assemblages (assemblages A, B, C, D, and E) except F were identified in cats (214). However, there was a high level of discrepancy in the genotyping results of animal specimens between the two genotyping tools used in the study.

To date, there are few data on genotypes of *G. duodenalis* in horses. Two assemblage A isolates established in suckling mice, Ad-159 and Ad-162, were initially obtained from Australian horses (263). In a study of the molecular epidemiology of giardiasis in horses, 10 isolates of *G. duodenalis* recovered from horses in Ithaca, NY, and Perth, Western Australia, were characterized at the SSU rRNA and *tpi* gene loci. The results indicated that 3, 1, and 6 horses were infected with assemblages AI, AII, and B, respectively (263). In a more recent study from Italy, however, all 20 isolates characterized belonged to assemblage E (285). In other domestic pets, assemblage B was found in one rabbit each in China and Sweden (154, 249), and assemblage A was found in three ferrets in Japan (3, 4). MLG analysis of the three ferret isolates suggested that they might belong to two host-adapted assemblage A subtypes (4).

Wild animals. It was the association between infected beavers and waterborne outbreaks of human giardiasis that led the World Health Organization to classify *Giardia* as a zoonotic parasite (257). Results of recent studies have shown that beavers are frequently infected with human-pathogenic *G. duodenalis* genotypes. In two studies, all 11 isolates from beavers in the United States belonged to assemblage B (78, 249). In another study, 12 of 113 (10.6%) beaver fecal specimens from southern Alberta, Canada, had assemblage A (16). Assemblage B was found in some muskrats in the United States, in addition to *G. microti* (249). In other rodents, assemblage A was found in a chinchilla in Germany (138), assemblage G was found in rats in Australia and Sweden (154, 178), assemblage E was found in one ash-gray mouse, and assemblages F and C were found in one bush rat in Australia (262). The finding of assemblages other than rodent-specific assemblage G in rodents may be due to the habit sharing of wildlife with domestic animals and humans (262).

Both assemblages A and B are commonly found in other wild mammals (Table 6). In wildlife in Europe, 74% of 172 samples belonged to assemblages A and B (247). In nonhuman primates, assemblage A was found in 16 southern brown howler monkeys in Brazil (288) and two gorillas in Uganda (104), whereas assemblage B was found in many other nonhuman primates, such as macaques, chimpanzees, and mandrills (20a, 37, 130). In ruminants, assemblage A was found in moose, fallow deer, reindeer, white-tailed deer, and roe deer (146, 154, 176, 219, 269, 282). Genotyping results indicated that *G. duodenalis* isolates infecting moose, reindeer, and fallow deer were either of assemblage AI or AIII. Surprisingly, assemblage E is largely absent in wild ruminants (Table 6). In carnivores, among Norwegian wild red foxes shot during a hunting season, assemblages A and B were found in five and two foxes, respectively (109). Among seven coyotes positive for *G. duodenalis* in the United States, one had assemblage B, three had assemblage C, and three had assemblage D isolates (268). In another study of *Giardia* infection in coyotes in Canada, assemblages A, C, and D were reported (258). Most of the 30 specimens from 30 captive and wild African painted dogs had assemblage B or A and B, with assemblages C and D rarely detected (20a). Since host-specific genotypes are not frequently seen in wildlife, it is unclear whether dogs were the likely contamination sources for assemblage C and D infection in some wild carnivores. The sources of assemblages A and B in wild mammals are also not clear.

In marine animals, assemblage B was found in a thresher

shark, and mixed infection of assemblages A and B was found in a Mako shark in the United States (150, 151). Although assemblage A was found in most dolphins and porpoises studied, mixed infections of assemblages A and B were also found in some of them (150, 151). Assemblages A, B, D, and H were found in seals (18, 86, 150, 151). In shore birds in the same marine system, almost all six gulls and three eiders studied had mixed infection of assemblages A and B (150), and one gull had mixed infection of assemblages B and H (151). Subtyping results indicate that either subassemblage AI or AII or mixed infection of both was found for most marine animals (37) (Table 10).

In one of the few studies conducted with marsupials, 41 isolates from various animal species had assemblage A and 8 had assemblage B found (255).

It appears that wild animals are commonly infected with zoonotic assemblages A and B (Table 10). Unlike *Cryptosporidium* spp., where many host-specific *Cryptosporidium* genotypes were found in wild animals (80), the numbers of host-specific *Giardia* assemblages or genotypes found in wild animals are quite limited, with assemblage H being found only in seals and gulls and the quenda genotype being found only in quendas. This is obviously due to the wide host range of assemblages A and B (Table 5) and highlights the potential role of wildlife in maintaining the zoonotic transmission cycle of giardiasis. Nevertheless, a recent MLG study indicated that assemblages A and B from captive mammals in a Croatian zoo are genetically different from isolates of human and domestic animal origins (26a).

Molecular Epidemiology of Giardiasis in Humans

Endemic giardiasis. An analysis of over 4,000 human isolates from different geographical locations by PCR amplification of DNA extracted directly from feces demonstrates that almost exclusively, only *G. duodenalis* assemblages A and B are associated with human infections (Tables 1 and 2). The distribution of these two assemblages in humans varies among studies, sometimes within the same country (42). Assemblage B seems to be slightly more common in both developing (708 cases) and developed (1,589 cases) countries than assemblage A (482 and 1,096 cases, respectively) (Tables 1 and 2). The number of molecular epidemiological studies of giardiasis in humans is still small and does not allow a clear detection of geographic or socioeconomic differences in the distribution of assemblages A and B or an assessment of the role of anthroponotic and zoonotic infections in human giardiasis.

Canine-specific assemblages C and D were reported for humans in Thailand in one study (264), but this might be the result of a poor genetic resolution of the SSU rRNA gene PCR products. A previous study by that same group identified assemblages C and D in humans in India using sequence analysis of the SSU rRNA gene. However, only assemblages A and B were detected by sequence analyses of the *tpi* and *ef1*α genes (265). Mixed infections of assemblages A and F were recently identified for seven humans in Ethiopia (87), but the presence of assemblage F could not be confirmed by sequence analyses of the SSU rRNA, *gdh*, or *tpi* gene. Likewise, the recent finding of assemblage E in three human cases by *tpi* gene sequencing needs confirmation by analyses of other loci (83). In a more recent study conducted in Europe, assemblage C was found in two human specimens, and assemblages D to F were each found in four specimens using sequence analysis of the *bg*, *gdh*, or *tpi* gene (247).

Subtyping results indicated that both subgroups AI and AII were found in humans, with subgroup AII being more common (Table 9). Many of these studies targeted only one or two loci; thus, it is not possible to accurately assign some of the less common sequence types to subassemblages in the context of MLGs. There are many more subtypes in assemblage B, and multiple subtypes are usually present in humans in one study area, although the significance of this is not clear (247).

Few studies have investigated the association between assemblage occurrence and the age of patients. In one study of 321 persons between 2 and 76 years old, children ≤12 years of age were at a higher risk of infection with assemblage B (165). The association between assemblage B infection and young age raises the question of whether the distribution of *Giardia* assemblages is affected by age. A comparison of data from studies in the Philippines, Ethiopia, Australia, and Spain showed that children were susceptible to both assemblages, with variable assemblage distributions among countries (87, 213, 228). The reasons behind the geographic variations in the distribution of the *G. duodenalis* assemblages are still unclear. It may be explained by the difference in transmission routes and infection sources. It is possible that assemblage A, with a wide range of animals as reservoir hosts, is more likely responsible for zoonotic transmission.

Several molecular epidemiological studies carried out in Ethiopia and the Philippines showed no significant difference between assemblage distribution and gender (87). However, a recent study in Malaysia showed that females were at a 2-fold-higher risk of acquiring giardiasis caused by assemblage B than males (165). Female gender as a risk factor for giardiasis could be related to the women's role as caretakers of children and direct contact with infected children and changing diapers. Nursing has been identified as a risk factor for giardiasis in communities where the infection rate is high in children (76). Thus, there might be some differences in the transmission routes of human giardiasis between males and females living in the same area.

The transmission of *G. duodenalis* through drinking and recreational waters is well documented (139). Despite this, little is known about the *Giardia* spp. or *G. duodenalis* assemblages present in water (240). As expected, assemblages A and E were detected in wastewater samples taken from a slaughterhouse (32). In contrast, only assemblages A and B have been found in urban wastewater samples examined to date, supporting a contamination of human origin (13a, 32, 38, 222, 223, 250). Not surprisingly, assemblage A was recently found in surface water in Portugal, Hungary, and Malaysia (157, 159, 204). There are no data available to make epidemiological linkages between *Giardia* genotypes/subtypes in source and/or drinking water and human giardiasis.

Outbreaks. Data on *G. duodenalis* genotypes and subtypes in outbreaks of human giardiasis are very limited. Only one person-to-person outbreak of giardiasis was investigated by genotyping. In April 2000, a nursery outbreak of giardiasis occurred in North Wales, United Kingdom, where children, child care workers, and parents had confirmed giardiasis. An analysis of

21 *tpi* PCR-positive specimens from the outbreak showed the presence of only *G. duodenalis* assemblage B. In contrast, an analysis of 35 specimens from sporadic giardiasis cases in England and Wales identified subgroup AII in nine specimens, assemblage B in 21 specimens, and both subgroup AII and assemblage B in three specimens (14).

One travel-associated giardiasis outbreak was investigated by genotyping. During 28 September to 4 October 2008, 19 travelers from Illinois, New Mexico, South Dakota, and Wisconsin embarked on a barge trip down the Rhone River in south France. Eighteen of the passengers (97%) reported symptoms consistent with giardiasis. Of the cases reported to the four states, 10 tested positive for *Giardia*. Specimens from two patients were genotyped as belonging to assemblage B. They produced *tpi* sequences identical to a subtype commonly found in several countries (strains reported under GenBank accession numbers AY368171 [raw wastewater from Milwaukee, WI], EU518582 [eider in Nantucket Island, MA], EU518572 and EU518561 [dolphins in Cape Cod, MA], and EU518566 [seal in Cape Cod, MA] and strains BAH34, AD-19, and M68, originating from Australian and Belgian residents) (L. Xiao, unpublished data).

Numerous food-borne outbreaks of giardiasis have been reported over the years. Unfortunately, only one food-borne outbreak was investigated by genotyping. The outbreak occurred in >30 school and church staff members who had lunch in the same restaurant in San Francisco, CA, in September 2001. Stool testing of restaurant employees identified three asymptomatically infected food handlers, including a cook, a waiter, and a counter assistant. Only five formalin-fixed stool specimens from school employees were available for molecular diagnosis, two of which were identified as having *G. duodenalis* assemblage B. They belonged to a common *tpi* subtype occurring in sporadic cases of giardiasis in India and Peru (249).

Only several waterborne outbreaks of giardiasis were investigated with genotyping tools. One was a large drinking water-associated outbreak during autumn and winter 2004 in Bergen, Norway, in which over 1,500 patients were diagnosed with giardiasis. One particular septic tank was thought to be the possible source of contamination. Analysis of specimens from the outbreak showed that *Giardia* cysts in the suspected septic tank belonged to assemblage A, whereas those in 21 patients belonged to assemblage B. Thus, the septic tank was exonerated as the contamination source. Sewage leakage from a residential area was subsequently considered the probable source of contamination (223).

Assemblage B was responsible for a waterborne outbreak of giardiasis in California in 2007, which occurred among attendees of a private recreational camp, with 26 laboratory-confirmed cases and 24 probable cases. A retrospective cohort study determined that showering was associated with illness. The camp had installed a slow-sand water filtration system 2 days before the outbreak. A review of historical water quality data identified substantially elevated total coliform and turbidity levels in sand-filtered spring water used for showering during the suspected exposure period. Unfiltered spring water tested at the same time had acceptable coliform and turbidity levels, implicating the filtration system as the most likely contamination source. Two PCR-positive stool specimens produced identical *tpi* sequences of assemblage B. The sequences obtained from the two patients were mostly identical to the WB6, S7, and B6 subtypes (GenBank accession numbers AY368167, AY228634, and GU564284), with only one nucleotide substitution (T to C) in the 530-bp region under analysis (140).

Assemblage B was responsible for another outbreak of giardiasis associated with a community drinking water system in New Hampshire in 2007, with at least 31 ill persons (59). The assemblage B subtype found in three human cases from this outbreak had substantial sequence differences in the *tpi* gene (five or more nucleotide substitutions within the 530-bp PCR target) from other known subtypes in humans but was identical to a subtype previously found in beavers in Massachusetts (GenBank accession number DQ789113) and a Barbary macaque in Italy (accession number EU637589) (see Fig. S7 in the supplemental material). Because specimens from two adult beavers near the water source were negative for *Giardia*, whether the beaver was the source of *Giardia* cysts could not be confirmed.

The only known outbreak of giardiasis involving assemblage A was a waterborne outbreak that occurred in Temagami, Ontario, Canada, in the spring of 1994 and was characterized by water *Giardia* cyst concentrations 2 to 3 orders of magnitude higher than normal and a significant increase in the number of cases of giardiasis in the community. Of the 10 human specimens analyzed, 6 had assemblage A isolates, whereas the remainder could not be amplified (283). Human sewage contamination was thought to be the likely contamination source.

