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When wildlife comes to town: interaction of sylvatic and domestic host animals in transmission of *Echinococcus* spp. in Namibia

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Summary

The present study was conducted in the isolated desert town of Oranjemund in the far south of Namibia. It is an extremely arid region where no livestock husbandry is practiced and only animals adapted to the desert can be found. However, in and around the city, artificial irrigation maintains lush green patches of grass that attract wild animals, in particular oryx antelopes (*Oryx gazella*). In 2015 four oryx antelopes were euthanised due to poor conditions and a post-mortem examination was conducted. Two were found positive for cystic echinococcosis and 16 cysts were collected for molecular analyses. In addition, faecal samples from black-backed jackals (n=5) and domestic dogs (n=9), which were regularly observed to feed on oryx carcasses, were collected and taeniid eggs isolated. Parasite species identification of the cysts and eggs was done by amplifying and sequencing the mitochondrial *nad1* gene. Both oryx antelopes were found infected with *E. ortleppi* and one co-infected with *E. canadensis* G6/7. Both *Echinococcus* species were able to develop fertile cysts in oryx, making oryx antelopes competent hosts for these parasites. Therefore, the analysis of faecal samples was of high interest and although the numbers were quite small, taeniid eggs were found in three out of five faecal samples of jackals and in all nine dog samples. However, species determination was only successful with two jackal and one dog sample. All three were positive for *E. canadensis* G6/7. The absence of *E. ortleppi* may be due to the low number of faecal samples examined. In our small study, we discovered a rather unique lifecycle of *Echinococcus* spp. between jackals and domestic dogs as definitive hosts and oryx antelopes as intermediate hosts. Here, the presence of *E. canadensis* G6/7 is of particular concern, as it is the second most important causative agent of CE in humans.

Keywords: *Echinococcus*; wildlife; Namibia; urban cycle; oryx; black-backed jackal

Introduction

The risk for echinococcoses posed by wildlife populations is well studied in Europe, mainly for *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis (AE), whose lifecycle is based on a transmission system between wild canids (mainly foxes) and arvicoline rodents (Romig *et al*., 2017). In contrast, out of the five currently recognized species within the *E. granulosus*

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sensu lato (s.l.) complex, causing cystic echinococcosis (CE), only one species (*E. felidis*) and one genotypic cluster (*E. canadensis* G8/G10) are assumed to be exclusively or for the latter predominantly transmitted by wild mammals (Romig *et al*., 2017). The remaining, globally distributed taxa of *E. granulosus* s.l. (i. e. *E. granulosus* sensu stricto (s.s.), *E. equinus*, *E. canadensis* G6/7, *E. ortleppi*) are well described from domestic lifecycles including domestic dogs and livestock, although for most species there seems to be a marginal involvement of wild mammals in some areas (Romig *et al*., 2017), and at least one secondary wildlife cycle is known to have developed in eastern Australia. There, the initial domestic dog-sheep lifecycle of *E. granulosus* s.s. has switched to a wildlife cycle between dingoes (*Canis lupus dingo*) and marsupials that is now apparently independent from domestic hosts (Jenkins & Macpherson, 2003). Such involvement of wildlife has an economic dimension by stabilising transmission of the parasites and thwarting control efforts, as control measures such as anthelmintic treatment, safe offal disposal or vaccination of intermediate hosts (ungulates) are not applicable to wild host species (Craig *et al*., 2017). In addition, there is a public health dimension, as two of the agents of human CE (*E. granulosus* s.s. and *E. canadensis* G6/7) contribute significantly to the global human disease burden (Alvarez-Rojas *et al*., 2014; Budke *et al*., 2006).

