Comparison of methods of sampling for *Toxocara* species and fecal coliforms in an outdoor day care environment

Hélène Carabin DVM MSc, Theresa W Gyorkos PhD, Evelyne Kokoskin ART MSc, Pierre Payment PhD, Lawrence Joseph PhD, Julio Soto MD PhD

H Carabin, TW Gyorkos, E Kokoskin, P Payment, L Joseph, J Soto. Comparison of methods of sampling for *Toxocara* species and fecal coliforms in an outdoor day care environment. Can J Infect Dis 1998;9(3):149-156.

OBJECTIVE: To compare three sampling methods and to pretest methods for the determination of fecal coliform (FC) counts and *Toxocara* species from sand in the day care outdoor environment.
DESIGN: The sand samples were obtained from the play area and the sandbox of a day care centre and examined for the presence of FC and *Toxocara* species, the common roundworm of dogs and cats. The sampling methods included random selection and two types of judgement methods. The latter included one method where domestic animals were judged to be likely to defecate and the other where children would be likely to be playing. In addition, to obtain a global estimate of contamination, the entire areas of both the sandbox and the play area were sampled on the last day.
SETTING: Outdoor day care environment.
MAIN RESULTS: The most representative levels of bacterial contamination and *Toxocara* species originated from the combined sample of the entire surface areas rather than from any separate random or judgement method of sampling. FCs were found in all sampled areas of the sandbox (median 910 FCs/g of sand) and of the play area (median 350 FCs/g of sand). *Toxocara* species were recovered from a number of areas in both the sandbox and the play area.
CONCLUSIONS: Research on environmental microbial contamination of outdoor day care settings would benefit from the application of standardized and validated sampling and laboratory methods.

Key Words: *Contamination, Day care centre, Environment, Fecal coliforms, Methodology,* Toxocara *species*

Comparaison des méthodes d'échantillonnage des espèces de *Toxocara* et des coliformes fécaux prélevés sur un terrain de jeu d'une garderie

OBJECTIF : Comparer trois méthodes d'échantillonnage et prétester des méthodes pour déterminer le nombre de coliformes fécaux et rechercher les espèces de *Toxocara* dans le sable d'une cour de garderie.
MODÈLE : Les échantillons de sable ont été prélevés dans le bac à sable et sur le terrain de jeu d'une garderie et analysés pour une recherche de coliformes fécaux (CF) et des espèces de *Toxocara*, le nématode commun des chiens et des chats. Les méthodes d'échantillonnage comprenaient une sélection au hasard et deux types d'échantillonnage au jugé, dont une estimant l'endroit où les animaux domestiques iraient probablement déféquer, et l'autre estimant l'endroit où les enfants iraient probablement jouer. De plus, pour obtenir une estimation globale de la contamination, des échantillons ont été prélevés dans tout le bac à sable et dans toutes les aires de jeu le dernier jour.
CONTEXTE : Terrain de jeu d'une garderie.
PRINCIPAUX RÉSULTATS : Les niveaux les plus représentatifs de contamination bactérienne et de contamination par les espèces de *Toxocara* provenaient de l'échantillon combiné prélevé sur les surfaces entières plutôt que d'une

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Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec
Correspondence and reprints: Dr Hélène Carabin, Division of Clinical Epidemiology, Montreal General Hospital, Room L10 321.1, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4. Telephone 514-937-6011 ext 4729, fax 514-934-8293, e-mail hcarab@po-box.mcgill.ca
Received for publication March 19, 1997. Accepted June 11, 1997

quelconque méthode d'échantillonnage distincte par sélection au hasard ou au jugé. On a décelé des CF dans toute les aires échantillonnées du bac à sable (médiane de 910 CF/g de sable) et des aires de jeux (médiane de 350 CF/g de sable). On a prélevé les espèces de *Toxocara* dans plusieurs endroits du bac à sable et du terrain de jeu.
CONCLUSIONS : La recherche sur la contamination microbienne environnementale touchant les terrains de jeu et les installations extérieures des garderies bénéficierait de l'application de méthodes d'échantillonnage et de laboratoire validées et normalisées.