***Giardia* genotypes and virulence.** Clinical manifestations of giardiasis are quite variable, ranging from the absence of symptoms to acute or chronic diarrhea, dehydration, abdominal pain, nausea, vomiting, and weight loss (221). The severity of disease is probably determined by the interplay between the virulence of the parasite, the developmental, nutritional, and immunological status of the host, the nature of intestinal microflora, and the presence or absence of other copathogens. Although different *G. duodenalis* assemblages may produce different toxins or metabolic products that may contribute to their pathogenicity (274), studies of the possible association between *G. duodenalis* assemblages and virulence (indicated by the likelihood of causing diarrhea and other clinical symptoms, as many *G. duodenalis* infections are asymptomatic) have thus far produced inconsistent results.

Some studies reported that assemblage A isolates were more virulent than assemblage B isolates. Assemblage A is often associated with the presence of symptoms, while assemblage B is not. Thus, in one case-control study of 322 giardiasis patients in Dhaka, Bangladesh, assemblage A, particularly subgroup AII, was strongly associated with the occurrence of diarrhea (111). In 267 cases of giardiasis in southwest London studied, both assemblages A and B caused similar illnesses, but assemblage A was significantly more frequently associated with fever than assemblage B, and all assemblage A strains subtyped were of subgroup AII (35). In contrast, assemblage B was more often associated with asymptomatic infection. A similar association was found for children less than 5 years of age in Spain; subassemblage AII was associated with symptomatic infection, and assemblage B was associated with asymptomatic infection (228). In a longitudinal study of giardiasis in children under 5 years of age in Australia, children infected with assemblage A

isolates were 26 times more likely to have diarrhea than those infected with assemblage B isolates (213). In agreement with these observations, in two studies of giardiasis in children less than 9 years old conducted in Peru and Turkey, assemblage A was preferentially found in symptomatic cases, whereas assemblage B was preferentially found in asymptomatic cases (21, 203).

The opposite conclusion was drawn from some other studies. In a study of giardiasis in persons of 8 to 60 years of age in the Netherlands, there was an association of assemblage A with intermittent diarrhea and an association of assemblage B with duodenal inflammation, nausea, and persistent symptoms (117). In a study of giardiasis in persons 2 to 76 years of age in Malaysia, assemblage B infection was significantly correlated with clinical symptoms of giardiasis (165). Likewise, in elementary school children in Saudi Arabia, all infections with assemblage B were symptomatic, while only subassemblages AI and AII were found in asymptomatic infections (12). It was also noticed that symptomatic giardiasis was more significantly associated with assemblage B in children and adults in Ethiopia (87) and elementary school children in Cuba (202).

One factor that was not considered was the intra-assemblage variation within assemblages, which could possibly account for the differences between the studies (180). Furthermore, it was suggested that in regions where a genotype is endemic, a new genotype might cause particularly severe symptoms when it first appears in the population, and dual infection with two different genotypes might produce a synergistic increase in pathology (221). Other parasite factors (such as the rate of multiplication, variable surface proteins expressed, resistance to pharmaceuticals, and ability to invade immune response) and their interplay with host factors has also been suggested to contribute to the pathophysiology observed for clinical giardiasis (221, 274). In addition, infection pressure may be one of the reasons for the different observations of the virulences of assemblages A and B in humans, as the infection dose and dominant *G. duodenalis* assemblage vary with different socioeconomic development and hygiene practices. On the other hand, host factors such as immune status and age might have played a role in the differences in observations of the virulences of assemblages A and B in humans. It was reported that symptomatic giardiasis was confined mainly to the young and the elderly (228, 274). The susceptibility of these age groups is believed to be the immaturity of the immune system in the former and the immune incompetence in the latter (100). Other potential host factors include history of exposure, diet, and concomitant intestinal microbiota (221).

CONCLUDING REMARKS

The use of molecular diagnostic tools has significantly changed our understanding of the zoonotic potential of *Giardia* spp. in various animals. Evidence accumulated over the past 2 decades has firmly established giardiasis as a zoonotic disease. We are beginning to use these tools to answer questions regarding the transmission of giardiasis in humans and domestic animals. Despite these progresses, we are still facing daunting challenges, especially regarding the disease burden of zoonotic infections, roles of various farm and companion animals in the transmission of human giardiasis, source and human-infective potential of *Giardia* cysts in source water and drinking water, and differences in biology, clinical manifestations, and outbreak potentials among *G. duodenalis* genotypes and subtypes. These issues can be addressed effectively only through improvements in molecular diagnostic tools, the more systematic use of these tools in well-designed epidemiological investigations in both endemic and epidemic settings, and a better understanding of the population genetics of *G. duodenalis* in various hosts under different socioeconomic and environmental conditions.

Currently, on the diagnosis front, MLG tools are used increasingly in characterizing *G. duodenalis* infections of humans and animals and comparisons of giardiasis transmission between them. This is especially important for resolving the question of the infectivity of some host-adapted assemblages (such as assemblages C, D, E, and F) in humans and in assessing the human-infective potential of assemblage A from animals and the disease burden of zoonotic infections. The latter is hampered by the lack of standardized subtype nomenclature and subtyping procedures, which would require coordinated efforts in thorough characterizations of a large number of reference strains and field isolates using multiple genetic markers and primer sets. New polymorphic markers are probably needed for subtyping isolates within assemblage A, as current tools can differentiate only a few subtypes within subassemblages AI, AII, and AIII. In contrast, other typing strategies may be needed for assemblage B because of the high genetic heterogeneity among isolates in most markers and the presence of apparent heterozygosity at some genetic loci (84, 144a, 154, 247, 254). This can include the use of whole-genome analysis employing the next-generation sequence technologies and single-nucleotide polymorphism (SNP) arrays.

A thorough understanding of the transmission of giardiasis in humans and animals also requires better characterizations of the population genetics of *G. duodenalis*. Thus far, studies of the population genetics of *G. duodenalis* are restricted largely to determinations of the presence of genetic recombination (57, 151a, 247, 254). Many other aspects of the population genetics of the parasites have not been addressed, such as population substructure, geographic and host segregations, and selection and dispersal of genetic lineages. Although urgently required, discriminatory subtyping tools based on microsatellites and microsatellite targets that are not under selection pressure are not currently available for *Giardia*. Sequences of the whole genome are now available for assemblages A, B, and E of *G. duodenalis* (6, 84, 135a, 276). It is now possible to "mine" the genomic databases to identify targets that are likely to show high-level sequence polymorphism. These population genetic tools would also be useful in addressing epidemiological issues related to the infection sources in humans and wildlife; the genetic basis for virulence and drug resistance; the temporal and geographic dispersal of assemblages A and B in humans, domestic animals, and wildlife; and the absence of assemblage E in some hosts (such as wild ruminants) and geographic areas (such as New Zealand).

Our understanding of giardiasis transmission can be improved through the systematic use of molecular diagnostic tools in well-designed epidemiological studies. The assessment of the disease burden attributable to zoonotic parasites re-

quires the case-control study design, extensive collection of epidemiological data using questionnaires, subtyping of specimens from both humans and animals, and more sophisticated statistical analyses (such as logistic regression and modeling) of research data. Likewise, the identification of the transmission of giardiasis between animals and humans requires longitudinal follow-up and subtyping of both humans and animals in the same focus of endemicity, which allows the establishment of the sequence of infections in humans and animals. Only through the integration of molecular diagnostic and epidemiological tools can we improve our understanding of the importance of zoonotic transmission in the epidemiology of human giardiasis and the transmission dynamics of giardiasis in various socioeconomic and geographic settings.

ACKNOWLEDGMENTS

This work was supported in part by the National Natural Science Foundation of China (grant no. 30771881, 30928019, and 81041078); fundamental research funds for the Central Universities, China (grant no. WB0914044); the Shanghai Science and Technology Committee (grant no. 09540704400); and the State Key Laboratory of Veterinary Etiological Biology at the Lanzhou Veterinary Research Institute.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES

1. **Abe, N., I. Kimata, and M. Iseki.** 2003. Identification of genotypes of *Giardia intestinalis* isolates from dogs in Japan by direct sequencing of the PCR amplified glutamate dehydrogenase gene. J. Vet. Med. Sci. **65:**29–33.
2. **Abe, N., I. Kimata, and M. Tokoro.** 2005. Genotyping of *Giardia* isolates from humans in Japan using the small subunit ribosomal RNA and glutamate dehydrogenase gene sequences. Jpn. J. Infect. Dis. **58:**57–58.
3. **Abe, N., C. Read, R. C. Thompson, and M. Iseki.** 2005. Zoonotic genotype of *Giardia intestinalis* detected in a ferret. J. Parasitol. **91:**179–182.
4. **Abe, N., T. Tanoue, E. Noguchi, G. Ohta, and H. Sakai.** 2010. Molecular characterization of *Giardia duodenalis* isolates from domestic ferrets. Parasitol. Res. **106:**733–736.
5. **Abou-Shady, O., M. S. El Raziky, M. M. Zaki, and R. K. Mohamed.** Impact of *Giardia lamblia* on growth, serum levels of zinc, copper, and iron in Egyptian children. Biol. Trace Elem. Res., in press.
6. **Adam, R. D., A. Nigam, V. Seshadri, C. A. Martens, G. A. Farneth, H. G. Morrison, T. E. Nash, S. F. Porcella, and R. Patel.** 2010. The *Giardia lamblia vsp* gene repertoire: characteristics, genomic organization, and evolution. BMC Genomics **11:**424.
7. **Adams, P. J., P. T. Monis, A. D. Elliot, and R. C. Thompson.** 2004. Cyst morphology and sequence analysis of the small subunit rDNA and ef1 alpha identifies a novel *Giardia* genotype in a quenda (*Isoodon obesulus*) from Western Australia. Infect. Genet. Evol. **4:**365–370.
8. **Al-Megrin, W. A.** 2010. Intestinal parasites infection among immunocompromised patients in Riyadh, Saudi Arabia. Pak. J. Biol. Sci. **13:**390–394.
9. **Almeida, A., E. Pozio, and S. M. Caccio.** 2010. Genotyping of *Giardia duodenalis* cysts by new real-time PCR assays for detection of mixed infections in human samples. Appl. Environ. Microbiol. **76:**1895–1901.
10. **Almeida, A. A., M. L. Delgado, S. C. Soares, A. O. Castro, M. J. Moreira, C. M. Mendonca, N. B. Canada, and J. M. Da Costa.** 2006. Genotype analysis of *Giardia* isolated from asymptomatic children in northern Portugal. J. Eukaryot. Microbiol. **53**(Suppl. 1)**:**S177–S178.
11. **Almerie, M. Q., M. S. Azzouz, M. A. Abdessamad, M. A. Mouchli, M. W. Sakbani, M. S. Alsibai, A. Alkafri, and M. T. Ismail.** 2008. Prevalence and risk factors for giardiasis among primary school children in Damascus, Syria. Saudi Med. J. **29:**234–240.
12. **Al-Mohammed, H. I.** Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. Parasitol. Res., in press.
13. **Aloisio, F., G. Filippini, P. Antenucci, E. Lepri, G. Pezzotti, S. M. Caccio, and E. Pozio.** 2006. Severe weight loss in lambs infected with *Giardia duodenalis* assemblage B. Vet. Parasitol. **142:**154–158.

13a. **Alonso, J. L., I. Amoros, and G. Cuesta.** 2010. LNA probes in a real-time TaqMan PCR assay for genotyping of *Giardia duodenalis* in wastewaters. J. Appl. Microbiol. **108:**1594–1601.

14. **Amar, C. F., P. H. Dear, S. Pedraza-Diaz, N. Looker, E. Linnane, and J. McLauchlin.** 2002. Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. J. Clin. Microbiol. **40:**446–452.
15. **Ang, L.** 2000. Outbreak of giardiasis in a daycare nursery. Commun. Dis. Public Health **3:**212–213.
16. **Appelbee, A., C. Thorlakson, and M. E. Olson.** 2002. Genotypic characterization of *Giardia* cysts isolated from wild beaver in southern Alberta, Canada, p. 299–300. *In* B. E. Olson, M. E. Olson, and P. M. Wallis (ed.), Giardia: the cosmopolitan parasite. CAB International, Wallingford, United Kingdom.
17. **Appelbee, A. J., L. M. Frederick, T. L. Heitman, and M. E. Olson.** 2003. Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. Vet. Parasitol. **112:**289–294.
18. **Appelbee, A. J., R. C. Thompson, L. M. Measures, and M. E. Olson.** 2010. *Giardia* and *Cryptosporidium* in harp and hooded seals from the Gulf of St. Lawrence, Canada. Vet. Parasitol. **173:**19–23.
19. **Appelbee, A. J., R. C. Thompson, and M. E. Olson.** 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife—current status and future needs. Trends Parasitol. **21:**370–376.
20. **Armson, A., R. Yang, J. Thompson, J. Johnson, S. Reid, and U. M. Ryan.** 2009. *Giardia* genotypes in pigs in Western Australia: prevalence and association with diarrhea. Exp. Parasitol. **121:**381–383.

20a. **Ash, A., A. Lymbery, J. Lemon, S. Vitali, and R. C. Thompson.** 2010. Molecular epidemiology of *Giardia duodenalis* in an endangered carnivore—the African painted dog. Vet. Parasitol. **174:**206–212.