Sub-Saharan Africa hosts the largest number of *Echinococcus* species causing CE of any continent. All four globally distributed species occur, with the addition of two taxa that seem to be geographically restricted to sub-Saharan Africa (Deplazes *et al*., 2017). One of these, *E. felidis,* obviously depends on the lion as definitive host and is therefore restricted to the remaining range of this predator (Romig *et al*., 2017), while a distinct genotype ('G Omo') related to, but not identical with *E. granulosus* s.s. was described from a human CE patient in southern Ethiopia, without any further information on the lifecycle available (Wassermann *et al*., 2016). All taxa, apart from *E. felidis* and 'G Omo', are well described from domestic definitive and/or intermediate hosts in Africa (Deplazes *et al*., 2017; Mulinge *et al*., 2023). Human CE cases are known predominantly from northern, eastern and southern Africa, mainly caused by *E. granulosus* s.s., to a lesser degree by *E. canadensis* G6/7, and rarely by *E. ortleppi* (lit. in Deplazes *et al*., 2017). Numerous species of wild carnivores and herbivores have been identified in Africa as hosts for *Echinococcus* (Aschenborn et al., 2023; Carmena & Cardona, 2014; Hüttner & Romig, 2009; Macpherson & Wachira, 1997). Most of this data is from the pre-molecular era, so the causative species of *Echinococcus* were not identified. Recent data show infection of lions (*Panthera leo*), spotted hyenas (*Crocuta crocuta*), warthogs (*Phacochoerus africanus*) and hippopotamus (*Hippopotamus amphibius*) with *E. felidis* in eastern and southern Africa (Halajian *et al*., 2017; Hüttner *et al*., 2008, 2009; Kagendo *et al*., 2014), lions, black-backed jackals (*Lupulella mesomelas*), plains zebras (*Equus quagga*) and white rhinoceros (*Ceratotherium simum*) with *E. equinus* in southern Africa (Wassermann *et al*., 2015; Zaffarano *et al*., 2021), lions, spotted hyenas, warthogs and wildebeest (*Connochaetes mearnsi*) with *E. granulosus* s.s. in eastern Africa (Hüttner *et al*., 2009; Kagendo *et al*., 2014), oryx antelopes (*Oryx gazella*) with *E. canadensis* G6/7 in Namibia (Addy *et al*., 2017; Aschenborn*et al*., 2023), and an unspecified species of zebra with *E. ortleppi* in Namibia (Obwaller *et al*., 2004). While the sketchy data that are currently available suggest, that *E. felidis* in East Africa and *E. equinus* in Namibia are propagated exclusively by wild mammals, a spill-over situation from domestic animals to wildlife is likely for *E. granulosus* s.s. in Kenya and Uganda. This may also be so for the single reported cases of *E. ortleppi* and *E. canadensis* G6/7 in Namibia, but far more information is needed to draw conclusions.

Compared to East African countries (particularly Kenya) and South Africa, studies on *Echinococcus* and CE have only recently begun in Namibia. In the first livestock survey for CE (Aschenborn *et al*., 2022), *E. ortleppi* was found to occur in commercially raised cattle at low prevalence of 1.65 % across the central and southern parts of the country, while a small number of samples indicates a far more intense transmission in the traditionally kept cattle in the north. Based on identification of three isolates, *E. canadensis* G6/7 occurs, at unknown prevalence, in domestic sheep in southern Namibia (Aschenborn *et al*., 2022). The reported wildlife cases may therefore be the result of some interaction between wildlife and domestic transmission in Namibia. In the context of an ongoing survey of *Echinococcus* in Namibian wildlife, we add evidence for such an interaction by describing a unique lifecycle that was recently discovered in the isolated desert town of Oranjemund in the far south of Namibia. Our research interest was triggered by reports from the town council of Oranjemund of a number of oryx antelopes being in poor condition and even found dead, carcasses containing large amounts of cystic structures.

Material and Methods

Study site

All samples were collected in the town of Oranjemund in the far southwest of Namibia. This town was founded in 1936 in the diamond restricted area and is home to an estimated 9000 inhabitants working either directly or indirectly for the diamond mine, or are their family members. Access to the restricted diamond area is limited through very strict control to the entire area and only people with special permits are allowed to enter. There are no agricultural activities in and around the town and the only domestic animals are household pets like dogs, cats and birds. Over the years, some of the dogs were abandoned when workers left the town and a population of feral dogs of unknown size has established. The annual rainfall for the area is less then 50 mm/a, but evergreen parks, sport fields, playing grounds and gardens are being maintained by artificial irrigation. Naturally occurring wildlife around Oranjemund is restricted to desert-adapted species like oryx antelopes, brown hyenas (*Hyaena brunnea*) and the highly adaptable black-backed jackals. Lush vegetation of irrigated parts of Oranjemund town has

attracted numerous oryx antelopes, which now have become a common sight in town. Also, numbers of jackals roam the town streets at night searching for anthropogenic food (e.g. garbage).