It is recognized that children who attend day care centres (DCCs) have a higher incidence of infectious diseases than children who do not attend DCCs (1-4). In particular, toddlers are considered to be the group at most elevated risk because their hygiene skills are not yet fully developed, they are in the 'oral' stage of their development and they are increasingly mobile (4-6). Black et al (7) have shown that children under three years of age put their hands or other objects into their mouths every 2 to 3 mins. This normal behaviour sometimes results in exposure to environmental contamination. It has been shown that fecal coliform (FC) contamination present in the indoor day care environment accounts for almost one-third of diarrhea in toddlers (5). These observations highlight the need to understand where and when microbial contamination is highest in the environment surrounding young children in order to initiate appropriate measures of prevention and control. Unfortunately, much remains unknown about the sources of microbial contamination, especially in the outdoor environment.

To date, only two studies have assessed sand- or soil-associated microorganism contamination in the outdoor environment of DCCs (ie, sandboxes and play areas). These studies, carried out in Canada (8) and in France (9), used the presence of *Toxocara* species as an indicator of domestic animal fecal contamination because they are a zoonosis and, therefore, of public health concern, and because they specifically represent contamination from domestic animal sources (dogs and cats). Both studies showed that this parasite is present in outdoor DCC play areas. Seasonality of contamination may occur, but in sandboxes of three nursery schools in Marseille (France), *Toxocara* species ova were recovered throughout the year (9). Animal feces can also contain viral (eg, rotavirus) and bacterial microorganisms (eg, *Escherichia coli*) (10). These microorganisms can remain viable in the environment for some time, especially in fecal matter (11). FC contamination has been reported in sandboxes in parks of the Angers region of France (12) and in lawns and sandboxes of parks in Poland (13). Birds may also be a potential source of contamination because they shed microorganisms in their droppings that can be infectious to humans (10). In Canada, one-third of seagulls in the Montreal area were shown to carry *Salmonella* species, *Listeria monocytogenes* and *Campylobacter* species in their cloacae (14). Transmission of these microorganisms to young children via the outdoor environment is thus possible, but the magnitude of risk remains unknown.

Guidelines regarding the prevention and control of contamination of sand and toys in outdoor DCC playgrounds have been established by public health authorities in Quebec (15), in Canada (16) and in the United States (17). However, these guidelines vary from one authority to another. Moreover, their efficacy and effectiveness have not been evaluated.

Numerous studies have assessed the presence of *Toxocara* species in sandpits, sandboxes and soil in public parks, kindergardens, schools, gardens and backyards. A comprehensive list of the results of these studies is shown in Table 1. The great variation in results is immediately apparent and highlights several issues. First, identification of *Toxocara* species from the outdoor environment is recognized internationally as an important indicator of potential pathogenic contamination. Second, there is a lack of documentation from day care centres, settings that may previously have been thought to present little risk of exposure. Third, sampling methodology differs greatly from one study to the next. Lastly, there is a large amount of information missing from the published reports.

The details of the various sampling and laboratory methodologies used in previous studies are shown in Table 2. The types of sampling most frequently used were random, systematic and two types of judgement: one, where children would play, and two, where domestic animals would be expected to defecate (eg, shaded areas, near walls). When reported, the depth and surface from which the sand or soil specimens were sampled and the weight of sample varied extensively. A similar observation was found with respect to laboratory methods used. Missing information combined with the great variation in methods provide insufficient evidence for an accurate assessment of the occurrence and/or intensity of microbial contamination reported in this literature.

Based on the above considerations, we designed a study with two objectives: to compare three of the most commonly used types of sampling methods (one random and two types of judgement sampling), and to pretest field and laboratory methods for the determination of *Toxocara* species and FC counts from sand.

METHODS

Selection of the study DCC: The sampling frame consisted of 10 DCCs located in the Montreal and Laval regions of Quebec. Participating DCCs had at least one outdoor sandbox and play area. One 100 g sample of sand from each DCC was examined for the presence of FCs. Of the 10 DCCs, six were found to have no FCs (or a coliform level below that detectable at the screening dilution). Contamination levels found at the other four centres were 1 FCs/g, 40 FCs/g, 660 FCs/g and 1600 FCs/g, respectively. The DCC having the highest number of FCs was selected for this study.

Sampling methods: Surfaces of both the sandbox and the play area were measured and divided into 25 areas of approximately equal size (Figure 1). The grid coordinates for the areas were indicated on the sides of the sandbox and the play area with a black marker. A total of 25 areas were, therefore, identified and numbered from 1 to 25.