21. **Aydin, A. F., B. A. Besirbellioglu, I. Y. Avci, M. Tanyuksel, E. Araz, and A. Pahsa.** 2004. Classification of *Giardia duodenalis* parasites in Turkey into groups A and B using restriction fragment length polymorphism. Diagn. Microbiol. Infect. Dis. **50:**147–151.
22. **Bajer, A.** 2008. *Cryptosporidium* and *Giardia* spp. infections in humans, animals and the environment in Poland. Parasitol. Res. **104:**1–17.
23. **Ballweber, L. R., L. Xiao, D. D. Bowman, G. Kahn, and V. A. Cama.** 2010. Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol. **26:**180–189.
24. **Barutzki, D., R. C. Thompson, C. Wielinga, U. Parka, and R. Schaper.** 2007. Observations on *Giardia* infection in dogs from veterinary clinics in Germany. Parasitol. Res. **101**(Suppl. 1)**:**153–156.
25. **Batchelor, D. J., S. Tzannes, P. A. Graham, J. M. Wastling, G. L. Pinchbeck, and A. J. German.** 2008. Detection of endoparasites with zoonotic potential in dogs with gastrointestinal disease in the UK. Transbound. Emerg. Dis. **55:**99–104.
26. **Becher, K. A., I. D. Robertson, D. M. Fraser, D. G. Palmer, and R. C. Thompson.** 2004. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections in dairy calves originating from three sources in Western Australia. Vet. Parasitol. **123:**1–9.

26a. **Beck, R., H. Sprong, I. Bata, S. Lucinger, E. Pozio, and S. M. Caccio.** 2011. Prevalence and molecular typing of *Giardia* spp. in captive mammals at the zoo of Zagreb, Croatia. Vet. Parasitol. **175:**40–46.

27. **Behera, B., B. R. Mirdha, G. K. Makharia, S. Bhatnagar, S. Dattagupta, and J. C. Samantaray.** 2008. Parasites in patients with malabsorption syndrome: a clinical study in children and adults. Dig. Dis. Sci. **53:**672–679.
28. **Berkman, D. S., A. G. Lescano, R. H. Gilman, S. L. Lopez, and M. M. Black.** 2002. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. Lancet **359:**564–571.
29. **Berrilli, F., D. Di Cave, C. D'Orazi, P. Orecchia, L. Xhelilaj, D. Bejko, P. Caca, D. Bebeci, F. Cenko, D. Donia, and M. Divizia.** 2006. Prevalence and genotyping of human isolates of *Giardia duodenalis* from Albania. Parasitol. Int. **55:**295–297.
30. **Berrilli, F., D. Di Cave, C. De Liberato, A. Franco, P. Scaramozzino, and P. Orecchia.** 2004. Genotype characterisation of *Giardia duodenalis* isolates from domestic and farm animals by SSU-rRNA gene sequencing. Vet. Parasitol. **122:**193–199.
31. **Bertrand, I., L. Albertini, and J. Schwartzbrod.** 2005. Comparison of two target genes for detection and genotyping of *Giardia lamblia* in human feces by PCR and PCR-restriction fragment length polymorphism. J. Clin. Microbiol. **43:**5940–5944.
32. **Bertrand, I., and J. Schwartzbrod.** 2007. Detection and genotyping of *Giardia duodenalis* in wastewater: relation between assemblages and faecal contamination origin. Water Res. **41:**3675–3682.
33. **Birky, C. W., Jr.** 2010. *Giardia* sex? Yes, but how and how much? Trends Parasitol. **26:**70–74.
34. **Bomfim, T. C., F. Huber, R. S. Gomes, and L. L. Alves.** 2005. Natural infection by *Giardia* sp. and *Cryptosporidium* sp. in dairy goats, associated with possible risk factors of the studied properties. Vet. Parasitol. **134:**9–13.
35. **Breathnach, A. S., T. D. McHugh, and P. D. Butcher.** 2010. Prevalence and clinical correlations of genetic subtypes of *Giardia lamblia* in an urban setting. Epidemiol. Infect. **138:**1459–1467.
36. Reference deleted.
37. **Caccio, S. M., R. Beck, M. Lalle, A. Marinculic, and E. Pozio.** 2008. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int. J. Parasitol. **38:**1523–1531.
38. **Caccio, S. M., M. De Giacomo, F. A. Aulicino, and E. Pozio.** 2003. *Giardia* cysts in wastewater treatment plants in Italy. Appl. Environ. Microbiol. **69:**3393–3398.

39. **Caccio, S. M., M. De Giacomo, and E. Pozio.** 2002. Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int. J. Parasitol. **32:**1023–1030.
40. **Caccio, S. M., L. Rinaldi, G. Cringoli, R. Condoleo, and E. Pozio.** 2007. Molecular identification of *Cryptosporidium parvum* and *Giardia duodenalis* in the Italian water buffalo (*Bubalus bubalis*). Vet. Parasitol. **150:**146–149.
41. **Caccio, S. M., and U. Ryan.** 2008. Molecular epidemiology of giardiasis. Mol. Biochem. Parasitol. **160:**75–80.
42. **Caccio, S. M., R. C. Thompson, J. McLauchlin, and H. V. Smith.** 2005. Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol. **21:**430–437.
43. **Campos Filho, P. C., L. M. Barros, J. O. Campos, I. M. Cazorla, G. R. Albuquerque, and S. M. Carvalho.** 2008. Zoonotic parasites in dog feces at public squares in the municipality of Itabuna, Bahia, Brazil. Rev. Bras. Parasitol. Vet. **17:**206–209. (In Portugese.)
44. **Capelli, G., A. F. di Regalbono, R. Iorio, M. Pietrobelli, B. Paoletti, and A. Giangaspero.** 2006. *Giardia* species and other intestinal parasites in dogs in north-east and central Italy. Vet. Rec. **159:**422–424.
45. **Carlin, E. P., D. D. Bowman, J. M. Scarlett, J. Garrett, and L. Lorentzen.** 2006. Prevalence of *Giardia* in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP *Giardia* test. Vet. Ther. **7:**199–206.
46. **Carvalho-Costa, F. A., A. Q. Goncalves, S. L. Lassance, L. M. Silva Neto, C. A. Salmazo, and M. N. Boia.** 2007. *Giardia lamblia* and other intestinal parasitic infections and their relationships with nutritional status in children in Brazilian Amazon. Rev. Inst. Med. Trop. Sao Paulo **49:**147–153.
47. **Castro-Hermida, J. A., A. Almeida, M. Gonzalez-Warleta, J. M. Da Costa, and M. Mezo.** 2006. Prevalence and preliminary genetic analysis of *Giardia* isolated from adult sheep in Galicia (northwest Spain). J. Eukaryot. Microbiol. **53**(Suppl. 1)**:**S172–S173.
48. **Castro-Hermida, J. A., A. Almeida, M. Gonzalez-Warleta, J. M. Correia da Costa, C. Rumbo-Lorenzo, and M. Mezo.** 2007. Occurrence of *Cryptosporidium parvum* and *Giardia duodenalis* in healthy adult domestic ruminants. Parasitol. Res. **101:**1443–1448.
49. **Castro-Hermida, J. A., C. Carro-Corral, M. Gonzalez-Warleta, and M. Mezo.** 2006. Prevalence and intensity of infection of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Galicia (NW Spain). J. Vet. Med. B Infect. Dis. Vet. Public Health **53:**244–246.
50. **Castro-Hermida, J. A., A. Delafosse, I. Pors, E. Ares-Mazas, and C. Chartier.** 2005. *Giardia duodenalis* and *Cryptosporidium parvum* infections in adult goats and their implications for neonatal kids. Vet. Rec. **157:**623–627.
51. **Celiksoz, A., M. Acioz, S. Degerli, Z. Cinar, N. Elaldi, and M. Erandac.** 2005. Effects of giardiasis on school success, weight and height indices of primary school children in Turkey. Pediatr. Int. **47:**567–571.
52. **Church, C., A. Neill, and A. Schotthoefer.** 2010. Seasonality of parasitic gut infections in humans in the Rocky Mountain region, United States. J. Parasitol. **96:**104–106.
53. **Claerebout, E., S. Casaert, A. C. Dalemans, N. De Wilde, B. Levecke, J. Vercruysse, and T. Geurden.** 2009. *Giardia* and other intestinal parasites in different dog populations in Northern Belgium. Vet. Parasitol. **161:**41–46.
54. **Coelho, W. M., A. F. do Amarante, R. V. de Soutello, M. V. Meireles, and K. D. Bresciani.** 2009. Occurrence of gastrointestinal parasites in fecal samples of cats in Andradina City, Sao Paulo. Rev. Bras. Parasitol. Vet. **18:**46–49. (In Portugese.)
55. **Coklin, T., J. Farber, L. Parrington, and B. Dixon.** 2007. Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada. Vet. Parasitol. **150:**297–305.
56. **Cook, D. M., R. C. Swanson, D. L. Eggett, and G. M. Booth.** 2009. A retrospective analysis of prevalence of gastrointestinal parasites among school children in the Palajunoj Valley of Guatemala. J. Health Popul. Nutr. **27:**31–40.
57. **Cooper, M. A., R. D. Adam, M. Worobey, and C. R. Sterling.** 2007. Population genetics provides evidence for recombination in *Giardia*. Curr. Biol. **17:**1984–1988.

57a. **Cooper, M. A., C. R. Sterling, R. H. Gilman, V. Cama, Y. Ortega, and R. D. Adam.** 2010. Molecular analysis of household transmission of *Giardia lamblia* in a region of high endemicity in Peru. J. Infect. Dis. **202:**1713–1721.

58. **Crotti, D., M. L. D'Annibale, G. Fonzo, M. Lalle, S. M. Caccio, and E. Pozio.** 2005. *Dientamoeba fragilis* is more prevalent than *Giardia duodenalis* in children and adults attending a day care centre in Central Italy. Parasite **12:**165–170.
59. **Daly, E. R., S. J. Roy, D. D. Blaney, J. S. Manning, V. R. Hill, L. Xiao, and J. W. Stull.** 2010. Outbreak of giardiasis associated with a community drinking-water source. Epidemiol. Infect. **138:**491–500.
60. **Daryani, A., M. Sharif, M. Meigouni, F. B. Mahmoudi, A. Rafiei, S. Gholami, A. Khalilian, S. Gohardehi, and A. M. Mirabi.** 2009. Prevalence of intestinal parasites and profile of CD4+ counts in HIV+/AIDS people in north of Iran, 2007-2008. Pak. J. Biol. Sci. **12:**1277–1281.
61. **Davies, A. P., B. Campbell, M. R. Evans, A. Bone, A. Roche, and R. M. Chalmers.** 2009. Asymptomatic carriage of protozoan parasites in children in day care centers in the United Kingdom. Pediatr. Infect. Dis. J. **28:**838–840.
62. **de Carvalho, T. B., L. R. de Carvalho, and L. M. Mascarini.** 2006. Occurrence of enteroparasites in day care centers in Botucatu (Sao Paulo State, Brazil) with emphasis on *Cryptosporidium* sp., *Giardia duodenalis* and *Enterobius vermicularis*. Rev. Inst. Med. Trop. Sao Paulo **48:**269–273.
63. **del Pilar Chaves, M., J. A. Fernandez, I. Ospina, M. C. Lopez, L. Moncada, and P. Reyes.** 2007. *Giardia duodenalis* prevalence and associated risk factors in preschool and school-age children of rural Colombia. Biomedica **27:**345–351. (In Spanish.)
64. **De Santis-Kerr, A. C., M. Raghavan, N. W. Glickman, R. J. Caldanaro, G. E. Moore, H. B. Lewis, P. M. Schantz, and L. T. Glickman.** 2006. Prevalence and risk factors for *Giardia* and coccidia species of pet cats in 2003-2004. J. Feline Med. Surg. **8:**292–301.
65. **Dib, H. H., S. Q. Lu, and S. F. Wen.** 2008. Prevalence of *Giardia lamblia* with or without diarrhea in South East, South East Asia and the Far East. Parasitol. Res. **103:**239–251.
66. **Di Giovanni, G. D., W. Q. Betancourt, J. Hernandez, N. W. Assadian, J. P. Flores Margez, and E. J. Lopez.** 2006. Investigation of potential zooanthroponotic transmission of cryptosporidiosis and giardiasis through agricultural use of reclaimed wastewater. Int. J. Environ. Health Res. **16:**405–418.

66a. **Dixon, B., L. Parrington, A. Cook, K. Pintar, F. Pollari, D. Kelton, and J. Farber.** The potential for zoonotic transmission of *Giardia duodenalis* and *Cryptosporidium* spp. from beef and dairy cattle in Ontario, Canada. Vet. Parasitol., in press.

