Short communication

A One Health approach to investigate bats as a potential source of zoonotic mycoses in selected areas of Mpumalanga province, the Republic of South Africa

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Abstract

A One Health approach pilot study was carried out in selected villages within the Mnisi Traditional Authority's area, Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station in Mpumalanga Province, the Republic of South Africa from July to December 2018. The study's main objectives were to identify positive and negative human-bat-environment interactions and microbiological screening of bats' faecal samples for zoonotic fungi. Thirty-three purposively selected participants were asked to complete a structured questionnaire with multiple-choice and open-ended questions, and a total of 55 faecal samples were collected, 25 from identified bat roosting sites and 30 from captured bats. Ninety seven percent of respondents were aware of the presence or absence of bats in their immediate surroundings. However, the majority of them (87.9%) were uneasy about the presence of bats in their buildings, and nearly half (48.5%) were unsure whether bats play a positive or negative role in the environment. Some respondents (15.2%) stated that bats play beneficial roles in the environment, such as pollinating plants, spreading seeds of indigenous plants, catching harmful insects, and so on. More than half of the respondents (66.7%) stated that bats can be a nuisance; 18.2% of those polled

reported contracting fungal diseases as a result of cleaning bat droppings without adequate protection. The analysis of faecal samples revealed that bats can harbour pathogenic fungi such as *Aspergillus fumigatus*, and *A.flavus*. We concluded that bats can harbour fungal pathogens that cause human diseases. Further research should be conducted to compile a complete list of fungi pathogens in bats in the study area.

Keywords. Bats; Faecal sample; One Health Approach; Questionnaire survey; Republic of South Africa; zoonotic fungi.

Introduction

Bats are mammals that are distributed everywhere in the world except in the hottest desert and Polar Regions. There are about 1200 species of bats worldwide that makeup one-fourth of all mammalian species (Gauteng & Northern Regions Bat Interest Group, 2020a). It is recorded that 59 species of bats occur in South Africa. Thirty-nine of them are found in Gauteng, Limpopo, Mpumalanga, and part of Northwest Province (Ecosolutions, 2018). Bats are involved in many ecosystem services that are important for the survival of a human being. At night, bats come out of their roosting sites and forage on various food items such as insects, nectars, fruits, seeds, frogs, fish, small mammals, and blood (Kunz *et al*., 2011).

Despite the above-mentioned positive roles in the ecosystem, bats are potentially a source of zoonotic diseases (Hayman *et al.,* 2013). They serve as a source of fungal diseases such as aspergillosis (Seelan *et al*., 2008), histoplasmosis, cryptococcosis, and blastomycoses (Taylor *et al*., 2000).

Although bats were identified as sources of highly pathogenic and zoonotic diseases (Kohl and Kurth, 2014), some fungal diseases with zoonotic potential have lacked adequate attention in international public health efforts leading to the lack of appropriate attention in the preventive strategies of the diseases (Seyedmousavi *et al.,* 2015). Therefore, this coupled with the lack of adequate knowledge on bats as a source of zoonotic diseases can pose a risk to the public that had direct or indirect contact with bats. Immunocompromised individuals are at a higher risk of developing a fungal infection than immunocompetent individuals. This is because immunocompromised individuals lack the basic mechanisms of cellular defence. The immunity of individuals can be compro-

mised due to prolonged treatment against malignant diseases, autoimmune diseases, immune-suppressive therapy, and immunosuppressive diseases such as leukaemia, lymphoma, or AIDS. Those individuals with deficient immunity are characterized by increased susceptibility to opportunistic infections (Silva, 2010). A review by Armstrong-James *et al.* (2014) showed that opportunistic fungal infections have been a primary driver for mortality from HIV infection since the first cases of AIDS were identified in San Francisco and New York in the early 1980s.In the Bushbuckridge community, HIV has a prevalence of over 25% in pregnant women, and human tuberculosis (TB) is a significant cause of death in the population (Bushbuckridge Local Municipality, 2016). Secondary infections with fungal diseases such as aspergillosis, histoplasmosis, cryptococcosis, or blastomycosis could thus pose a serious risk in the immunocompromised population of Mnisi Traditional Authority, Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station in the Republic of South Africa.