TABLE 1
Reported prevalence of *Toxocara* species in outdoor environments by country

Reference	Year	Country	Number of sites and type	Number of samples per site (total)	Prevalence number (%)	
					Per site	Per sample
18	1984	Australia	6 parks	? (?)	0	0
19	1990	Australia	41 sandpits in 30 kindergardens	2-3 (?)	0	0
20	1994	Brazil	39 parks	5 (195)	9 (23)	?
21	1976	Canada	10 parks	1-5	6 (60)	14 (33)
			33 sandboxes in 10 parks			7 (18)
22	1986	Canada	21 playgrounds in parks	? (510)	11 parks (5)	8 (2)
8	1994	Canada	10 play areas in 10 DCCs	10 (100)	2 (20)	?
23	1980	France	17 parks	?	11 (65)	?
24	1982	France	15 sandboxes in 8 parks	4-11 (58)	2 (13) in 1 park	4 (7)
9	1986	France	13 sandboxes: 10 parks, 3 DCCs	?	8 (62):2 DCCs	?
25	1994	France	5 sandboxes: 3 parks, 2 kindergardens	10 (50)	4 (80)	17 (34)
26	1984	Germany	31 sandpits: ?	4-10 (562)	27 (87)	?
27	1987	Germany	18 sandboxes	? (86)	4 (22)	4 (5)
28	1990	Germany	52 sandpits in playground	4 (208)	29 (56)	51 (25)
29	1991	Ireland	26 gardens	?	10 (38)	?
			17 parks	2-6 (53)	2 (12)	3 (6)
30	1994	Ireland	9 playgrounds	12-40 (228)	8 (89)	35 (15)
31	1993	Japan	24 sandpits in parks	5 (120)	21 (80)	?
			22 sandpits in kindergardens	5 (110)	8 (36)	?
32	1993	Japan	13 sandpits in parks	5-8 (?)	12 (92)	?
33	1989	Jordan	? schools	? (86)	?	5 (6)
			? public places	? (94)	?	7 (8)
34	1986	La Réunion*	13 playgrounds: park and school	1	6 (46)	6 (46)
35	1993	Netherlands	27 parks	6 (162)	?	13 (8)
			? sandboxes	2 (61)	?	15 (25)
36	1980	Scotland	? parks	? (234)	?	17 (7)
37	1989	Spain	132 urban park, street	1 (132)	6 (5)	6 (5)
			310 rural play areas	1 (310)	28 (9)	28 (9)
38	1973	United Kingdom	10 parks	40 (400)	10 (100)	93 (23)
39	1987	United Kingdom	5 play areas in 5 parks	Vary (226)	5 (100)	147 (65)
			5 parks	Vary (277)	5 (100)	169 (61)
40	1991	United Kingdom	8 parks	8-229 (521)	7 (88)	33 (6)
41	1975	United States	2 parks	42 and 48 (90)	2 (100)	26 (29)
42	1979	United States	23 swing areas in 10 parks	1 (23)	4 (17)	4 (17)
			23 sandboxes in 10 parks	1 (23)	9 (39)	9 (39)
43	1980	United States	32 play areas in parks	Vary (285)	1 (3)	1 (0.4)
44	1984	United States	20 parks	? (1529)	4 (20)	6 (0.4)
45	1985	United States	146 backyards	3 (438)	16 (11)	?
46	1988	United States	23 play areas in parks	Vary (135)	11 (48)	22 (16)
47	1989	United States	3 parks	13-53 (114)	2 (67)	22 (19)
48	1983	Yugoslavia	10 parks	10 (100)	8 (80)	27 (27)

**La Réunion is an Overseas French Department (France). ? Indicates that this information is not provided in the publication. DCC Day care centre*

On each day over a nine-day period, five areas from the sandbox and five areas from the play area were sampled for a total of 90 sand samples. The areas sampled varied from day to day according to the method used, random or by judgement (two types). The methods are described below.

Random method (R): Five numbers from 1 to 25 were selected at random using a table of random numbers. The areas corresponding to the selected numbers were sampled. The numbers selected for the sandbox differed from the ones selected for the play area.