67. **Dixon, B., L. Parrington, M. Parenteau, D. Leclair, M. Santin-Duran, and R. Fayer.** 2008. *Giardia duodenalis* and *Cryptosporidium* spp. in the intestinal contents of ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) in Nunavik, Quebec, Canada. J. Parasitol. **94:**1161–1163.
68. **Dubna, S., I. Langrova, J. Napravnik, I. Jankovska, J. Vadlejch, S. Pekar, and J. Fechtner.** 2007. The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. Vet. Parasitol. **145:**120–128.
69. **El Guamri, Y., D. Belghyti, A. Achicha, M. Tiabi, N. Aujjar, A. Barkia, K. El Kharrim, H. Barkia, E. El-Fellaki, R. Mousahel, H. Bouachra, and A. Lakhal.** 2009. Epidemiological retrospective survey intestinal parasitism in the Provincial Hospital Center (Kenitra, Morocco): review of 10 years (1996-2005). Ann. Biol. Clin. (Paris) **67:**191–202. (In French.)
70. **Eligio-Garcia, L., A. Cortes-Campos, S. Cota-Guajardo, S. Gaxiola, and E. Jimenez-Cardoso.** 2008. Frequency of *Giardia intestinalis* assemblages isolated from dogs and humans in a community from Culiacan, Sinaloa, Mexico using beta-giardin restriction gene. Vet. Parasitol. **156:**205–209.
71. **Eligio-Garcia, L., A. Cortes-Campos, and E. Jimenez-Cardoso.** 2005. Genotype of *Giardia intestinalis* isolates from children and dogs and its relationship to host origin. Parasitol. Res. **97:**1–6.
72. **El Kettani, S., E. M. Azzouzi, and A. Maata.** 2006. Prevalence of *Giardia intestinalis* in a farming population using sewage water in agriculture, Settat, Morocco. Med. Mal. Infect. **36:**322–328.
73. **Epe, C., N. Coati, and T. Schnieder.** 2004. Results of parasitological examinations of faecal samples from horses, ruminants, pigs, dogs, cats, hedgehogs and rabbits between 1998 and 2002. Dtsch. Tierarztl. Wochenschr. **111:**243–247. (In German.)
74. **Epe, C., G. Rehkter, T. Schnieder, L. Lorentzen, and L. Kreienbrock.** 2010. *Giardia* in symptomatic dogs and cats in Europe—results of a European study. Vet. Parasitol. **173:**32–38.
75. **Erickson, M. C., and Y. R. Ortega.** 2006. Inactivation of protozoan parasites in food, water, and environmental systems. J. Food Prot. **69:**2786–2808.
76. **Espelage, W., M. an der Heiden, K. Stark, and K. Alpers.** 2010. Characteristics and risk factors for symptomatic *Giardia lamblia* infections in Germany. BMC Public Health **10:**41–50.
77. **Fayer, R., P. A. Fair, G. D. Bossart, and M. Santin.** 2008. Examination of naturally exposed bottlenose dolphins (*Tursiops truncatus*) for microsporidia, *Cryptosporidium*, and *Giardia*. J. Parasitol. **94:**143–147.
78. **Fayer, R., M. Santin, J. M. Trout, S. DeStefano, K. Koenen, and T. Kaur.** 2006. Prevalence of microsporidia, Cryptosporidium spp., and *Giardia* spp. in beavers (*Castor canadensis*) in Massachusetts. J. Zoo Wildl. Med. **37:**492–497.
79. **Fayer, R., M. Santin, J. M. Trout, and J. P. Dubey.** 2006. Detection of *Cryptosporidium felis* and *Giardia duodenalis* assemblage F in a cat colony. Vet. Parasitol. **140:**44–53.
80. **Feng, Y.** 2010. *Cryptosporidium* in wild placental mammals. Exp. Parasitol. **124:**128–137.
81. **Feng, Y., Y. Ortega, V. Cama, J. Terrel, and L. Xiao.** 2008. High intragenotypic diversity of *Giardia duodenalis* in dairy cattle on three farms. Parasitol. Res. **103:**87–92.
82. **Fontanarrosa, M. F., D. Vezzani, J. Basabe, and D. F. Eiras.** 2006. An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns. Vet. Parasitol. **136:**283–295.
83. **Foronda, P., M. D. Bargues, N. Abreu-Acosta, M. V. Periago, M. A. Valero, B. Valladares, and S. Mas-Coma.** 2008. Identification of genotypes of

Giardia intestinalis of human isolates in Egypt. Parasitol. Res. **103:**1171–1181.

84. **Franzen, O., J. Jerlstrom-Hultqvist, E. Castro, E. Sherwood, J. Ankarklev, D. S. Reiner, D. Palm, J. O. Andersson, B. Andersson, and S. G. Svard.** 2009. Draft genome sequencing of *Giardia intestinalis* assemblage B isolate GS: is human giardiasis caused by two different species? PLoS Pathog. **5:**e1000560.
85. **Gates, M. C., and T. J. Nolan.** 2009. Endoparasite prevalence and recurrence across different age groups of dogs and cats. Vet. Parasitol. **166:**153–158.
86. **Gaydos, J. K., W. A. Miller, C. Kreuder-Johnson, H. Zornetzer, A. C. Melli, A. E. Packham, S. J. Jeffries, M. M. Lance, and P. A. Conrad.** 2008. Novel and canine genotypes of *Giardia duodenalis* in harbor seals (*Phoca vitulina richardsi*). J. Parasitol. **94:**1264–1268.
87. **Gelanew, T., M. Lalle, A. Hailu, E. Pozio, and S. M. Caccio.** 2007. Molecular characterization of human isolates of *Giardia duodenalis* from Ethiopia. Acta Trop. **102:**92–99.
88. **Geurden, T., E. Claerebout, L. Dursin, A. Deflandre, F. Bernay, V. Kaltsatos, and J. Vercruysse.** 2006. The efficacy of an oral treatment with paromomycin against an experimental infection with *Giardia* in calves. Vet. Parasitol. **135:**241–247.
89. **Geurden, T., E. Claerebout, and J. Vercruysse.** 2005. Protozoan infection causes diarrhea in calves. Tijdschr. Diergeneeskd. **130:**734–737. (In Dutch.)
90. **Geurden, T., E. Claerebout, J. Vercruysse, and D. Berkvens.** 2004. Estimation of diagnostic test characteristics and prevalence of *Giardia duodenalis* in dairy calves in Belgium using a Bayesian approach. Int. J. Parasitol. **34:**1121–1127.
91. **Geurden, T., P. Geldhof, B. Levecke, C. Martens, D. Berkvens, S. Casaert, J. Vercruysse, and E. Claerebout.** 2008. Mixed *Giardia duodenalis* assemblage A and E infections in calves. Int. J. Parasitol. **38:**259–264.
92. **Geurden, T., E. Goossens, B. Levecke, F. Vercammen, J. Vercruysse, and E. Claerebout.** 2009. Occurrence and molecular characterization of *Cryptosporidium* and *Giardia* in captive wild ruminants in Belgium. J. Zoo Wildl. Med. **40:**126–130.
93. **Geurden, T., B. Levecke, S. M. Caccio, A. Visser, G. De Groote, S. Casaert, J. Vercruysse, and E. Claerebout.** 2009. Multilocus genotyping of *Cryptosporidium* and *Giardia* in non-outbreak related cases of diarrhoea in human patients in Belgium. Parasitology **136:**1161–1168.
94. **Geurden, T., R. Somers, N. T. Thanh, L. V. Vien, V. T. Nga, H. H. Giang, P. Dorny, H. K. Giao, and J. Vercruysse.** 2008. Parasitic infections in dairy cattle around Hanoi, northern Vietnam. Vet. Parasitol. **153:**384–388.
95. **Geurden, T., P. Thomas, S. Casaert, J. Vercruysse, and E. Claerebout.** 2008. Prevalence and molecular characterisation of *Cryptosporidium* and *Giardia* in lambs and goat kids in Belgium. Vet. Parasitol. **155:**142–145.
96. **Geurden, T., E. Vandenhoute, H. Pohle, S. Casaert, N. De Wilde, J. Vercruysse, and E. Claerebout.** 2010. The effect of a fenbendazole treatment on cyst excretion and weight gain in calves experimentally infected with *Giardia duodenalis*. Vet. Parasitol. **169:**18–23.
97. **Giangaspero, A., F. Berrilli, and O. Brandonisio.** 2007. *Giardia* and *Cryptosporidium* and public health: the epidemiological scenario from the Italian perspective. Parasitol. Res. **101:**1169–1182.
98. **Giangaspero, A., B. Paoletti, R. Iorio, and D. Traversa.** 2005. Prevalence and molecular characterization of *Giardia duodenalis* from sheep in central Italy. Parasitol. Res. **96:**32–37.
99. **Gomez-Munoz, M. T., C. Navarro, M. M. Garijo-Toledo, M. A. Dea-Ayuela, S. Fernandez-Barredo, M. T. Perez-Gracia, M. V. Dominguez-Marquez, and R. Borras.** 2009. Occurrence and genotypes of *Giardia* isolated from lambs in Spain. Parasitol. Int. **58:**297–299.
100. **Gorczynski, R. M., and E. Terzioglu.** 2008. Aging and the immune system. Int. Urol. Nephrol. **40:**1117–1125.
101. **Goto, R., C. G. Mascie-Taylor, and P. G. Lunn.** 2009. Impact of anti-*Giardia* and anthelminthic treatment on infant growth and intestinal permeability in rural Bangladesh: a randomised double-blind controlled study. Trans. R. Soc. Trop. Med. Hyg. **103:**520–529.
102. **Gow, A. G., D. J. Gow, E. J. Hall, D. Langton, C. Clarke, and K. Papasouliotis.** 2009. Prevalence of potentially pathogenic enteric organisms in clinically healthy kittens in the UK. J. Feline Med. Surg. **11:**655–662.
103. **Gow, S., and C. Waldner.** 2006. An examination of the prevalence of and risk factors for shedding of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves from western Canadian cow-calf herds. Vet. Parasitol. **137:**50–61.
104. **Graczyk, T. K., J. Bosco-Nizeyi, B. Ssebide, R. C. Thompson, C. Read, and M. R. Cranfield.** 2002. Anthropozoonotic *Giardia duodenalis* genotype (assemblage) infections in habitats of free-ranging human-habituated gorillas, Uganda. J. Parasitol. **88:**905–909.
105. **Guy, R. A., P. Payment, U. J. Krull, and P. A. Horgen.** 2003. Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage. Appl. Environ. Microbiol. **69:**5178–5185.
106. **Guy, R. A., C. Xiao, and P. A. Horgen.** 2004. Real-time PCR assay for detection and genotype differentiation of *Giardia lamblia* in stool specimens. J. Clin. Microbiol. **42:**3317–3320.
107. **Hamnes, I. S., B. Gjerde, and L. Robertson.** 2006. Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway. Vet. Parasitol. **140:**204–216.
108. **Hamnes, I. S., B. K. Gjerde, T. Forberg, and L. J. Robertson.** 2007. Occurrence of *Cryptosporidium* and *Giardia* in suckling piglets in Norway. Vet. Parasitol. **144:**222–233.
109. **Hamnes, I. S., B. K. Gjerde, T. Forberg, and L. J. Robertson.** 2007. Occurrence of *Giardia* and *Cryptosporidium* in Norwegian red foxes (*Vulpes vulpes*). Vet. Parasitol. **143:**347–353.
110. **Hamnes, I. S., B. K. Gjerde, and L. J. Robertson.** 2007. A longitudinal study on the occurrence of *Cryptosporidium* and *Giardia* in dogs during their first year of life. Acta Vet. Scand. **49:**22.
111. **Haque, R., S. Roy, M. Kabir, S. E. Stroup, D. Mondal, and E. R. Houpt.** 2005. *Giardia* assemblage A infection and diarrhea in Bangladesh. J. Infect. Dis. **192:**2171–2173.
112. **Heitman, T. L., L. M. Frederick, J. R. Viste, N. J. Guselle, U. M. Morgan, R. C. Thompson, and M. E. Olson.** 2002. Prevalence of *Giardia* and *Cryptosporidium* and characterization of *Cryptosporidium* spp. isolated from wildlife, human, and agricultural sources in the North Saskatchewan River Basin in Alberta, Canada. Can. J. Microbiol. **48:**530–541.
113. **Hellard, M. E., M. I. Sinclair, G. G. Hogg, and C. K. Fairley.** 2000. Prevalence of enteric pathogens among community based asymptomatic individuals. J. Gastroenterol. Hepatol. **15:**290–293.

113a. **Himsworth, C. G., S. Skinner, B. Chaban, E. Jenkins, B. A. Wagner, N. J. Harms, F. A. Leighton, R. C. Thompson, and J. E. Hill.** 2010. Multiple zoonotic pathogens identified in canine feces collected from a remote Canadian indigenous community. Am. J. Trop. Med. Hyg. **83:**338–341.