Therefore, the objectives of this study were to determine positive and negative human-bats-environment interactions and microbiological screening of bat faecal samples for zoonotic fungi.

Materials and methods

Study area

The study was conducted in selected villages within Mnisi Traditional Authority's area (i.e., Clare A, Share, and Utah), Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station, Mpumalanga Province in the Republic of South Africa from July to December 2018. Mpumalanga Province is located at latitude: 25° 33' 55.2096" S and longitude: 30° 31' 40.476" E. The province has an elevation of 1401.096 meters above sea level (Country Coordinate, 2018)

The Mnisi Traditional Authority's area is located in the Mpumalanga Province in the north-eastern corner of the Bushbuck-ridge Local Municipality and it is the core research and engagement area of the Mnisi Community Programme, Faculty of Veterinary Science, University of Pretoria. The Mnisi Community Programme is a multidisciplinary platform for research, teaching, learning, and community engagement within the 'One Health' philosophy (Berrian *et al*., 2016; UP, 2022).

Manyeleti Game Reserve is situated adjacent to the Mnisi community and has open access to Kruger National Park. Hans Hoheisen Wildlife Research Station is situated at Orpen gate of Kruger National Park. Manyeleti Game Reserve and Hans Hoheisen Wildlife Research Station are thus part of the Great Limpopo Trans Fontier Conservation Area (Berrian *et al.*, 2016).

Study design

A cross-sectional study was conducted on three focus groups: households and public buildings in selected villages within the Mnisi Traditional Authority's area, lodges and tourist facilities in the Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station buildings. These areas were chosen because there was a high risk of bats and humans coming into contact, either directly or indirectly. The sample size for environmental samples (bat faeces) and the questionnaire survey was determined on purpose based on the sample, and respondents' availability, respectively. Similarly, the number of bats sampled in this study was determined purposively in line with approval from the Animal Ethics Committee of the University of Pretoria under project number V062-18, Section 20 of the animal diseases act 1984 (act no. 35 of 1984), and a bat catch permit obtained from Mpumalanga Tourism and Park Agency (permit number MPB. 5622), the Republic of South Africa.

Study animals

Bats from Mnisi Traditional Authority, Manyeleti Game Reserve, and Hans Hoheisen Wildlife Research Station were included in the study.

Study methodology

Questionnaire

To identify suitable sites for sample collection, a structured questionnaire comprised of multiple-choice and open-ended questions was used with a total of thirty-three purposively selected participants in all three focus groups. Participants were also asked about their perceptions of bats and their role in disease risks, whether positive (e.g., mosquito control) or negative (e.g., pathogen source), as well as their current control measures for bat populations in their immediate environment, if any. The questionnaire also looked into potential

risk factors related to human activity at the study sites that could promote pathogen transmission.

Faecal sample collection

Faecal samples were directly collected from the 30 captured bats across four different sites: Manyeleti game reserve, Share village (household), Share village (church), and Hans Hoheisen Wildlife Research Station. Furthermore, about 25 faecal samples (2 – 5 faecal pellets) were collected from the environment at identified bat roosting sites. The environmental faecal samples were placed in solid plastic sample bottles with leak-proof lids and zip-locked plastic bags separately and transported to the Hans Hoheisen Wildlife Research Station Laboratory in a cold-chain.