TABLE 2
Methodologies used in the recovery of *Toxocara* species ova from sand and soil

	Sampling methods				Laboratory method		
Reference	Type*	Depth (cm)	Surface	Weight (g)	Pretreatment	Flotation	Sieving
18	?	?	?	?	?	?	?
19	Children	10	?	250	?	?	?
20	?	5	?	?	?	$MgSO_4$ + KI	No
21	Random	Various	100 cm^2	200	NaCl + water	Brine	No
22	Children	1	15 cm^2	?	?	$ZnSO_4$ + NaOH	No
8	?	12	?	75	Water	$ZnSO_4$	No
23	Animal	?	?	450-2350	Water	NA	Yes
24	Random	8-10	3- 4 cm†	500-600	Water	NA	Yes
9	?	40	3.5 cm†	250-300	Water	NaCl	No
25	Children	15	?	1000	Water	KIHg	No
26	Systematic	10	?	250-300	Water + mesh	Saline	No
27	Animal	10	?	250	Hypochlorite sodium	NaCl	No
28	Systematic	Surface	?	1000	Tween 80	Sugar	Yes
29	Children	2	130 cm^2	250	None	$NaNO_3$	No
30	Random	1	1 m^2	450	Tween 80	$NaNO_3$	No
31	Systematic	Upper	1000 cm^2	?	Water + mesh	$NaNO_3$	No
32	Systematic	3	6 cm†	100-150	?	Sucrose	No
33	?	10	?	250-300	None	$ZnSO_4$	No
34	?	Surface	?	10	?	$ZnSO_4$	No
35	?	5	?	10	Teepol + sieve	$ZnSO_4$	Yes
36	?	3	?	?	Tween 80	$MgSO_4$ + KI	No
37	?	3	100 cm^2	?	Tween 60	$MgSO_4$ + KI	No
38	Systematic	?	?	250	Water	$ZnSO_4$	No
39	Systematic	3	?	200	Tween 60	$ZnSO_4$	No
40	?	?	?	50	Tween 80	$MgSO_4$	No
41	?	0.5	15×15 cm^2	?	Tween 60	$NaNO_3$	No
42	Systematic	0.5-1	930 cm^2	250	NaOH	$ZnSO_4$	No
43	?	Upper	?	?	?	$ZnSO_4$	No
44	Children	?	?	?	Tween 40	$NaNO_3$	No
45	Animal	1	?	250	Tween 60	$ZnSO_4$	No
46	Systematic	?	?	50	Tween 40	$NaNO_3$	No
47	Systematic	1-2	?	40	Tween 40	$NaNO_3$	No
48		0.5-1	500 cm^2	250	N NaOH	$ZnSO_4$	No

**Method by which the sample was taken: children – from areas where children play; animals – from areas where animals are expected to defecate; systematic; random. †Diameter of sample. ? Indicates that this information is not provided in the publication. KI Potassium Iodide; KI Potassium Iodine; KIHg Mercury potassium iodide; $MgSO_4$ Magnesium sulphate; $NaNO_3$ Sodium nitrate; NaOH Sodium hydroxide; $ZnSO_4$ Zinc sulphate*

Judgement 1 method (J1): In order to assess soil contamination by sand or soil-associated microorganisms, the World Health Organization (WHO) recommends that sand be sampled in shaded areas and near trees (49). Therefore, this judgement method focused on covered areas, places where traces of cats were visible, areas near walls and shaded areas.

Judgement 2 method (J2): Areas where children were the most likely to play were sampled in the sandbox and in the play area. These areas were identified by observing children at play.

The nine-day period was divided into three blocks of three days each. The three methods were each used once in each block. For each three-day block, the order of the sampling method used each day was chosen at random in order to avoid an order effect. The order of the sampling methods is described in Table 3.

All 25 areas of the sandbox and all 25 areas of the play area were sampled on day 10.

Sand sampling for laboratory analysis: The sampling took place every morning before the arrival of the children at the DCC. In each selected area, 100 g of sand were obtained, to a depth of 10 cm, with a 4 cm diameter sterile container for bac-