Sample Collection

In October 2015, field post-mortem examination was conducted on four adult oryx antelopes (*Oryx gazella),* one male and three female, that were euthanised due to their poor condition. Two oryx had multiple cysts in the lungs and/or liver; a total of 16 cysts were collected for molecular examination and stored in 75 % pure grade ethanol. In addition, five faecal samples were collected at a garbage dump 1 km outside of town, where approx. 80 – 120 black-backed jackals (*Lupulella mesomelas*), were observed feeding on oryx carcasses. In the town, nine faeces of domestic dogs (*Canis lupus familiaris*) were collected from playing grounds, parks (n=6) and from home gardens (n=3). To ensure that only dog faeces were collected, only large-sized faeces were collected since there are no other carnivores in the city from which large-sized faeces could originate. Dog faeces were collected in different parts of the town, to avoid collecting multiple faeces from one dog. However, for both dog and jackal samples, it cannot be completely excluded that multiple samples are from the same animal. Faecal samples were frozen between -4 and -20°C and transported to the laboratory. For safety reasons faecal samples were stored at -80°C for at least 7 days before processing in the laboratory.

Sample preparation

Taeniid eggs were retrieved from faecal material through zinc chloride flotation (Mathis *et al*., 1996). This was done by suspending 2 cm^3 of faecal material in 1 x PBS and 0.3 % Tween20. The suspension was centrifuged for 10 min at 1600 g. The supernatant was discarded, and the pellet re-suspended in 15 ml zinc chloride solution with the specific gravity of 1.45 g/cm^3 after which the new suspension was centrifuged for 30 min at 400 g. The resulting supernatant was passed through sieves with mesh size 50 µm and 20 µm respectively (Mathis *et al*., 1996). The eggs passed the 50 µm sieve and were retained by the 20 µm sieve. The captured particles were washed off the latter sieve with distilled water and collected in a 50 ml tube. The suspension was centrifuged for 10 min at 1600 g, the supernatant discarded, the pellet suspended in 2 ml distilled water and transferred to 2 ml tubes. The samples were examined for taeniid eggs under an inverse microscope. To identify double or multiple infection with *Echinococcus* spp. or other taeniid species, single taeniid eggs were analysed. For this purpose, single taeniid eggs were transferred via pipette in a volume of 1 µl into 9 µl of 0.02 M NaOH solution and lysed at 95°C for 10 min (Nakao *et al*., 2003). Cysts collected from intermediate hosts were examined for the presence of protoscoleces under microscopy. Single protoscoleces were transferred into 10 µl of 0.02 M NaOH solution. In case of non-fertile cysts, a small piece of germinal layer (0.5 mm^2) was transferred to 30 µl of 0.02 M NaOH solution. Protoscoleces and tissue were lysed at 95°C for 15 min. The lysates were used directly as template in the following PCRs. DNA of cysts, which gave negative results after PCR, were extracted via proteinase K digestion, phenol-chloroform extraction and EtOH precipitation as described previously (Dinkel *et al*., 1998).

DNA amplification and sequencing

Due to the minute amounts of DNA especially in the taeniid eggs, a nested PCR was necessary to amplify the complete NADH dehydrogenase subunit 1 (*nad1*) gene, resulting in a ~1080 bp long PCR product. In cases were the PCR for the complete *nad1* failed, another PCR was conducted targeting a smaller fragment of ~180 bp of the *nad1* gene. Primer combinations are shown in Table 1 (Hüttner *et al*., 2008). The reaction mixture for the first PCR had a volume of 25 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTPs, 6.25 pmol of each primer and 0.625 U of Taq polymerase. One microliter of the egg, protoscolex or tissue lysate or extracted DNA was added to the PCR-mixture as template. The volume of the nested PCR reaction mixture was 50 µl and consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTPs, 12.5 pmol of each primer and 1.25 U of Taq polymerase and 2 µl of the primary PCR product as template.