Laboratory analysis of faecal samples

In a BSL2+ cabinet (Healforce, IMP), the samples were plated onto potato dextrose agar (Selecta media, #510159), and plate with antibiotics (chloramphenicol) (Selecta media, # 510184), then were incubated at 37^{0}C. The plates were sealed with Parafilm (Parafilm® M All-Purpose Laboratory Film, 100MM x 75M, Lasec). Plates were checked regularly for growth. Once growth was present, a smear was made and stained with Lactophenol cotton blue (Merck, 1137410100) and examined under a microscope (Primo star, Zeiss) with the 40x objective. The plates were then returned to the incubator (IB 25G, Labotec) for a further six weeks until final examination of the plates was done and discarded. Fungal species was confirmed based on the morphology of the fungal growth on culture plates, the temperature at which the fungi grew as well as the results of the lactophenol cotton blue stain (Quinn *et al.,* 2011).

Data management and analysis

All questionnaire and laboratory results data were recorded on a specially designed sheet and then entered into Excel spreadsheet. R statistical software was used for the analysis (R Development Core Team, 2008). For analysis, a precision of 5% and a confidence interval of 95% were used. The proportion analysis result was described using descriptive analysis.

Results

Questionnaire results

Demographic information of participants

During this pilot study, 33 people were interviewed from Hans Hoheisen Wildlife Research Station, Manyeleti Game Reserve, Share, Utah, and Clare A villages (Table 1).

Table 1. Frequency distribution of study participants

Locations	Number of participants (n)	Percent (%)
Hans Hohenstein Wildlife Research Station	6	18.2
Manyeleti Game Reserve	6	18.2
Share	8	24.2
Utah	3	9.1
Clare A	10	30.3
Total	**33**	100

Perception of participants on the presence and positive roles of bats

Almost all (97.0%) of those polled were aware of the presence or absence of bats in their immediate surroundings. However, the majority of them (87.9%) were not comfortable with the presence of these animals in their buildings, and nearly half of the respondents (48.5%) were unsure whether bats play a positive or negative role in the environment. However, some respondents (15.2%) stated that bats play beneficial roles in the environment, such as pollinating plants, spreading seeds of indigenous plants, catching harmful insects (such as mosquitoes), and aiding in research (Table 2).

Table 2. Perception of participants on the presence of bats

Variables	Category	Frequency (n)	Percent (%)
Do bats live in or around your building?	Yes	25	75.8
	No	7	21.2
	I don't know	1	3.0
Are you comfortable with the presence of bats in your building?	Yes	4	12.1
	No	29	87.9
Do bats have a positive role in your area?	Yes	5	15.2
	No	12	36.4
	I don't know	16	48.5
Do you know any problems caused by bats?	Yes	22	66.7
	No	4	12.1
	I don't know	7	21.2

Knowledge of participants in the management of bats and their droppings

In terms of bat management in buildings, the majority of respondents (84%) did nothing to remove them. However, some respondents (16%) stated that they tried to remove bats with brooms or by closing the opening where they came in. In terms of bat droppings management, 60% of participants stated that they did not use any type of protective clothing when cleaning droppings. When cleaning bat droppings, staff at Hans Hoheisen Wildlife Research Station and Manyeleti Game Reserve wear protective clothing such as rubber gloves and/or masks (Table 3).

Table 3. Knowledge of participants on the management of bats and their droppings (n=25)

Variables	Category	Frequency (n)	Percent (%)
How do you manage the bats population (if any) that dwells in your building?	I do nothing	21	84.0
	I hire an exterminator	0	0.0
	I attempt to remove them	4	16.0
Do you regularly clean bats dropping?	Yes	19	76.0
	No	6	24.0
Do you use any protective wearing when you clean bats dropping?	Always	9	36.0
	Sometimes	1	4.0
	Never	15	60.0

Knowledge of participants on bats as a source of diseases

Approximately 78.8% of those polled were unfamiliar with diseases that affect both bats and humans. Only 21.2% of respondents were aware of diseases shared by bats and humans (such as rabies). Furthermore, the majority of respondents (75.8%) were found to not know the source of diseases common to bats and humans.