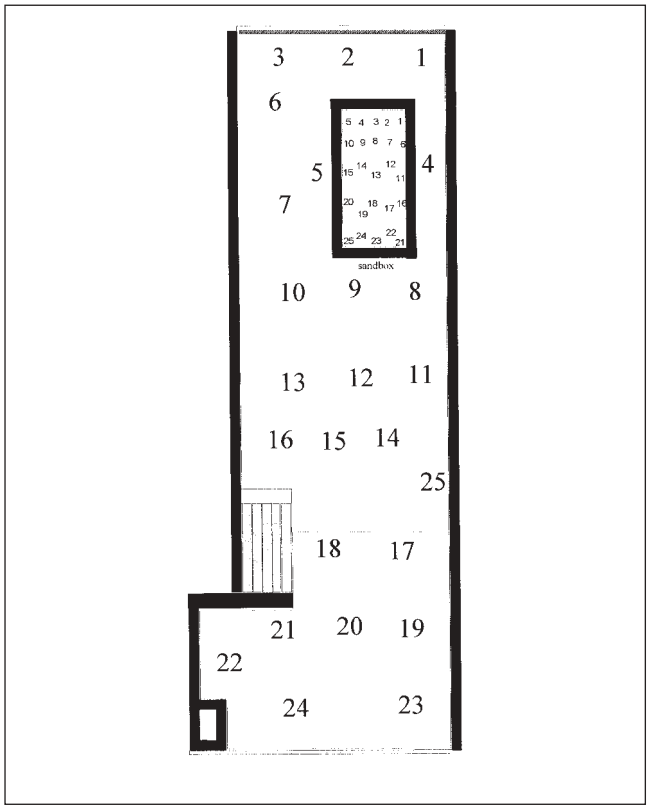

Figure 1) *Division of sandbox and play area into 25 areas for sampling*

TABLE 3
Order in which areas at day care centre where sampled

Block	1			2			3		
Method	J2	R	J1	J1	R	J2	R	J1	J2

J1 Judgement 1 method; J2 Judgement 2 method; R Random method

teriological analysis. The container was placed at 4°C until it was transported to the laboratory, where it was immediately processed (within 1 h of sampling). Another 100 g of sand, similarly obtained, was placed in a container filled with sodium-acetic acid-formalin (SAF) for the parasitological analysis.

Method of quantification of the FCs: Bacteriological analyses were performed at the Centre de recherche en virologie, Institut Armand-Frappier, **location**. A membrane filtration method for the identification of FCs was used (50). First, the sand was shaken to homogenize the sample. Then, 10 g was weighed and placed in a solution of 100 mL phosphate-buffered saline 10×. The samples were left at 4°C for 24 h. The sample was filtered, placed on m-FC medium and incubated at 44°C for 24 h. Blue colonies with metallic sheen were counted.

Recovery methods for *Toxocara* species: Parasitological analyses were performed at the Centre for Tropical Diseases at the Montreal General Hospital, Montreal, Quebec. Recovery of *Toxocara* species ova from sand included a pretreatment stage to homogenize the sand and to 'unstick' the ova from the sand particles. A flotation-centrifugation method was used to separate and collect the ova from the sand sediment.

TABLE 4
Fecal coliforms (FCs) recovered from the study sandbox and play area

	Number of FCs (number of colonies/g sand) from sandbox Block			Number of FCs (number of colonies/g sand) from play area Block		
Method*	**1**	**2**	**3**	**1**	**2**	**3**
Random	200	200	260	460	250	96
	1020	2800	115	1	2100	750
	100	10,500	690	2	24	0
	16	18	149	34	10	70
	87	18	4640	152	24,800	130
Average	285	2707	1171	130	5437	1046
Judgement 1 'animal'	21	140,000	1050	240	25,000	1090
	7	17	5	50	620	610
	270	380	1680	3840	530	230
	40	30	7200	0	1	4110
	4	7	54	4	22	80
Average	68	28,087	1998	827	5235	779
Judgement 2 'children'	800	430	1440	48	28	215
	4360	370	460	260	610	2480
	2810	1770	900	570	230	3870
	790	210	680	330	4110	210
	170	68	1000	12	8	13
Average	2186	570	896	244	997	1358

**See text for explanation of methods used*

One millilitre of Tween 80 solution (Anachema, Quebec) was added to the sand sample (100 g) diluted in SAF to obtain a 0.1% solution and then shaken for 1 min. This solution was poured into 15 mL centrifuge tubes and centrifuged for 2 mins at 700 *g*. The supernatant was discarded. The sediment was then suspended in a solution of zinc sulphate (specific gravity 1.2) (51) and centrifuged for 2 mins at ×700 *g*. A small amount of the supernatant was pipetted and placed on a microscope slide. The slide was examined promptly at 40× magnification.

