Swimming Pool–Associated *Vittaforma*-Like Microsporidia Linked to Microsporidial Keratoconjunctivitis Outbreak, Taiwan

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We analyzed 2 batches of environmental samples after a microsporidial keratoconjunctivitis outbreak in Taiwan. Results indicated a transmission route from a parking lot to a foot washing pool to a swimming pool and suggested that accumulation of mud in the foot washing pool during the rainy season might be a risk factor.

Microsporidia are obligate intracellular parasites that are generally found in aquatic environments (*1*,*2*). The main symptoms of microsporidiosis are keratoconjunctivitis, diarrhea, muscular infection, and acalculous cholecystitis, among which keratoconjunctivitis and diarrhea are the most common (*3*,*4*). As a result of improved diagnostic methods and increased awareness, microsporidia are now considered emergent pathogens worldwide (*4*,*5*).

Vittaforma corneae has been considered a major risk factor for ocular microsporidiosis. In a previous study, we provided molecular evidence for the presence of *V. corneae* in hot springs in Taiwan (*6*). However, recent evidence indicates that ocular microsporidiosis might be underreported in keratoconjunctivitis (*5*,*7*). In our previous study, we hypothesized that *V. corneae* or *Vittaforma*-like microsporidia might spread from adjacent land environments (e.g., soil or mud) to aquatic environments (*2*).

In June 2017, the New Taipei City Health Bureau (New Taipei City, Taiwan) was notified of a keratoconjunctivitis outbreak at a resort. The patients, healthy teenagers from a high school wrestling team, were found to contain DNA and spores from *V. corneae*, thus indicating that it was a microsporidial keratoconjunctivitis (MK) outbreak (*8*). Water contamination at the pool was suspected to be responsible for the outbreak. To identify the source of the pathogen, the transmission route, and the risk factors, water samples from various facilities at the swimming resort were collected for further evaluation. Moreover, because past studies have indicated that soil exposure is an important risk factor for MK, both soil and water samples were collected in a follow-up field survey. We describe results based on the 2 field surveys and provide information on the important risk factors for MK.

The Study

This study was initially conducted because of a request of the New Taipei City Health Bureau in response to the MK outbreak (*8*). The swimming pools at the resort had been filled with tap water. Unfortunately, we received information about the MK outbreak 1 day after cleaning and disinfection of the swimming pools had taken place (*8*). Before water samples were collected, the resort was temporarily closed by the health authorities, and the facility was cleaned and disinfected as recommended by the health authorities (i.e., treatment with 5 ppm free available chlorine for 3 hours). The cleaning and disinfection procedures included draining of all water reservoirs, pools, and tubs, followed by surface scrubbing to remove any potential biofilms and disinfection with sodium hypochlorite. The pretreatment methods of samples, PCR conditions, phylogenetic analysis, and all protocols were performed as described in our previous studies (*2*,*6*).

We collected 19 water samples and 8 soil samples from the swimming resort and its surrounding environment (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/25/11/18-1483-App1.pdf). We sequenced all 17 amplicons of the 15 test-positive samples, analyzed them by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and compared them with reference species from GenBank to determine the most closely related species. All the amplicons were homologous to microsporidia, with identity ranging from 89% to 99% (Table). Only 5 (29.4%) amplicons showed a high degree of homology (>97% identity) to the reference strain.

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Table. Comparison of BLAST results from environmental strains in a field study conducted after a microsporidial keratoconjunctivitis outbreak, Taiwan*