Amplification conditions for both PCRs were an initial denaturation of 94°C for 5 min followed by 35 cycles with denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, elongation at 72°C for 60 sec (complete *nad1* gene) or 30 sec (fragment of *nad1*) and a final elongation at 72°C for 5 min. Amplification results were visualized on a 1.5 % agarose gel stained with GelRed™. Resulting amplicons were purified using the High Pure PCR Purification Kit (Roche) and sent for sequencing (GATC Biotech AG, Konstanz, Germany). The sequences were analysed using GENtle V1.9.4 program (Manske M., 2003, University of Cologne, Germany) and compared with GenBank entries using the NCBI basic local alignment search tool (BLAST) for identification the causative species.

Table 1. Primer pairs used for PCRs (Hüttner *et al*., 2008).

Target	Primers for first PCR (5'-3')	Primers for nested PCR (5'-3')
Complete *nad1* gene	F: TGGAACTCAGTTTGAGCTTTACTA	F: TATTAAAAATATTGAGTTTGCGTC
	R: ATATCAAAGTAACCTGCTATGCAG	R: TCTTGAAGTTAACAGCATCACGAT
~190 bp fragment of *nad1*	F: TGTTTTTGAGATCAGTTCGGTGTG	F: CAGTTCGGTGTGCTTTTGGGTCTG
	R: CATAATCAAACGGAGTACGATTAG	R: GAGTACGATTAGTCTCACACAGCA

F – forward primer, R – reverse primer

Table 2. Number of eggs and *Echinococcus* species identified from faecal samples.

Host species	Number of isolated taeniid eggs	Number of successful amplified and sequenced eggs	species identified (number of eggs)
Dog A	2	-	-
Dog B	10	4	*E. canadensis* G6/7 (4)
Dog C	7	-	-
Dog D	10	-	-
Dog E	2	-	-
Dog F	3	-	-
Dog G	2	-	-
Dog H	7	-	-
Dog I	2	-	-
Jackal A	-	-	-
Jackal B	30	6	*E. canadensis* G6/7 (6)
Jackal C	-	-	-
Jackal D	19	2	*E. canadensis* G6/7 (2)
Jackal E	10	-	-

Ethical Approval and/or Informed Consent

Research permits were issued for the work by the Ministry of Environment, Forestry and Tourism (Permit No.: 1740/2012) as well as the National Commission on Research, Science and Technology (Authorization No.: AN202101126) in line with Namibian regulations.

Results

In most dog and jackal faeces samples, only very few eggs could be detected. Depending on the sample, up to 30 single eggs were isolated and subjected to a PCR. Eggs of *E. canadensis* G6/7 were found in 1/9 faecal samples from domestic dogs and in 2/5 faecal samples from black-backed jackals (Table 2). Other taeniid species were not detected. Other parasite eggs discovered belonged to various nematodes, mainly of the family Toxocaridae and Trichuridae.

Upon post-mortem examination, two of the four euthanized oryx had cysts. Oryx A had approximately 15 large cysts of 2 to 6 cm maximum diameter in the lungs and approximately 30 small cysts (<1 cm diameter) in the liver. Eleven lung cysts and one liver cyst were collected from this animal. Of these twelve cyst samples, ten fertile lung cysts were identified as *E. canadensis* G6/7, one fertile lung cyst as *E. ortleppi* and from the small sterile liver cyst no DNA could be amplified. Oryx B had approximately 15 small (<1 cm diameter), sterile cysts in the liver. Of four collected cysts, two were identified as *E. ortleppi* while from the other two no DNA could be amplified (Table 3).

Discussion

From the results of our small study we hypothesize, that the permanent availability of food matter, mainly grass on the irrigated town parts of Oranjemund, attracts herbivorous oryx antelopes from the surrounding arid land, leading to an animal density far above that in natural habitats, while parasite eggs accumulate on this moist, shaded ground and retain viability for a prolonged time. This causes massive infection of the ungulates leading to high morbidity and mortality, while carcasses of succumbed animals are being scavenged by domestic and wild canids, completing and enhancing the lifecycles of *Echinococcus* species and other parasites. The involvement of other animal species in the lifecycle can be excluded, as no domestic animals apart from dogs and cats are present in Oranjemund.