Statistical analyses: The results are described by sampling method and by block. A logarithmic transformation ($\log_{10}$) was used because the data were not normally distributed. Ninety-five per cent confidence intervals were calculated for the difference in FC counts in the play area and the sandbox. SAS software (Statistical Analysis Systems Institute Inc, North Carolina) was used to obtain summary statistics (quartiles, 95% CI, median, ranges).

RESULTS

FCs: FC counts by method and by block for the sandbox and the play area are shown in Table 4. The FC counts varied extensively from area to area and from day to day. All three methods almost constantly underestimated the overall contamination found on the last day of sampling. Only in one instance did the J2 method (where children play) provide a FC count higher than

TABLE 5
Presence of *Toxocara* species in the study sandbox and play area

	Sandbox Block			Play area Block		
Method	**1**	**2**	**3**	**1**	**2**	**3**
Random	–	–	–	–	–	–
	–	–	–	–	–	–
	–	–	–	–	+	–
	–	–	–	–	–	–
	–	–	–	–	–	–
Judgement 1 'animal'	–	+	–	–	–	–
	–	–	–	–	–	–
	–	–	+	–	–	–
	–	–	–	+	–	–
	–	+	–	–	–	–
Judgement 2 'children'	–	–	+	–	–	+
	–	–	–	–	–	–
	–	–	–	–	–	–
	–	–	–	–	+	–
	–	–	–	–	–	–

– No Toxocara *species present in sample; +* Toxocara *species present in sample*

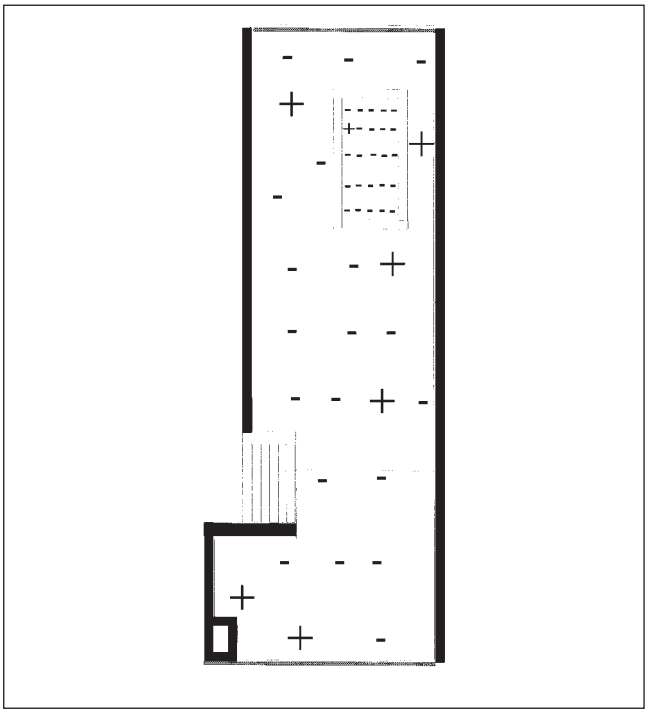

Figure 2) *Spatial distribution of the presence of* Toxocara *species (+) on the last day of sampling. – No* Toxocara *species present*

the count on day 10. However, because of the extreme variation in the FC levels both within the same day and between days, there was insufficient power to conduct any meaningful parametric or nonparametric test. Therefore, it was not possible to identify a method that was superior to the other methods.

On the last day of sampling (day 10), the average counts were 3036±7700 FCs/g and 915±930 FCs/g of sand for the play area and the sandbox, respectively. These counts were not normally distributed. The median counts of FC were 910 FCs/g (interquartile range [IQR]=1050) and 350 FCs/g (IQR=1160 of sand for the sandbox and the play area, respectively). The difference in the log transformed FC counts between the play area and sandbox was 0.0093 with a 95% CI of –0.5589 to 0.5776.

***Toxocara* species:** The areas where *Toxocara* species ova were found using the different sampling methods for the three blocks are shown in Table 5. Because very few ova were recovered in each sample, a qualitative measure was used to describe the presence or absence of *Toxocara* species. No differentiation was made between *Toxocara canis* and *Toxocara cati*. The recovery varied considerably from one sampling area and day to another. Figure 2 shows the areas in which toxocara ova were recovered on the last day of sampling. Toxocara ova were recovered in one area of the sandbox and in six areas of the play area. The presence of toxocara ova was not associated with any particular region of the play area.