Strain	Sample source	Top BLAST hit (GenBank accession no.)	Maximum identity, study strain/reference (%)	Reference source
Xindian_1_Water	Standard swimming pool	*Vittaforma corneae* strain HotSpring-E1-o (KY245918)	470/473 (99)	Environmental
Xindian_3_Water	Standard swimming pool	*Vittaforma corneae* strain HotSpring-E1-o (KY245918)	467/473 (99)	Environmental
Xindian_10_Water	Foot wash pool	Microsporidium sp. BVOR4 (FJ756182)	438/472 (93)	Environmental
Xindian_14_Water	River	*Enterocytospora artemiae* isolate Ea_monica20 (JX915755)	477/483 (99)	*Artemia franciscana*
Xindian_15_Soil	Park near the resort	*Vittaforma corneae* strain HotSpring-C3-o (KY245925)	430/467 (92)	Environmental
Xindian_16_Water	Sink in the park	Uncultured microsporidia clone Chula_Myositis 1 (JN619406)	420/469 (90)	Clinical
Xindian_17_Soil	Park near the resort	*Nosema* sp. FCG-1468 (LC033883)	429/450 (95)	Honey bee
Xindian_18_Soil	Park near the resort	*Sporanauta perivermis* (KC172651)	432/485 (89)	Marine nematode
Xindian_20_Water	Wastewater from the resort	*Unikaryonidae* sp. JI-2011 (JF960137)	456/486 (94)	Curculionidae
Xindian_21_Water	Wastewater from the resort	Uncultured microsporidia clone Chula_Myositis 1 (JN619406)	425/474 (90)	Clinical
Xindian_22_Soil(U)	Soil in the wastewater flow	*Vittaforma corneae* strain HotSpring-F2-o (KY245925)	453/473 (96)	Environmental
Xindian_22_Soil(D)	Soil in the wastewater flow	*Vittaforma corneae* strain HotSpring-F2-o (KY245925)	450/472 (95)	Environmental
Xindian_23_Soil(U)	Park near the resort	*Vittaforma corneae* strain HotSpring-F3-o (KY245925)	458/472 (97)	Environmental
Xindian_23_Soil(D)	Park near the resort	*Vittaforma corneae* strain HotSpring-F2-o (KY245925)	448/474(95)	Environmental
Xindian_24_Soil	Parking lot near the resort	*Vittaforma corneae* strain LVPEI.BP235FR_11 (KP099409)	453/475 (95)	Clinical
Xindian_25_Water	Pavement rainwater	*Endoreticulatus* sp. Melnik (KU900486)	455/471 (97)	*Euproctis chrysorrhoea*
Xindian_26_Water	Foot wash pool	*Vittaforma corneae* strain LVPEI.BP235FR_11 (KP099409)	453/475 (95)	Clinical
Sw_MK_outbreak	Patient	*Vittaforma corneae* strain LVPEI.BP235FR_11 (KP099409)	472/472(100)	Clinical

*Top BLAST hit indicates the closest reference species that matched the environmental strains using BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Maximum identity represents the percentage identity between the environmental strains and the closest reference species. The fifth column shows the isolated source of the closest reference species.

In the initial survey (10 days after site disinfection), *V. corneae* was not detected. However, other *Vittaforma*-like microsporidia were identified in the standard swimming pool and foot washing pool. According to the Enforcement Rules for Swimming Pool Management in Taiwan, swimming pool water should be chlorinated (with a concentration of free available chlorine of ≈0.3–0.7 ppm) to prevent the spread of waterborne diseases. The presence of these other *Vittaforma*-like microsporidia indicates that some problems might have occurred during the disinfection process.

According to the New Taipei City Health Bureau, all pools, water source tanks, waterlines, and tubs in this facility were drained and scrubbed during the cleanup and disinfection process. The source of *V. corneae* infection remains debatable. Chlorine disinfection studies have shown that residual chlorine is capable of inactivating and reducing the number of microsporidians. However, microsporidian spores are relatively resistant to the typical concentrations of chlorine used for swimming pool disinfection (depending on the species, spores are inactivated after an exposure time of 10–120 min [i.e., inactivation of *Encephalitozoon intestinalis* spores does not reach 100% under 5 ppm of free available chlorine even after 120 minutes of treatment]) (*9*–*11*). Many clinical studies have indicated that soil or mud exposure, visits to hot springs, and outdoor activities, especially after rainfall, are all risk factors for ocular microsporidiosis (*12*,*13*). In addition, our previous study provided evidence for the presence of *V. corneae* in hot springs, thus indicating that pools in outdoor environments were associated with the presence of *V. corneae* (*6*). Therefore, we considered that the contaminating microsporidia in the swimming resort might have been brought to the resort from outside by human activities.