Table 3. Cysts identified from oryx antelopes (EC = *E. canadensis* G6/7, EO = *E. ortleppi,* na = not amplifiable).

	n cysts examined	*Echinococcus* species
Oryx A	11 (lungs, fertile) 1 (liver, sterile)	EC (n=10), EO (n=1) na (n=1)
Oryx B	4 (liver, sterile)	EO (n=2), na (n=2)

Fig. 1. Aerial view of the town of Oranjemund. A lush green setting in an otherwise desert environment that attracts and establishes resident wildlife populations. (Source of picture www.wikipedia.com; CC BY-SA 4.0)

Personal observation and information provided by the Ministry of Environment, Forestry and Tourism (personal communication W. Handley), it is estimated that about 30 oryx residing permanently inside the town area, concentrating on the irrigated greens, which measure only approximately 35 ha (Fig. 1). Like all larger mammals in this extremely arid area, oryx antelopes are adapted to very dry conditions, and are classified as non-water dependant antelopes (Nagy, 1994). In search of food and water, they cover extremely large distances and are rarely resident for prolonged periods in a given area (Lehmann, 2015). Their distribution in Namibia is limited to the arid and semi-arid parts of the country, while they are absent from moister regions (e.g. the northeast) where they are apparently outcompeted by other species of wild ruminants. The precise reason for this is not known, but may have to do with decreased resistance to soil-transmitted pathogens. Natural habitats of oryx are characterized by limited biomass and low density of large mammals. Long period without precipitation can be interrupted by short heavy rains, often localized, that result in rapid plant growth and an accumulation of large numbers of wildlife, both ungulates and predators, over a short period of time in small areas. However, after grazing off the plant material, animals disperse leaving bare, desiccated ground with high soil temperature. Under such conditions most pathogens have a short survival time. Concerning *Echinococcus*, it was shown that egg survival on exposed ground was less than 2 hours in the arid Turkana area of Kenya with similar climatic conditions (Wachira *et al*., 1991). Despite this, transmission of *Echinococcus* spp. is obviously successful even under arid and hot conditions. This is explained by infection of intermediate hosts via eggs from carnivores being limited to short, moist periods, while the parasite survives the long hot and dry spells (which may persist even for years in some regions) as long-lived cysts in the intermediate host ungulates (Massolo *et al*., 2022).

The artificial conditions in the sport fields, playgrounds and parks of Oranjemund have created a resident population of oryx, while permanently moist greens provide a suitable environment for parasite egg survival; *Echinococcus* eggs were shown to survive for more than one year under moist and cool conditions (Sweatman & Williams, 1963; Veit *et al*., 1995).

Apart from our findings of multiple *Echinococcus* cysts in two oryx, the impact of soil-transmitted parasites as a cause of oryx mortality is corroborated by reports of local authorities in Oranjemund. During June to October 2015 increased mortality of oryx were observed. Post-mortem examinations revealed high frequencies

and parasite loads of CE as well as *Taenia hydatigena*, *Taenia multiceps*, *Trichuris* spp., *Dictyocaulus* spp. and other gastro-intestinal helminths. Following information of the town council and the mining company management on the public health implications of an *Echinococcus* transmission cycle inside town, entry permission was granted to the veterinary section of the Ministry of Environment, Forestry and Tourism which eventually led to the necropsy of four oryx antelopes and the examination of carnivore faeces reported here.

Apart from a number of small sterile cysts in the liver of the oryx, we found fertile lung cysts of *E. canadensis* G6/7 and *E. ortleppi* in two of the necropsied oryx. Number and size of the cysts make it unlikely, that *Echinococcus* was the sole cause of death, but the presence of eleven large lung cysts in one of the oryx certainly caused a significant reduction of fitness. *Echinococcus canadensis* G6/7 was also found in faeces of a domestic dog and two black-backed jackals. There was no faecal detection of *E. ortleppi*, but this can be considered as an artefact due to the small sample size and the failure to amplify DNA from many eggs found in the faeces: all of the nine dog faeces and three of the five jackal faeces contained taeniid eggs which might have been *Echinococcus*. The high prevalence of taeniids in both dogs and jackals indicates a high rate of scavenging on ungulate carcasses, and the only available source in Oranjemund, and in abundance, is carcasses of oryx. We do not speculate about the introduction route of both *Echinococcus* species to Oranjemund. Both species have been reported from cattle (*E. ortleppi*) and sheep (*E. canadensis* G6/7) in Namibia, and interestingly, the sheep isolates of *E. canadensis* G6/7 from Mariental abattoir in southern Namibia belonged to identical mt DNA haplotypes as the isolates from our oryx samples, which had been characterized in previous studies (Addy *et al*., 2017; Aschenborn *et al*., 2022). The haplotype variant of the *E. ortleppi* samples is widely distributed throughout sub-Saharan Africa (Addy *et al*., 2017).