114. **Hoar, B. R., R. R. Paul, J. Siembieda, M. G. Pereira, and E. R. Atwill.** 2009. *Giardia duodenalis* in feedlot cattle from the central and western United States. BMC Vet. Res. **5:**37.
115. **Hoffmann, S., P. Fischbeck, A. Krupnick, and M. McWilliams.** 2007. Using expert elicitation to link foodborne illnesses in the United States to foods. J. Food Prot. **70:**1220–1229.
116. **Homan, W. L., M. Gilsing, H. Bentala, L. Limper, and F. van Knapen.** 1998. Characterization of *Giardia duodenalis* by polymerase-chain-reaction fingerprinting. Parasitol. Res. **84:**707–714.
117. **Homan, W. L., and T. G. Mank.** 2001. Human giardiasis: genotype linked differences in clinical symptomatology. Int. J. Parasitol. **31:**822–826.
118. **Hopkins, R. M., B. P. Meloni, D. M. Groth, J. D. Wetherall, J. A. Reynoldson, and R. C. Thompson.** 1997. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. J. Parasitol. **83:**44–51.
119. **Hoque, M. E., V. T. Hope, T. Kjellstrom, R. Scragg, and R. Lay-Yee.** 2002. Risk of giardiasis in Aucklanders: a case-control study. Int. J. Infect. Dis. **6:**191–197.
120. **Hoque, M. E., V. T. Hope, R. Scragg, and T. Kjellstrom.** 2003. Children at risk of giardiasis in Auckland: a case-control analysis. Epidemiol. Infect. **131:**655–662.
121. **Horman, A., H. Korpela, J. Sutinen, H. Wedel, and M. L. Hanninen.** 2004. Meta-analysis in assessment of the prevalence and annual incidence of *Giardia* spp. and *Cryptosporidium* spp. infections in humans in the Nordic countries. Int. J. Parasitol. **34:**1337–1346.
122. **Hsu, B. M., H. Y. Wun, and P. C. Hsu.** 2007. Prevalence and genotyping of *Giardia* in husbandry systems in Taiwan. Parasitol. Res. **101:**275–280.
123. **Huang, D. B., and A. C. White.** 2006. An updated review on *Cryptosporidium* and *Giardia*. Gastroenterol. Clin. North Am. **35:**291–314.
124. **Huber, F., T. C. Bomfim, and R. S. Gomes.** 2005. Comparison between natural infection by *Cryptosporidium* sp., *Giardia* sp. in dogs in two living situations in the West Zone of the municipality of Rio de Janeiro. Vet. Parasitol. **130:**69–72.
125. **Huetink, R. E., J. W. van der Giessen, J. P. Noordhuizen, and H. W. Ploeger.** 2001. Epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* on a dairy farm. Vet. Parasitol. **102:**53–67.
126. **Huh, J. W., S. G. Moon, and Y. H. Lim.** 2009. A survey of intestinal protozoan infections among gastroenteritis patients during a 3-year period (2004-2006) in Gyeonggi-do (province), South Korea. Korean J. Parasitol. **47:**303–305.
127. **Hunt, C. L., G. Ionas, and T. J. Brown.** 2000. Prevalence and strain differentiation of *Giardia intestinalis* in calves in the Manawatu and Waikato regions of North Island, New Zealand. Vet. Parasitol. **91:**7–13.
128. **Hussein, A. I., T. Yamaguchi, K. Nakamoto, M. Iseki, and M. Tokoro.** 2009. Multiple-subgenotype infections of *Giardia intestinalis* detected in Palestinian clinical cases using a subcloning approach. Parasitol. Int. **58:**258–262.
129. **Inpankaew, T., R. Traub, R. C. Thompson, and Y. Sukthana.** 2007. Canine parasitic zoonoses in Bangkok temples. Southeast Asian J. Trop. Med. Public Health **38:**247–255.
130. **Itagaki, T., S. Kinoshita, M. Aoki, N. Itoh, H. Saeki, N. Sato, J. Uetsuki, S. Izumiyama, K. Yagita, and T. Endo.** 2005. Genotyping of *Giardia intestinalis* from domestic and wild animals in Japan using glutamete dehydrogenase gene sequencing. Vet. Parasitol. **133:**283–287.

130a. **Itoh, N., T. Itagaki, T. Kawabata, T. Konaka, N. Muraoka, H. Saeki, K. Kanai, S. Chikazawa, Y. Hori, F. Hoshi, and S. Higuchi.** Prevalence of

intestinal parasites and genotyping of *Giardia intestinalis* in pet shop puppies in east Japan. Vet. Parasitol., in press.
131. **Itoh, N., K. Kanai, Y. Hori, F. Hoshi, and S. Higuchi.** 2009. Prevalence of *Giardia intestinalis* and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in Aomori prefecture, Japan in 1997, 2002 and 2007. J. Vet. Sci. **10:**305–308.
132. **Itoh, N., N. Muraoka, J. Kawamata, M. Aoki, and T. Itagaki.** 2006. Prevalence of *Giardia intestinalis* infection in household cats of Tohoku district in Japan. J. Vet. Med. Sci. **68:**161–163.
133. **Itoh, N., N. Muraoka, H. Saeki, M. Aoki, and T. Itagaki.** 2005. Prevalence of *Giardia intestinalis* infection in dogs of breeding kennels in Japan. J. Vet. Med. Sci. **67:**717–718.
134. Reference deleted.
135. **Jager, M., M. Gauly, C. Bauer, K. Failing, G. Erhardt, and H. Zahner.** 2005. Endoparasites in calves of beef cattle herds: management systems dependent and genetic influences. Vet. Parasitol. **131:**173–191.
135a. **Jerlstrom-Hultqvist, J., O. Franzen, J. Ankarklev, F. Xu, E. Nohynkova, J. O. Andersson, S. G. Svard, and B. Andersson.** 2010. Genome analysis and comparative genomics of a *Giardia intestinalis* assemblage E isolate. BMC Genomics **11:**543.
136. **Johargy, A., H. Ghazi, and A. Mumenah.** 2010. Frequency of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Saudi Arabia. J. Pak. Med. Assoc. **60:**456–459.
137. **Johnston, A. R., T. R. Gillespie, I. B. Rwego, T. L. McLachlan, A. D. Kent, and T. L. Goldberg.** 2010. Molecular epidemiology of cross-species *Giardia duodenalis* transmission in western Uganda. PLoS Negl. Trop. Dis. **4:**e683.
138. **Karanis, P., and P. L. Ey.** 1998. Characterization of axenic isolates of *Giardia intestinalis* established from humans and animals in Germany. Parasitol. Res. **84:**442–449.
139. **Karanis, P., C. Kourenti, and H. Smith.** 2007. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J. Water Health **5:**1–38.
140. **Karon, A. E., K. D. Hanni, J. C. Mohle-Boetani, R. A. Beretti, V. R. Hill, M. Arrowood, S. P. Johnston, and L. Xiao.** Giardiasis outbreak at a camp after installation of a slow-sand filtration water treatment system. Epidemiol. Infect., in press.
141. **Katagiri, S., and T. C. Oliveira-Sequeira.** 2008. Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in Sao Paulo State, Brazil. Zoonoses Public Health **55:**406–413.
142. **Katz, D. E., D. Heisey-Grove, M. Beach, R. C. Dicker, and B. T. Matyas.** 2006. Prolonged outbreak of giardiasis with two modes of transmission. Epidemiol. Infect. **134:**935–941.
143. **Klimpel, S., J. Heukelbach, D. Pothmann, and S. Ruckert.** 2010. Gastrointestinal and ectoparasites from urban stray dogs in Fortaleza (Brazil): high infection risk for humans? Parasitol. Res. **107:**713–719.
144. **Korkes, F., F. U. Kumagai, R. N. Belfort, D. Szejnfeld, T. G. Abud, A. Kleinman, G. M. Florez, T. Szejnfeld, and P. P. Chieffi.** 2009. Relationship between intestinal parasitic infection in children and soil contamination in an urban slum. J. Trop. Pediatr. **55:**42–45.
144a. **Kosuwin, R., C. Putaporntip, U. Pattanawong, and S. Jongwutiwes.** 2010. Clonal diversity in *Giardia duodenalis* isolates from Thailand: evidences for intragenic recombination and purifying selection at the beta giardin locus. Gene **449:**1–8.
145. **Lalle, M., F. Bruschi, B. Castagna, M. Campa, E. Pozio, and S. M. Caccio.** 2009. High genetic polymorphism among *Giardia duodenalis* isolates from Sahrawi children. Trans. R. Soc. Trop. Med. Hyg. **103:**834–838.
146. **Lalle, M., A. F. di Regalbono, L. Poppi, G. Nobili, D. Tonanzi, E. Pozio, and S. M. Caccio.** 2007. A novel *Giardia duodenalis* assemblage A subtype in fallow deer. J. Parasitol. **93:**426–428.
147. **Lalle, M., E. Jimenez-Cardosa, S. M. Caccio, and E. Pozio.** 2005. Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay. J. Parasitol. **91:**203–205.
148. **Lalle, M., E. Pozio, G. Capelli, F. Bruschi, D. Crotti, and S. M. Caccio.** 2005. Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int. J. Parasitol. **35:**207–213.
149. **Langkjaer, R. B., H. Vigre, H. L. Enemark, and C. Maddox-Hyttel.** 2007. Molecular and phylogenetic characterization of *Cryptosporidium* and *Giardia* from pigs and cattle in Denmark. Parasitology **134:**339–350.
150. **Lasek-Nesselquist, E., A. L. Bogomolni, R. J. Gast, D. M. Welch, J. C. Ellis, M. L. Sogin, and M. J. Moore.** 2008. Molecular characterization of *Giardia intestinalis* haplotypes in marine animals: variation and zoonotic potential. Dis. Aquat. Organ. **81:**39–51.
151. **Lasek-Nesselquist, E., D. M. Welch, and M. L. Sogin.** 2010. The identification of a new *Giardia duodenalis* assemblage in marine vertebrates and a preliminary analysis of *G. duodenalis* population biology in marine systems. Int. J. Parasitol. **40:**1063–1074.
151a. **Lasek-Nesselquist, E., D. M. Welch, R. C. Thompson, R. F. Steuart, and M. L. Sogin.** 2009. Genetic exchange within and between assemblages of *Giardia duodenalis*. J. Eukaryot. Microbiol. **56:**504–518.
152. **Learmonth, J. J., G. Ionas, A. B. Pita, and R. S. Cowie.** 2003. Identification and genetic characterisation of *Giardia* and *Cryptosporidium* strains in humans and dairy cattle in the Waikato Region of New Zealand. Water Sci. Technol. **47:**21–26.
153. **Lebbad, M., J. Ankarklev, A. Tellez, B. Leiva, J. O. Andersson, and S. Svard.** 2008. Dominance of *Giardia* assemblage B in Leon, Nicaragua. Acta Trop. **106:**44–53.
154. **Lebbad, M., J. G. Mattsson, B. Christensson, B. Ljungstrom, A. Backhans, J. O. Andersson, and S. G. Svard.** 2010. From mouse to moose: multilocus genotyping of *Giardia* isolates from various animal species. Vet. Parasitol. **168:**231–239.
155. **Leonhard, S., K. Pfister, P. Beelitz, C. Wielinga, and R. C. Thompson.** 2007. The molecular characterisation of *Giardia* from dogs in southern Germany. Vet. Parasitol. **150:**33–38.
156. **Levecke, B., P. Geldhof, E. Claerebout, P. Dorny, F. Vercammen, S. M. Caccio, J. Vercruysse, and T. Geurden.** 2009. Molecular characterisation of *Giardia duodenalis* in captive non-human primates reveals mixed assemblage A and B infections and novel polymorphisms. Int. J. Parasitol. **39:**1595–1601.
157. **Lim, Y. A., S. D. Ramasame, M. A. Mahdy, W. Y. Sulaiman, and H. V. Smith.** 2009. Detection and molecular characterization of *Giardia* isolated from recreational lake water in Malaysia. Parasitol. Res. **106:**289–291.
158. **Little, S. E., E. M. Johnson, D. Lewis, R. P. Jaklitsch, M. E. Payton, B. L. Blagburn, D. D. Bowman, S. Moroff, T. Tams, L. Rich, and D. Aucoin.** 2009. Prevalence of intestinal parasites in pet dogs in the United States. Vet. Parasitol. **166:**144–152.
158a. **Liu, J., S. E. Lee, and K. H. Song.** 2008. Prevalence of canine giardiosis in South Korea. Res. Vet. Sci. **84:**416–418.
159. **Lobo, M. L., L. Xiao, F. Antunes, and O. Matos.** 2009. Occurrence of *Cryptosporidium* and *Giardia* genotypes and subtypes in raw and treated water in Portugal. Lett. Appl. Microbiol. **48:**732–737.
160. **Londono, A. L., S. Mejia, and J. E. Gomez-Marin.** 2009. Prevalence and risk factors associated with intestinal parasitism in preschool children from the urban area of Calarca, Colombia. Rev. Salud Publica (Bogota) **11:**72–81. (In Spanish.)
161. **Lopez, J., K. Abarca, P. Paredes, and E. Inzunza.** 2006. Intestinal parasites in dogs and cats with gastrointestinal symptoms in Santiago, Chile. Rev. Med. Chil. **134:**193–200. (In Spanish.)
162. **Lu, S., J. Wen, J. Li, and F. Wang.** 2002. DNA sequence analysis of the triose phosphate isomerase gene from isolates of *Giardia lamblia*. Chin. Med. J. (Engl.) **115:**99–102.
163. **Maddox-Hyttel, C., R. B. Langkjaer, H. L. Enemark, and H. Vigre.** 2006. *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs—occurrence and management associated risk factors. Vet. Parasitol. **141:**48–59.
164. **Mahdy, A. K., J. Surin, A. Mohd-Adnan, K. L. Wan, and Y. A. Lim.** 2009. Molecular characterization of *Giardia duodenalis* isolated from Semai Pahang Orang Asli (Peninsular Malaysia aborigines). Parasitology **136:**1237–1241.
165. **Mahdy, A. K. M., J. Surin, K. L. Wan, A. Mohd-Adnan, M. S. Al-Mekhlafi, and Y. A. Lim.** 2009. *Giardia intestinalis* genotypes: risk factors and correlation with clinical symptoms. Acta Trop. **112:**67–70.
166. **Maia, M. M., M. A. Fausto, E. L. Vieira, M. L. Benetton, and M. Carneiro.** 2009. Intestinal parasitic infection and associated risk factors, among children presenting at outpatient clinics in Manaus, Amazonas state, Brazil. Ann. Trop. Med. Parasitol. **103:**583–591.
167. **Manzardo, C., B. Trevino, J. Gomez i Prat, J. Cabezos, E. Mongui, I. Claveria, J. Luis Del Val, E. Zabaleta, F. Zarzuela, and R. Navarro.** 2008. Communicable diseases in the immigrant population attended to in a tropical medicine unit: epidemiological aspects and public health issues. Travel Med. Infect. Dis. **6:**4–11.
168. **Marangi, M., F. Berrilli, D. Otranto, and A. Giangaspero.** Genotyping of *Giardia duodenalis* among children and dogs in a closed socially deprived community from Italy. Zoonoses Public Health, in press.
169. **Martinez-Moreno, F. J., S. Hernandez, E. Lopez-Cobos, C. Becerra, I. Acosta, and A. Martinez-Moreno.** 2007. Estimation of canine intestinal parasites in Cordoba (Spain) and their risk to public health. Vet. Parasitol. **143:**7–13.
170. **Matsubayashi, M., I. Kimata, and N. Abe.** 2005. Identification of genotypes of *Giardia intestinalis* isolates from a human and calf in Japan. J. Vet. Med. Sci. **67:**337–340.
171. **McAllister, T. A., M. E. Olson, A. Fletch, M. Wetzstein, and T. Entz.** 2005. Prevalence of *Giardia* and *Cryptosporidium* in beef cows in southern Ontario and in beef calves in southern British Columbia. Can. Vet. J. **46:**47–55.
172. **Meireles, P., F. Montiani-Ferreira, and V. Thomaz-Soccol.** 2008. Survey of giardiosis in household and shelter dogs from metropolitan areas of Curitiba, Parana state, Southern Brazil. Vet. Parasitol. **152:**242–248.
173. **Mendonca, C., A. Almeida, A. Castro, M. de Lurdes Delgado, S. Soares, J. M. da Costa, and N. Canada.** 2007. Molecular characterization of *Cryptosporidium* and *Giardia* isolates from cattle from Portugal. Vet. Parasitol. **147:**47–50.
174. **Minvielle, M. C., N. B. Molina, D. Polverino, and J. A. Basualdo.** 2008. First