Our data show that many clade IV microsporidia were present in the soil and water samples from the resort site (Appendix Figure 2). Most of the microsporidia in clade IV are of terrestrial origin, according to small subunit rRNA gene phylogenetic analysis (*14*,*15*). Therefore, given that previous studies have shown that rainfall is an important risk factor for ocular microsporidiosis (*5*,*12*,*13*), we believe that water contamination might originate from soil environments after rainfall. Rainfall occurred near the sampling location during June 15–19 and again during July 1–4 (as recorded by the Taiwan Central Weather Bureau). We conducted a follow-up site survey after the July 1–4 rainfall and found *Vittaforma*-like microsporidians were found in pavement rainwater and the parking lot of the resort. Hence, we hypothesized that the swimming pool water was contaminated through soil or water brought in by human activity during the rainfall. *Vittaforma*-like microsporidians were found in the swimming pool and foot washing pool in the initial survey, which was conducted after a careful disinfection procedure, but microsporidia were not found in tap water or other pools. Therefore, these results suggest that the contamination was not from the waterlines or water sources, and the pools may have been contaminated from an outside source owing to human activities and poor facility configuration. In the follow-up survey, we found 100% identical amplicons in the parking lot and foot washing pool, suggesting a possible transmission pathway for *Vittaforma*-like microsporidians from the outside environment to the swimming pool (Appendix Figure 1).

Conclusions

Our study demonstrated the presence of *Vittaforma*-like microsporidia in a swimming resort and nearby environments in Taiwan. Human activities, rainy weather, and soil-rich or park environments might have been possible sources of microsporidia in the waters at the facility. The foot washing pool and shoe cabinet area are possible contamination areas and might facilitate transmission of microsporidia throughout the swimming resort. We suggest several precautions, including improving the frequency and efficacy of disinfection procedures at the facility, using a continuous water flow facility in foot washing pools, and paying attention to the disinfection and cleaning of the shoe cabinet area, especially during the rainy season. In addition, for swimming resorts that are located in a park, enhanced monitoring of the environment surrounding the swimming pool is warranted.

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About the Author

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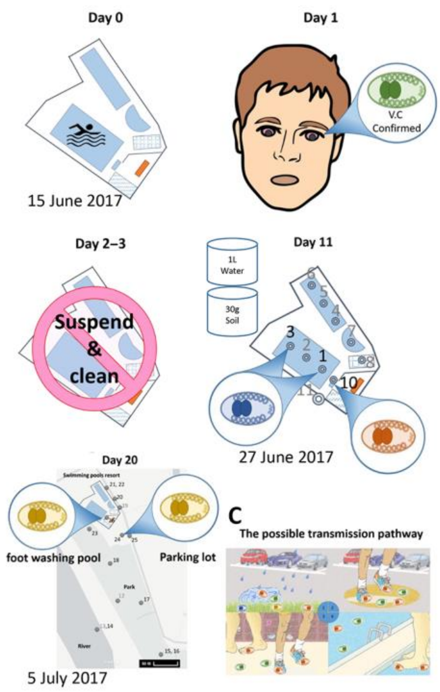
Appendix