Both oryx and black-backed jackals are able to travel large distances and could have introduced the parasites from elsewhere; black-backed jackals have previously been identified as carriers of both *E. ortleppi* and *E. canadensis* G6/7 on central Namibian farmland (Aschenborn *et al*., 2023). Alternatively, introduction in the past through livestock transported from elsewhere for slaughter in Oranjemund cannot be excluded.

Observations by the first author support the hypothetical lifecycle between oryx and dogs or jackals: numerous dog and jackal faeces are seen on the grass, left unattended until decomposed. Oryx carcasses are removed to the communal garbage site on the outskirts of the town, where they should be burned. During the visit it was observed that the burning effort had little effect on the inner organs, leaving them attractive for scavengers. Jackals were observed for five consecutive days feeding on the carcasses during day and night. Remnants of a carcass were found just next to a sports field, where the animal had died approximately two weeks earlier, and local inhabitants reported that dogs had been feeding on it. Around the irrigated golf course, 4.4 km from the town, a total of 21 carcasses were seen in various stages of decomposition. Here, due to the long distance to the rubbish dump, carcasses were just pulled off the field and left out of sight.

This abundant food source in an otherwise nutrient poor environment could have the same effect on the spatial ecology of black backed jackals as was observed around the Cape Cross seal colony in Namibia. There, jackals travelled as far as 20.2 km from their territory to abundant focal food sources (Jenner *et al*., 2011). In Etosha National Park a similar situation was observed where jackals commute to carcasses that are extremely common during the annual anthrax outbreaks. Thus, over 60 jackals have been observed there at a single carcass, and commuting distances in excess of 20 km to their territories were recorded (Bellan *et al*., 2012; Bellan *et al*. unpublished). The above factors result in very high jackal densities in and around the focal food point with resultant high faecal contamination. Looking at Oranjemund, it follows that the environmental contamination with *Echinococcus* eggs is not limited to the small area of the town, but can be carried far by animals visiting from distant territories. In this wildlife cycle not only the definitive host is very mobile, a study on oryx in the Kunene region of Namibia, a similar environment to that of the area around Oranjemund, found the home ranges of eight GPS collared oryx to vary from 683 to 11,399 ha (Lehmann, 2015). The role of free roaming domestic dogs and their interactions with wildlife are difficult to quantify. Based on a recent review, 50 % of publications on this subject were concerned with direct predation on wildlife and 20 % with disease transmission between dogs and wildlife (Hughes & Macdonald, 2013). Both of these interactions are seen in Oranjemund. Inhabitants reported that groups of free roaming domestic dogs occasionally hunt and kill oryx. However, scavenging seems to be the more important source of dog infection, as the oryx killed by dogs are normally calves or very young animals which would not yet have developed fertile cysts at this early age. In consequence, the removal and safe destruction of carcasses in and around the town would stop infection of domestic dogs and, thus, a large part of the human infection risk. Here, *E. canadensis* G6/7 is of particular concern, as it is the second most important causative agent of CE in humans (Alvarez-Rojas *et al*., 2014) which, on the wildlife-domestic-animal-human interface, can have serious public health implications. The close contact between wildlife and humans in Oranjemund poses a real risk of spill over into the human population especially as dogs are also involved, that may carry eggs directly into human homes. Another area of potential infection are the numerous playing grounds and sport fields in the town and with the constant watering of the fields, egg survival is promoted, bringing humans into direct contact with high concentrations of eggs. Human activity and uninformed management in Oranjemund has created the perfect conditions for the establishment and maintenance of a truly urban cycle of one, probably two agents of human CE.

Conflict of Interest

Authors state no conflict of interest.

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