DISCUSSION

The number of FCs and the presence of *Toxocara* species recovered from the play area and the sandbox of the study day care centre varied extensively, both in time and space. Environmental factors, such as temperature and humidity, and physical factors, such as the shifting of sand by children or animals, and the presence of domestic and small wild animals or birds defecating in different areas of the playground on different days may partially explain the observed variation.

In Quebec, the presence of animals in DCCs is prohibited (52). In addition, outdoor playgrounds of DCCs must be surrounded by a fence of at least 1.2 m in height (52). However, no mention is made of the spacing between the ground and the lower perimeter of the fence or fence maintenance. Small animals such as dogs, cats and raccoons consequently may have access to the playgrounds. In a 1994 study of 10 DCCs from three different geographical regions of Quebec (Quebec City, Trois-Rivière and Montreal), the presence of dogs, cats, raccoons, pigeons and mice during the night was reported by four DCC directors (8).

Due to the magnitude of the random variation in bacterial and parasite contamination observed, a statistical comparison among the three sampling methods was not possible. To best represent the overall level of contamination in the outdoor DCC environment (sandbox and play area), results from the sampling of all squares (as observed from the results obtained on day 10) were determined to be the most useful.

To evaluate the importance of the level of contamination found, we obtained the microbiological standards (for total and FC contamination) established by Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec for the interpretation of the results of food analyses (53). For example, 30 FCs/g is the upper 'acceptable' limit of contamination in molluscs prepared for human consumption; a norm of 1000 total coliforms is the upper limit in ice milk. The proportion of

FCs to total coliforms varies considerably from one medium to another (personal communication) but to our knowledge, no standards have been established for sand. Standards used by the Ministère de l'Environnement du Québec in assessing beach water contamination are 200 FCs/100 mL (55). The results we obtained (medians of 350 and 910 FCs/g of sand from the sandbox and play area, respectively) are clearly higher than these standards. Indoor FC contamination from surfaces, toys, and from children's and staff's hands has been reported at (median) levels between 0 and 39.8 FCs (5). Although our results from sand cannot be directly compared with results from food, water, indoor surfaces or hands, they indicate significant fecal contamination.

Based on data obtained over a two-week period, 65 children age one to four years were reported to have ingested a median of 40 mg of soil per day in a DCC setting in the United States (55). One child in this study had ingested 5 to 8 g of soil per day. Using our data, it is possible, therefore, that children playing in a play area contaminated with an average of 1000 FCs/g of sand, could ingest a median of 40 FCs per day.

Our study confirms previous reports documenting the presence of *Toxocara* species ova in the outdoor DCC environment. This result may have been missed if the sampling method had been limited to one method only. It is impossible to know whether this is due to a poor recovery rate due to the method itself, because the sand is moved by children and animals each day or because the samples were not taken exactly at the same place from day to day. The percentage recovery of toxocara ova in experimental studies is reported to range from 0% to 70%, but this can vary with the number of grams processed, the level of contamination, the pretreatment techniques, the type of flotation solution used and the type of sand/soil examined (36,56-60). Consequently, the prevalence of toxocara observed can only be an underestimate of the true level of contamination.

CONCLUSION

The contamination levels found in this study indicate a risk of potentially pathogenic bacterial and parasite contamination in the outdoor day care environment. The most representative levels of bacterial contamination were found in a combined sample of the total surface area rather than from a random or judgement sampling method. Research on environmental microbial contamination of outdoor day care settings would benefit from the application of standardized and validated sampling and laboratory methods.

ACKNOWLEDGEMENTS: This work was supported by the Animal Branch of Pfizer Canada and, in part, by the National Health Research and Development Program (NHRDP) through a National Health Research Scholar Award to Theresa W Gyorkos; by NHRDP and the Fonds pour la formation de Chercheurs et l'Aide à la Recherche (FCAR) through a doctoral fellowship to Hélène Carabin; and by the Fonds de la Recherche en Santé du Québec (FRSQ) through a Chercheur boursier to Lawrence Joseph. Denis Minville provided expert assistance with the bacteriological analyses. The authors gratefully acknowledge all the day care centres that participated in this study, particularly the day care centre in which the complete series of sampling methods were studied.

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