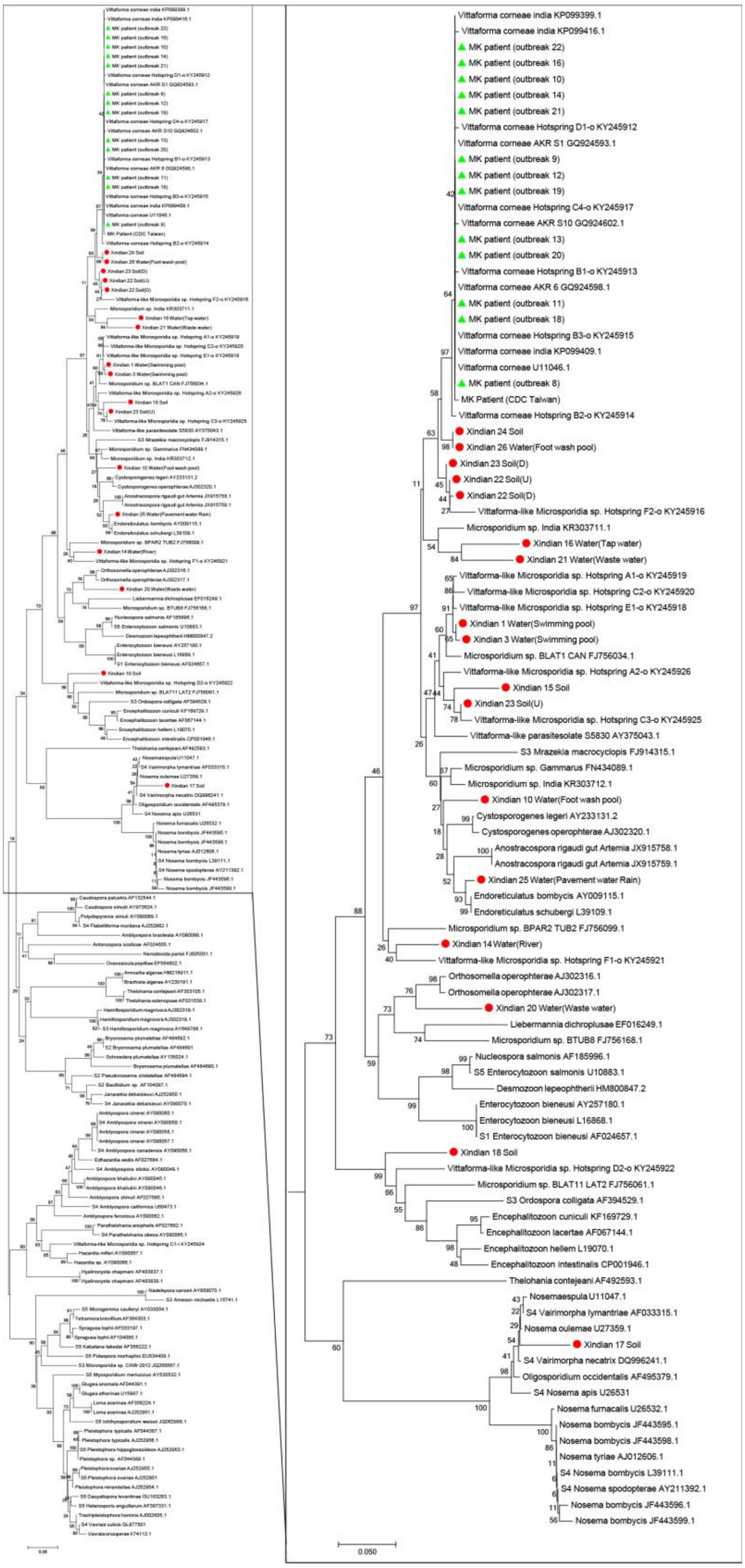
A

	Sample No.	Sampling Type
Initial survey	1	Standard swimming pool (Water)
	2	Standard swimming pool (Water)
	3	Standard swimming pool (Water)
	4	Spa pool
	5	Spa pool
	6	Spa pool
	7	Warm spa pool
	8	Bathing room
	9	Tap water source
	10	Foot wash pool (initial survey)
	11	Tap water source
Follow-up survey	12	Soil in the Park nearby the resort
	13	River (Soil)
	14	River (Water)
	15	Soil in the Park nearby the resort
	16	Sink in the park
	17	Soil in the Park nearby the resort
	18	Soil in the Park nearby the resort
	19	Wastewater from Rain flow
	20	Wastewater from the resort
	21	Wastewater from the resort
	22	Soil in the wastewater flow
	23	Soil in the Park nearby the resort
	24	Parking lot nearby the resort
	25	Pavement Rain water
	26	Foot wash pool(follow-up survey)