genotyping of *Giardia lamblia* from human and animal feces in Argentina, South America. Mem. Inst. Oswaldo Cruz **103:**98–103.
175. **Miro, G., M. Mateo, A. Montoya, E. Vela, and R. Calonge.** 2007. Survey of intestinal parasites in stray dogs in the Madrid area and comparison of the efficacy of three anthelmintics in naturally infected dogs. Parasitol. Res. **100:**317–320.
176. **Miska, K. B., M. Jenkins, J. Trout, M. Santin, and R. Fayer.** 2009. Detection and comparison of *Giardia* virus (GLV) from different assemblages of *Giardia duodenalis*. J. Parasitol. **95:**1197–1200.
177. **Mohsen, A., and H. Hossein.** 2009. Gastrointestinal parasites of stray cats in Kashan, Iran. Trop. Biomed. **26:**16–22.
178. **Monis, P. T., R. H. Andrews, G. Mayrhofer, and P. L. Ey.** 2003. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. Infect. Genet. Evol. **3:**29–38.
179. **Monis, P. T., R. H. Andrews, G. Mayrhofer, J. Mackrill, J. Kulda, J. L. Isaac-Renton, and P. L. Ey.** 1998. Novel lineages of *Giardia intestinalis* identified by genetic analysis of organisms isolated from dogs in Australia. Parasitology **116**(Pt. 1)**:**7–19.
180. **Monis, P. T., S. M. Caccio, and R. C. Thompson.** 2009. Variation in *Giardia*: towards a taxonomic revision of the genus. Trends Parasitol. **25:**93–100.
181. **Monis, P. T., and R. C. Thompson.** 2003. *Cryptosporidium* and *Giardia*-zoonoses: fact or fiction? Infect. Genet. Evol. **3:**233–244.
182. **Moriarty, E. M., L. W. Sinton, M. L. Mackenzie, N. Karki, and D. R. Wood.** 2008. A survey of enteric bacteria and protozoans in fresh bovine faeces on New Zealand dairy farms. J. Appl. Microbiol. **105:**2015–2025.
183. **Moro, D., M. A. Lawson, R. P. Hobbs, and R. C. Thompson.** 2003. Pathogens of house mice on arid Boullanger Island and subantarctic Macquarie Island, Australia. J. Wildl. Dis. **39:**762–771.
184. **Mukherjee, A. K., P. Chowdhury, M. K. Bhattacharya, M. Ghosh, K. Rajendran, and S. Ganguly.** 2009. Hospital-based surveillance of enteric parasites in Kolkata. BMC Res. Notes **2:**110.
185. **Mundim, M. J., L. A. Rosa, S. M. Hortencio, E. S. Faria, R. M. Rodrigues, and M. C. Cury.** 2007. Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in dogs from different living conditions in Uberlandia, Brazil. Vet. Parasitol. **144:**356–359.
186. **Nematian, J., A. Gholamrezanezhad, and E. Nematian.** 2008. Giardiasis and other intestinal parasitic infections in relation to anthropometric indicators of malnutrition: a large, population-based survey of schoolchildren in Tehran. Ann. Trop. Med. Parasitol. **102:**209–214.
187. **Ng, C. T., C. A. Gilchrist, A. Lane, S. Roy, R. Haque, and E. R. Houpt.** 2005. Multiplex real-time PCR assay using Scorpion probes and DNA capture for genotype-specific detection of *Giardia lamblia* on fecal samples. J. Clin. Microbiol. **43:**1256–1260.
188. **Nikolic, A., S. Dimitrijevic, S. Katic-Radivojevic, I. Klun, B. Bobrc, and O. Djurkovic-Djakovic.** 2008. High prevalence of intestinal zoonotic parasites in dogs from Belgrade, Serbia. Acta Vet. Hung. **56:**335–340.
189. **Nolan, M. J., A. R. Jex, A. Pangasa, N. D. Young, A. J. Campbell, M. Stevens, and R. B. Gasser.** 2010. Analysis of nucleotide variation within the triose-phosphate isomerase gene of *Giardia duodenalis* from sheep and its zoonotic implications. Electrophoresis **31:**287–298.
190. **O'Handley, R. M., C. Cockwill, M. Jelinski, T. A. McAllister, and M. E. Olson.** 2000. Effects of repeat fenbendazole treatment in dairy calves with giardiosis on cyst excretion, clinical signs and production. Vet. Parasitol. **89:**209–218.
191. **O'Handley, R. M., and M. E. Olson.** 2006. Giardiasis and cryptosporidiosis in ruminants. Vet. Clin. North Am. Food Anim. Pract. **22:**623–643.
192. **O'Handley, R. M., M. E. Olson, D. Fraser, P. Adams, and R. C. Thompson.** 2000. Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. Vet. Parasitol. **90:**193–200.
193. **Oksenhendler, E., L. Gerard, C. Fieschi, M. Malphettes, G. Mouillot, R. Jaussaud, J. F. Viallard, M. Gardembas, L. Galicier, N. Schleinitz, F. Suarez, P. Soulas-Sprauel, E. Hachulla, A. Jaccard, A. Gardeur, I. Theodorou, C. Rabian, and P. Debre.** 2008. Infections in 252 patients with common variable immunodeficiency. Clin. Infect. Dis. **46:**1547–1554.
194. **Olson, M. E., N. J. Leonard, and J. Strout.** 2010. Prevalence and diagnosis of *Giardia* infection in dogs and cats using a fecal antigen test and fecal smear. Can. Vet. J. **51:**640–642.
195. **Overgaauw, P. A., L. van Zutphen, D. Hoek, F. O. Yaya, J. Roelfsema, E. Pinelli, F. van Knapen, and L. M. Kortbeek.** 2009. Zoonotic parasites in fecal samples and fur from dogs and cats in the Netherlands. Vet. Parasitol. **163:**115–122.
196. **Palmer, C. S., R. J. Traub, I. D. Robertson, G. Devlin, R. Rees, and R. C. Thompson.** 2008. Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats. Vet. Parasitol. **154:**142–147.
197. **Paoletti, B., R. Iorio, G. Capelli, O. A. Sparagano, and A. Giangaspero.** 2008. Epidemiological scenario of giardiosis in dogs from central Italy. Ann. N. Y. Acad. Sci. **1149:**371–374.
197a. **Paoletti, B., D. Otranto, S. Weigl, A. Giangaspero, A. D. Cesare, and D. Traversa.** Prevalence and genetic characterization of *Giardia* and *Cryptosporidium* in cats from Italy. Res. Vet. Sci., in press.
198. **Papazahariadou, M., A. Founta, E. Papadopoulos, S. Chliounakis, K. Antoniadou-Sotiriadou, and Y. Theodorides.** 2007. Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece. Vet. Parasitol. **148:**170–173.
199. **Papini, R., G. Cardini, B. Paoletti, and A. Giangaspero.** 2007. Detection of *Giardia* assemblage A in cats in Florence, Italy. Parasitol. Res. **100:**653–656.
200. **Papini, R., G. Giuliani, G. Gorini, and G. Cardini.** 2007. Survey of feline giardiasis by ELISA test in Italy. Vet. Res. Commun. **31:**297–303.
201. **Paziewska, A., M. Bednarska, H. Nieweglowski, G. Karbowiak, and A. Bajer.** 2007. Distribution of *Cryptosporidium* and *Giardia* spp. in selected species of protected and game mammals from North-Eastern Poland. Ann. Agric. Environ. Med. **14:**265–270.
202. **Pelayo, L., F. A. Nunez, L. Rojas, E. F. Hansen, B. Gjerde, H. Wilke, B. Mulder, and L. Robertson.** 2008. *Giardia* infections in Cuban children: the genotypes circulating in a rural population. Ann. Trop. Med. Parasitol. **102:**585–595.
203. **Perez Cordon, G., O. Cordova Paz Soldan, F. Vargas Vasquez, J. R. Velasco Soto, L. Sempere Bordes, M. Sanchez Moreno, and M. J. Rosales.** 2008. Prevalence of enteroparasites and genotyping of *Giardia lamblia* in Peruvian children. Parasitol. Res. **103:**459–465.
204. **Plutzer, J., P. Karanis, K. Domokos, A. Torokne, and K. Marialigeti.** 2008. Detection and characterisation of *Giardia* and *Cryptosporidium* in Hungarian raw, surface and sewage water samples by IFT, PCR and sequence analysis of the SSUrRNA and GDH genes. Int. J. Hyg. Environ. Health **211:**524–533.
205. **Pollock, K. G., H. V. Smith, D. Young, C. N. Ramsay, and W. J. Reilly.** 2005. *Giardia* surveillance in Scotland, 1988-2003. Eur. J. Clin. Microbiol. Infect. Dis. **24:**571–573.
206. **Ponce-Macotela, M., M. N. Martinez-Gordillo, R. M. Bermudez-Cruz, P. M. Salazar-Schettino, G. Ortega-Pierres, and P. L. Ey.** 2002. Unusual prevalence of the *Giardia intestinalis* A-II subtype amongst isolates from humans and domestic animals in Mexico. Int. J. Parasitol. **32:**1201–1202.
207. **Poxleitner, M. K., M. L. Carpenter, J. J. Mancuso, C. J. Wang, S. C. Dawson, and W. Z. Cande.** 2008. Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. Science **319:**1530–1533.
208. **Prado, M. S., S. Cairncross, A. Strina, M. L. Barreto, A. M. Oliveira-Assis, and S. Rego.** 2005. Asymptomatic giardiasis and growth in young children; a longitudinal study in Salvador, Brazil. Parasitology **131:**51–56.
209. **Quihui, L., G. G. Morales, R. O. Mendez, J. G. Leyva, J. E. Romero, and M. E. Valencia.** 2010. Could giardiasis be a risk factor for low zinc status in schoolchildren from northwestern Mexico? A cross-sectional study with longitudinal follow-up. BMC Public Health **10:**85–91.
210. **Ralston, B. J., T. A. McAllister, and M. E. Olson.** 2003. Prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in range beef calves and their dams. Vet. Parasitol. **114:**113–122.
211. **Ramesh, M. A., S. B. Malik, and J. M. Logsdon, Jr.** 2005. A phylogenomic inventory of meiotic genes; evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. Curr. Biol. **15:**185–191.
212. **Ratanapo, S., M. Mungthin, S. Soontrapa, C. Faithed, S. Siripattanapipong, R. Rangsin, T. Naaglor, P. Piyaraj, P. Taamasri, and S. Leelayoova.** 2008. Multiple modes of transmission of giardiasis in primary schoolchildren of a rural community, Thailand. Am. J. Trop. Med. Hyg. **78:**611–615.
213. **Read, C., J. Walters, I. D. Robertson, and R. C. Thompson.** 2002. Correlation between genotype of *Giardia duodenalis* and diarrhoea. Int. J. Parasitol. **32:**229–231.
214. **Read, C. M., P. T. Monis, and R. C. Thompson.** 2004. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect. Genet. Evol. **4:**125–130.
215. **Rimhanen-Finne, R., H. L. Enemark, J. Kolehmainen, P. Toropainen, and M. L. Hanninen.** 2007. Evaluation of immunofluorescence microscopy and enzyme-linked immunosorbent assay in detection of *Cryptosporidium* and *Giardia* infections in asymptomatic dogs. Vet. Parasitol. **145:**345–348.
216. **Rinaldi, L., M. P. Maurelli, V. Musella, V. Veneziano, S. Carbone, A. Di Sarno, M. Paone, and G. Cringoli.** 2008. *Giardia* and *Cryptosporidium* in canine faecal samples contaminating an urban area. Res. Vet. Sci. **84:**413–415.
217. **Robertson, L. J., T. Forberg, and B. K. Gjerde.** 2008. *Giardia* cysts in sewage influent in Bergen, Norway 15-23 months after an extensive waterborne outbreak of giardiasis. J. Appl. Microbiol. **104:**1147–1152.
218. **Robertson, L. J., T. Forberg, L. Hermansen, B. K. Gjerde, and N. Langeland.** 2007. Molecular characterisation of *Giardia* isolates from clinical infections following a waterborne outbreak. J. Infect. **55:**79–88.
219. **Robertson, L. J., T. Forberg, L. Hermansen, I. S. Hamnes, and B. Gjerde.** 2007. *Giardia duodenalis* cysts isolated from wild moose and reindeer in Norway: genetic characterization by PCR-RFLP and sequence analysis at two genes. J. Wildl. Dis. **43:**576–585.
220. **Robertson, L. J., B. K. Gjerde, and E. F. Hansen.** 2010. The zoonotic potential of *Giardia* and *Cryptosporidium* in Norwegian sheep: a longitudinal investigation of 6 flocks of lambs. Vet. Parasitol. **171:**140–145.
221. **Robertson, L. J., K. Hanevik, A. A. Escobedo, K. Morch, and N. Langeland.** 2010. Giardiasis—why do the symptoms sometimes never stop? Trends Parasitol. **26:**75–81.
222. **Robertson, L. J., L. Hermansen, and B. K. Gjerde.** 2006. Occurrence of