B

Sample type	First field survey (in resort)		Follow-up survey (around resort)	
	Total Samples	Number of detections	Total Samples	Number of detections
Standard swimming pool (Water)	3	2(66.7%)	0	--
Spa pool	3	0	0	--
Tap water source	2	0	0	--
Warm spa pool	1	0	0	--
Foot wash pool	1	1(100%)	1	1(100%)
Bathing room	1	0	0	--
River (Soil)	0	--	1	0
River (Water)	0	--	1	1(100%)
Sink in the park	0	--	1	1(100%)
Wastewater from the resort	0	--	2	2(100%)
Soil in the wastewater flow	0	--	1	1(100%)
Wastewater from Rain flow	0	--	1	0
Soil in the Park nearby the resort	0	--	5	4(80%)
Parking lot nearby the resort	0	--	1	1(100%)
Pavement Rain water	0	--	1	1(100%)
Total	**11**	**3(27.3%)**	**15**	**12(80%)**

Appendix Figure 1. Graphical abstract for this study. All patients had engaged in swimming activities at the resort on June 15 (Day 0). Some of the patients presented with keratoconjunctivitis symptom on June 16 (Day 1). The resort was temporally suspended, and full disinfection commenced (5 ppm free available chlorine for 3 hours) from June 17 to 18 (Days 2–3). The initial sample collection was performed on June 27 (Day 11). A follow-up sampling survey was conducted on July 5 (Day 20). Part (a) are the sampling locations of the swimming resort associated with microsporidial keratoconjunctivitis outbreak and its surrounding environment in Taiwan. Sampling location numbers 1 to 11 were in the swimming resort from the initial survey, and number 26 was in the swimming resort from the follow-up survey. The others were located at the park near the swimming resort from the follow-up survey. Part (b) is the occurrence of *Vittaforma*-like microsporidia in the water and soil samples from the two field surveys. Part (c) is the possible transmission pathway of microsporidians in the swimming resort. The illustration is presented from left to right and top to bottom. A person who goes swimming during or after a period of rainfall might carry microsporidian-contaminated soil or mud from the outside environment of the park or other locations. Subsequently, when this person takes off his or her shoes and places them in the shoe cabinet, the cabinet might then be potentially contaminated. Then, the person with contaminated bare feet enters the foot washing pool, causing the foot washing pool to accumulate more contamination, especially as the foot washing pool in this resort had a low water flow rate. Finally, the standard swimming pool near the foot washing pool is contaminated by microsporidia, thus providing multiple sources for an ocular microsporidiosis outbreak.



Appendix Figure 2. Phylogenetic analysis of the strains from this surveys. The phylogenetic tree was constructed based on the ≈472 bp SSU-rRNA gene sequences. Bootstrap values (%) involving 1000 pseudo-replicates for the major lineage are presented at each branch node. The major microsporidian clades are labeled by the black line on the left side of the figure. The right part mostly consists of clade IV microsporidian sequences including our amplicons. The symbols -U, and -D indicate the amplicon position in the electrophoresis lane. The water or soil samples from this study are labeled by red circle and the microsporidial keratoconjunctivitis outbreak patients are labeled by green triangle. The reference strains were downloaded from GenBank with their accession numbers. Many reference strains cited in the literature are associated with five major clades of microsporidian sequences. The reference strains with symbol S before the species name are cited from Stentiford et al. (2013) (*1*) and are associated with aquatic animals' diseases according to GenBank. Number S1 represents fish-infecting taxa; S2 represents aquatic-arthropod–infecting taxa; S3 denotes non-arthropod invertebrate–, free-living protist–, and hyper-parasite–infecting taxa; S4 represents insect-infecting taxa; and S5 means human-infecting taxa.

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