Cryptosporidium oocysts and *Giardia* cysts in sewage in Norway. Appl. Environ. Microbiol. **72:**5297–5303.

223. **Robertson, L. J., L. Hermansen, B. K. Gjerde, E. Strand, J. O. Alvsvag, and N. Langeland.** 2006. Application of genotyping during an extensive outbreak of waterborne giardiasis in Bergen, Norway, during autumn and winter 2004. Appl. Environ. Microbiol. **72:**2212–2217.
224. **Rodriguez-Garcia, R., L. M. Rodriguez-Guzman, M. I. Sanchez-Maldonado, A. Gomez-Delgado, and R. Rivera-Cedillo.** 2002. Prevalence and risk factors associated with intestinal parasitoses in pregnant women and their relation to the infant's birth weight. Ginecol. Obstet. Mex. **70:**338–343. (In Spanish.)
225. **Ruiz, A., P. Foronda, J. F. Gonzalez, A. Guedes, N. Abreu-Acosta, J. M. Molina, and B. Valladares.** 2008. Occurrence and genotype characterization of *Giardia duodenalis* in goat kids from the Canary Islands, Spain. Vet. Parasitol. **154:**137–141.
226. **Ryan, U. M., C. Bath, I. Robertson, C. Read, A. Elliot, L. McInnes, R. Traub, and B. Besier.** 2005. Sheep may not be an important zoonotic reservoir for *Cryptosporidium* and *Giardia* parasites. Appl. Environ. Microbiol. **71:**4992–4997.
227. **Sagebiel, D., T. Weitzel, K. Stark, and K. Leitmeyer.** 2009. Giardiasis in kindergartens: prevalence study in Berlin, Germany, 2006. Parasitol. Res. **105:**681–687.
228. **Sahagun, J., A. Clavel, P. Goni, C. Seral, M. T. Llorente, F. J. Castillo, S. Capilla, A. Arias, and R. Gomez-Lus.** 2008. Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. Eur. J. Clin. Microbiol. Infect. Dis. **27:**81–83.
229. **Samie, A., R. L. Guerrant, L. Barrett, P. O. Bessong, E. O. Igumbor, and C. L. Obi.** 2009. Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarroeal human stools from Vhembe district, South Africa. J. Health Popul. Nutr. **27:**739–745.
230. **Sanchez-Vega, J. T., J. Tay-Zavala, A. Aguilar-Chiu, D. Ruiz-Sanchez, F. Malagon, J. A. Rodriguez-Covarrubias, J. Ordonez-Martinez, and L. Calderon-Romero.** 2006. Cryptosporidiosis and other intestinal protozoan infections in children less than one year of age in Mexico City. Am. J. Trop. Med. Hyg. **75:**1095–1098.
231. **Santin, M., J. M. Trout, and R. Fayer.** 2009. A longitudinal study of *Giardia duodenalis* genotypes in dairy cows from birth to 2 years of age. Vet. Parasitol. **162:**40–45.
232. **Santin, M., J. M. Trout, and R. Fayer.** 2007. Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* species and genotypes in sheep in Maryland. Vet. Parasitol. **146:**17–24.
233. **Santin, M., J. M. Trout, J. A. Vecino, J. P. Dubey, and R. Fayer.** 2006. *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in cats from Bogota (Colombia) and genotyping of isolates. Vet. Parasitol. **141:**334–339.
234. **Santos, S. A., and L. S. Merlini.** 2010. Prevalence of enteroparasitosis in the population of Maria Helena, Parana State. Cien. Saude Colet. **15:**899–905. (In Portugese.)
235. **Scaramozzino, P., D. Di Cave, F. Berrilli, C. D'Orazi, A. Spaziani, S. Mazzanti, F. Scholl, and C. De Liberato.** 2009. A study of the prevalence and genotypes of *Giardia duodenalis* infecting kennelled dogs. Vet. J. **182:** 231–234.

235a. **Shoorijeh, S. J., S. M. Sadjjadi, A. Asheri, and K. Eraghi.** 2008. *Giardia* spp. and *Sarcocystis* spp. status in pet dogs of Shiraz, Southern part of Iran. Trop. Biomed. **25:**154–159.

236. **Shukla, R., P. Giraldo, A. Kraliz, M. Finnigan, and A. L. Sanchez.** 2006. *Cryptosporidium* spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario. Can. Vet. J. **47:**1179–1184.
237. **Silva, R. R., C. A. da Silva, C. A. de Jesus Pereira, R. L. de Carvalho Nicolato, D. Negrao-Correa, J. A. Lamounier, and M. Carneiro.** 2009. Association between nutritional status, environmental and socio-economic factors and *Giardia lamblia* infections among children aged 6-71 months in Brazil. Trans. R. Soc. Trop. Med. Hyg. **103:**512–519.
238. **Simsek, Z., F. Y. Zeyrek, and M. A. Kurcer.** 2004. Effect of *Giardia* infection on growth and psychomotor development of children aged 0-5 years. J. Trop. Pediatr. **50:**90–93.

238a. **Singh, A., L. Janaki, W. A. Petri, Jr., and E. R. Houpt.** 2009. *Giardia intestinalis* assemblages A and B infections in Nepal. Am. J. Trop. Med. Hyg. **81:**538–539.

239. **Siwila, J., I. G. Phiri, H. L. Enemark, M. Nchito, and A. Olsen.** 2010. Intestinal helminths and protozoa in children in pre-schools in Kafue district, Zambia. Trans. R. Soc. Trop. Med. Hyg. **104:**122–128.
240. **Smith, H. V., S. M. Caccio, A. Tait, J. McLauchlin, and R. C. Thompson.** 2006. Tools for investigating the environmental transmission of *Cryptosporidium* and *Giardia* infections in humans. Trends Parasitol. **22:**160–167.
241. **Snel, S. J., M. G. Baker, and K. Venugopal.** 2009. The epidemiology of giardiasis in New Zealand, 1997-2006. N. Z. Med. J. **122:**62–75.
242. **Solarczyk, P., and A. C. Majewska.** 2010. A survey of the prevalence and genotypes of *Giardia duodenalis* infecting household and sheltered dogs. Parasitol. Res. **106:**1015–1019.
243. **Solarczyk, P., A. Werner, and A. C. Majewska.** 2010. Genotype analysis of *Giardia duodenalis* isolates obtained from humans in west-central Poland. Wiad. Parazytol. **56:**171–177. (In Polish.)
244. **Soriano, S. V., N. B. Pierangeli, I. Roccia, H. F. Bergagna, L. E. Lazzarini, A. Celescinco, M. S. Saiz, A. Kossman, P. A. Contreras, C. Arias, and J. A. Basualdo.** 2010. A wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquen, Patagonia, Argentina. Vet. Parasitol. **167:**81–85.
245. **Sousa, M. C., J. B. Morais, J. E. Machado, and J. Poiares-da-Silva.** 2006. Genotyping of *Giardia lamblia* human isolates from Portugal by PCR-RFLP and sequencing. J. Eukaryot. Microbiol. **53**(Suppl. 1)**:**S174–S176.
246. **Souza, S. L., S. M. Gennari, L. J. Richtzenhain, H. F. Pena, M. R. Funada, A. Cortez, F. Gregori, and R. M. Soares.** 2007. Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of Sao Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene. Vet. Parasitol. **149:**258–264.
247. **Sprong, H., S. M. Caccio, and J. W. van der Giessen.** 2009. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl. Trop. Dis. **3:**e558.
248. **Stuart, J. M., H. J. Orr, F. G. Warburton, S. Jeyakanth, C. Pugh, I. Morris, J. Sarangi, and G. Nichols.** 2003. Risk factors for sporadic giardiasis: a case-control study in southwestern England. Emerg. Infect. Dis. **9:**229–233.
249. **Sulaiman, I. M., R. Fayer, C. Bern, R. H. Gilman, J. M. Trout, P. M. Schantz, P. Das, A. A. Lal, and L. Xiao.** 2003. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. **9:**1444–1452.
250. **Sulaiman, I. M., J. Jiang, A. Singh, and L. Xiao.** 2004. Distribution of *Giardia duodenalis* genotypes and subgenotypes in raw urban wastewater in Milwaukee, Wisconsin. Appl. Environ. Microbiol. **70:**3776–3780.

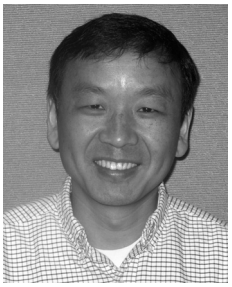
250a. **Suzuki, J., R. Murata, S. Kobayashi, K. Sadamasu, A. Kai, and T. Takeuchi.** Risk of human infection with *Giardia duodenalis* from cats in Japan and genotyping of the isolates to assess the route of infection in cats. Parasitology, in press.

251. **Szenasi, Z., S. Marton, I. Kucsera, B. Tanczos, K. Horvath, E. Orosz, Z. Lucacs, and Z. Szeidemann.** 2007. Preliminary investigation of the prevalence and genotype distribution of *Giardia intestinalis* in dogs in Hungary. Parasitol. Res. **101:**S145–S152.
252. **Tashima, N. T., M. J. Simoes, C. Q. Leite, A. Fluminhan, M. A. Nogueira, and A. C. Malaspina.** 2009. Classic and molecular study of *Giardia duodenalis* in children from a daycare center in the region of Presidente Prudente, Sao Paulo, Brazil. Rev. Inst. Med. Trop. Sao Paulo **51:**19–24.
253. **Teixeira, J. C., L. Heller, and M. L. Barreto.** 2007. *Giardia duodenalis* infection: risk factors for children living in sub-standard settlements in Brazil. Cad. Saude Publica **23:**1489–1493. (In Portugese.)
254. **Teodorovic, S., J. M. Braverman, and H. G. Elmendorf.** 2007. Unusually low levels of genetic variation among *Giardia lamblia* isolates. Eukaryot. Cell **6:**1421–1430.
255. **Thompson, J., R. Yang, M. Power, J. Hufschmid, I. Beveridge, S. Reid, J. Ng, A. Armson, and U. Ryan.** 2008. Identification of zoonotic *Giardia* genotypes in marsupials in Australia. Exp. Parasitol. **120:**88–93.
256. **Thompson, R. C.** 2000. Giardiasis as a re-emerging infectious disease and its zoonotic potential. Int. J. Parasitol. **30:**1259–1267.
257. **Thompson, R. C.** 2004. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. Vet. Parasitol. **126:**15–35.
258. **Thompson, R. C., D. D. Colwell, T. Shury, A. J. Appelbee, C. Read, Z. Njiru, and M. E. Olson.** 2009. The molecular epidemiology of *Cryptosporidium* and *Giardia* infections in coyotes from Alberta, Canada, and observations on some cohabiting parasites. Vet. Parasitol. **159:**167–170.
259. **Thompson, R. C., R. M. Hopkins, and W. L. Homan.** 2000. Nomenclature and genetic groupings of *Giardia* infecting mammals. Parasitol. Today **16:** 210–213.
260. **Thompson, R. C., and P. T. Monis.** 2004. Variation in *Giardia*: implications for taxonomy and epidemiology. Adv. Parasitol. **58:**69–137.
261. **Thompson, R. C., C. S. Palmer, and R. O'Handley.** 2008. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. Vet. J. **177:**18–25.
262. **Thompson, R. C., A. Smith, A. J. Lymbery, S. Averis, K. D. Morris, and A. F. Wayne.** 2010. *Giardia* in Western Australian wildlife. Vet. Parasitol. **170:**207–211.
263. **Traub, R., S. Wade, C. Read, A. Thompson, and H. Mohammed.** 2005. Molecular characterization of potentially zoonotic isolates of *Giardia duodenalis* in horses. Vet. Parasitol. **130:**317–321.
264. **Traub, R. J., T. Inpankaew, S. A. Reid, C. Sutthikornchai, Y. Sukthana, I. D. Robertson, and R. C. Thompson.** 2009. Transmission cycles of *Giardia duodenalis* in dogs and humans in temple communities in Bangkok—a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop. **111:**125–132.
265. **Traub, R. J., P. T. Monis, I. Robertson, P. Irwin, N. Mencke, and R. C. Thompson.** 2004. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology **128:**253–262.
266. **Traub, R. J., I. D. Robertson, P. Irwin, N. Mencke, P. Monis, and R. C. Thompson.** 2003. Humans, dogs and parasitic zoonoses—unravelling the

relationships in a remote endemic community in northeast India using molecular tools. Parasitol. Res. **90**(Suppl. 3)**:**S156–S157.
267. **Trout, J. M., M. Santin, and R. Fayer.** 2008. Detection of assemblage A, *Giardia duodenalis* and *Eimeria* spp. in alpacas on two Maryland farms. Vet. Parasitol. **153:**203–208.
268. **Trout, J. M., M. Santin, and R. Fayer.** 2006. *Giardia* and *Cryptosporidium* species and genotypes in coyotes (*Canis latrans*). J. Zoo Wildl. Med. **37:** 141–144.
269. **Trout, J. M., M. Santin, and R. Fayer.** 2003. Identification of assemblage A *Giardia* in white-tailed deer. J. Parasitol. **89:**1254–1255.
270. **Trout, J. M., M. Santin, and R. Fayer.** 2007. Prevalence of *Giardia duodenalis* genotypes in adult dairy cows. Vet. Parasitol. **147:**205–209.
271. **Trout, J. M., M. Santin, E. Greiner, and R. Fayer.** 2005. Prevalence and genotypes of *Giardia duodenalis* in post-weaned dairy calves. Vet. Parasitol. **130:**177–183.
272. **Trout, J. M., M. Santin, E. Greiner, and R. Fayer.** 2004. Prevalence of *Giardia duodenalis* genotypes in pre-weaned dairy calves. Vet. Parasitol. **124:**179–186.
273. **Trout, J. M., M. Santin, E. C. Greiner, and R. Fayer.** 2006. Prevalence and genotypes of *Giardia duodenalis* in 1-2 year old dairy cattle. Vet. Parasitol. **140:**217–222.
274. **Tungtrongchitr, A., N. Sookrung, N. Indrawattana, S. Kwangsi, J. Ongrotchanakun, and W. Chaicumpa.** 2010. *Giardia intestinalis* in Thailand: identification of genotypes. J. Health Popul. Nutr. **28:**42–52.
275. **Uehlinger, F. D., H. W. Barkema, B. R. Dixon, T. Coklin, and R. M. O'Handley.** 2006. *Giardia duodenalis* and *Cryptosporidium* spp. in a veterinary college bovine teaching herd. Vet. Parasitol. **142:**231–237.
276. **Upcroft, J. A., K. G. Krauer, A. G. Burgess, L. A. Dunn, N. Chen, and P. Upcroft.** 2009. Sequence map of the 3-Mb *Giardia duodenalis* assemblage A chromosome. Chromosome Res. **17:**1001–1014.
277. **Upcroft, J. A., and P. Upcroft.** 1993. Drug resistance and *Giardia*. Parasitol. Today **9:**187–190.
278. **Upjohn, M., C. Cobb, J. Monger, T. Geurden, E. Claerebout, and M. Fox.** 2010. Prevalence, molecular typing and risk factor analysis for *Giardia duodenalis* infections in dogs in a central London rescue shelter. Vet. Parasitol. **172:**341–346.
279. **Upton, S. J., and C. A. Zien.** 1997. Description of a *Giardia varani*-like flagellate from a water monitor, Varanus salvator, from Malaysia. J. Parasitol. **83:**970–971.
280. **Usluca, S., T. Inceboz, L. Over, S. Tuncay, G. Yalcin, S. S. Arcak, S. Ozkoc, U. Aksoy, and C. Akisu.** 2010. The distribution of intestinal parasites detected in the Dokuz Eylul University Medical Faculty Hospital between 2005 and 2008. Turkiye Parazitol. Derg. **34:**27–31. (In Turkish.)
281. **Utzinger, J., S. Botero-Kleiven, F. Castelli, P. L. Chiodini, H. Edwards, N. Kohler, M. Gulletta, M. Lebbad, M. Manser, B. Matthys, K. N′Goran, E. Tannich, P. Vounatsou, and H. Marti.** 2010. Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories. Clin. Microbiol. Infect. **16:**267–273.
282. **van der Giessen, J. W., A. de Vries, M. Roos, P. Wielinga, L. M. Kortbeek, and T. G. Mank.** 2006. Genotyping of *Giardia* in Dutch patients and animals: a phylogenetic analysis of human and animal isolates. Int. J. Parasitol. **36:**849–858.
283. **van Keulen, H., P. T. Macechko, S. Wade, S. Schaaf, P. M. Wallis, and S. L. Erlandsen.** 2002. Presence of human *Giardia* in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for giardiasis. Vet. Parasitol. **108:**97–107.
284. **Vasilopulos, R. J., L. G. Rickard, A. J. Mackin, G. T. Pharr, and C. L. Huston.** 2007. Genotypic analysis of *Giardia duodenalis* in domestic cats. J. Vet. Intern. Med. **21:**352–355.
285. **Veronesi, F., F. Passamonti, S. Caccio, M. Diaferia, and D. P. Fioretti.** 2010. Epidemiological survey on equine *Cryptosporidium* and *Giardia* infections in Italy and molecular characterization of isolates. Zoonoses Public Health **57:**510–517.
286. **Vijgen, S., M. Mangen, L. Kortbeek, Y. van Duijnhoven, and A. Havelaar.** 2007. Disease burden and related costs of cryptosporidiosis and giardiasis in the Netherlands. RIVM report 330081001/2007. RIVM, Bilthoven, Netherlands.
287. **Volotao, A. C., L. M. Costa-Macedo, F. S. Haddad, A. Brandao, J. M. Peralta, and O. Fernandes.** 2007. Genotyping of *Giardia duodenalis* from human and animal samples from Brazil using beta-giardin gene: a phylogenetic analysis. Acta Trop. **102:**10–19.
288. **Volotao, A. C., J. C. Souza, Jr., C. Grassini, J. M. Peralta, and O. Fernandes.** 2008. Genotyping of *Giardia duodenalis* from Southern brown howler monkeys (*Alouatta clamitans*) from Brazil. Vet. Parasitol. **158:**133–137.
289. **Wang, R., X. Zhang, H. Zhu, L. Zhang, Y. Feng, F. Jian, C. Ning, M. Qi, Y. Zhou, K. Fu, Y. Wang, Y. Sun, Q. Wang, and L. Xiao.** Genetic characterizations of *Cryptosporidium* spp. and *Giardia duodenalis* in humans in Henan, China. Exp. Parasitol., in press.
290. **Wang, Z. Q., and J. Cui.** 2005. Epidemiology of giardiasis. Foreign Med. Sci. Parasit. Dis. **32:**99–105.
291. **Weiss, J. B., H. van Keulen, and T. E. Nash.** 1992. Classification of subgroups of *Giardia lamblia* based upon ribosomal RNA gene sequence using the polymerase chain reaction. Mol. Biochem. Parasitol. **54:**73–86.
292. **Welch, T. P.** 2000. Risk of giardiasis from consumption of wilderness water in North America: a systematic review of epidemiologic data. Int. J. Infect. Dis. **4:**100–103.
293. **Wielinga, C. M., and R. C. Thompson.** 2007. Comparative evaluation of *Giardia duodenalis* sequence data. Parasitology **134:**1795–1821.
294. **Winkworth, C. L., J. J. Learmonth, C. D. Matthaei, and C. R. Townsend.** 2008. Molecular characterization of *Giardia* isolates from calves and humans in a region in which dairy farming has recently intensified. Appl. Environ. Microbiol. **74:**5100–5105.
295. **Winkworth, C. L., C. D. Matthaei, and C. R. Townsend.** 2008. Prevalence of *Giardia* and *Cryptosporidium* spp in calves from a region in New Zealand experiencing intensification of dairying. N. Z. Vet. J. **56:**15–20.
296. **Xiao, L., and R. Fayer.** 2008. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int. J. Parasitol. **38:**1239–1255.
297. **Yamamoto, N., M. Kon, T. Saito, N. Maeno, M. Koyama, K. Sunaoshi, M. Yamaguchi, Y. Morishima, and M. Kawanaka.** 2009. Prevalence of intestinal canine and feline parasites in Saitama Prefecture, Japan. Kansenshogaku Zasshi **83:**223–228. (In Japanese.)
298. **Yang, R., C. Jacobson, C. Gordon, and U. Ryan.** 2009. Prevalence and molecular characterisation of *Cryptosporidium* and *Giardia* species in preweaned sheep in Australia. Vet. Parasitol. **161:**19–24.
299. **Yang, R., J. Lee, J. Ng, and U. Ryan.** 2009. High prevalence *Giardia duodenalis* assemblage B and potentially zoonotic subtypes in sporadic human cases in Western Australia. Int. J. Parasitol. **40:**293–297.
300. **Yang, R., A. Reid, A. Lymbery, and U. Ryan.** 2009. Identification of zoonotic *Giardia* genotypes in fish. Int. J. Parasitol. **40:**779–785.
301. **Yason, J. A., and W. L. Rivera.** 2007. Genotyping of *Giardia duodenalis* isolates among residents of slum area in Manila, Philippines. Parasitol. Res. **101:**681–687.
302. **Yoder, J. S., C. Harral, and M. J. Beach.** 2010. Giardiasis surveillance—United States, 2006-2008. MMWR Surveill. Summ. **59:**15–25.
303. **Yong, T., K. Han, H. Yang, and S. Park.** 2002. PCR-RFLP analysis of *Giardia intestinalis* using a *Giardia*-specific gene, GLORF-C4. Parasite **9:**65–70.
304. **Yong, T. S., S. J. Park, U. W. Hwang, H. W. Yang, K. W. Lee, D. Y. Min, H. J. Rim, Y. Wang, and F. Zheng.** 2000. Genotyping of *Giardia lamblia* isolates from humans in China and Korea using ribosomal DNA sequences. J. Parasitol. **86:**887–891.

Continued next page

Yaoyu Feng obtained her B.S. and M.S. at Nankai University, China. After working at Tianjin University, China, as a lecturer for 6 years, she obtained her Ph.D. degree in 1999. She received postdoctoral training and worked as a research fellow at the National University of Singapore. During her work at Tongji University, China, as a professor between 2004 and 2007, she was a guest researcher at the Centers for Disease Control and Prevention as an International EID Fellow for 1 year. She is currently a professor at the East China University of Science and Technology, China. Dr. Feng's earlier research interest was mainly on applied microbiology. Since 2003, she has been working on the molecular epidemiology, pathogenesis, transmission, and environmental ecology of waterborne and food-borne pathogens, such as *Cryptosporidium*, *Giardia*, and microsporidia. Dr. Feng has published over 50 original papers, invited reviews, and book chapters.



Lihua Xiao received his veterinary education in China. After his M.S. in veterinary parasitology and 2 years of teaching at the Northeast Agricultural University in China, he did his Ph.D. work in veterinary parasitology at the University of Maine and received postdoctoral training at The Ohio State University College of Veterinary Medicine. In 1993, he moved to the Centers for Disease Control and Prevention, first as a guest researcher and then a senior staff scientist. Dr. Xiao's earlier research interests were in the epidemiology and pathogenesis of gastrointestinal nematodes, cryptosporidiosis and giardiasis of farm animals, immunopathogenesis of and vaccine development for malaria, and immunopathogenesis of HIV. For the last 15 years, he has largely focused on the taxonomy, molecular epidemiology, and environmental biology of *Cryptosporidium*, *Giardia*, microsporidia, and other zoonotic protists in humans and animals. Dr. Xiao has published over 200 original papers, invited reviews, and book chapters.