Some respondents (18.2%) claimed to have contracted fungal diseases as a result of cleaning bat droppings without adequate protection. They also stated that the proximity of bats and humans, a lack of care when cleaning bat droppings, and the nature of the pathogens shared by bats and humans were major factors in disease transmission (Table 4).

Table 4. Knowledge of participants on bats as a source of zoonotic disease transmission (n=33)

Variables	Category	Frequency (n)	Percent (%)
Do you know of any disease that affects both bats and humans?	Yes	7	21.2
	No	26	78.8
Who do you think is the source of the disease common to bats and humans?	Bats	4	12.1
	Humans	1	3.0
	Other animals	1	3.0
	I don't know	27	81.8
Have you contracted any fungal disease due to cleaning droppings?	Yes	6	18.2
	No	27	81.8
Do you know what the treatment is?	Yes	7	21.2
	No	26	78.8
What do you think of the risk factors for the transmission of diseases between bats and humans?	The proximity between bats and humans	4	12.1
	Lack of care when cleaning bat dropping	3	9.1
	Nature of pathogen shared	2	6.1
	All of the above	5	15.2
	I don't know	19	57.6

Laboratory result for faecal sample analysis

Culture analysis of faecal samples revealed that bats can harbour pathogenic fungi. In this study, we isolated two species of *Aspergillus* from faeces collected in Share village and Manyeleti Game Reserve from households, churches, and directly from bats (Table 5).

Table 5. Fungal pathogens isolated from faecal samples of bats at share village and Manyeleti game reserve

Type of sample	Villages	Site of collection	Positive samples (n)	Percent (%)	Isolated pathogen
Faeces collected from captured bats (n=30)	Manyeleti game reserve	Dormitory	1	3.33	*A. flavus*
	Share village	Church	2	6.67	*A. flavus*
	Share village	Church	1	3.33	*A. flavus* and *A. niger*
Faeces collected from the environment (n=25)	Share village	Household	1	4.0	*A. niger*
		Church	2	8.0	*A. flavus*

Discussion

The current result was in line with the findings of Kunz *et al*. (2011), Banskar *et al*. (2016), and Mahandran *et al.* (2018) who reported the roles played by bats in arthropod control, seed dispersal, and pollination. In addition, the perception of participants on the presence of bats in their environment was found to be similar to the perception of western cultures described by Lewis and Oliver (1996). In contrast, Nabhan *et al*. (2010) indicated that bats were considered symbols of fortune in China. The high proportion of public discomfort due to the presence of bats in their immediate environment in our current pilot study could be due to a lack of awareness of the positive role of bats, bad smelling associated with their urine and faeces, and damages they caused to infrastructures such as roof and wall of buildings.

There are various reasons to remove bats that dwell in buildings. For example, bat colonies that live in the building may sometimes be noisy or create unpleasant odours, or some people just don't want them in their houses (due to bat phobia) (Gauteng & Northern Regions Bat Interest Group, 2020b). There is little reason to remove bats from buildings where they are not causing a nuisance. South African bats do not feed on blood, and bats are not aggressive and will not attack people. However, bats should be prevented from entering human living quarters (Gauteng & Northern Regions Bat Interest Group, 2020c) because they can serve as a potential source of zoonotic diseases (Hayman *et al.,* 2013). In addition, all necessary protective clothing should be worn when

cleaning bat droppings, as faecal materials from these animals may contain pathogenic microorganisms (De Mandal *et al.*, 2015; Yinda *et al.*, 2016).

A review done by Kohl and Kurth (2014) indicated that European bats harbour highly pathogenic viruses that have been implicated in human deaths. Similarly, we isolated 12.77% pathogenic fungi (*A. favus* and *A. niger*) from captured bats and their dropping. These fungi are known for their potent mycotoxins and cause infections in immunocompromised individuals (Ráduly *et al.,* 2020).

Regarding fungal pathogens isolated from faecal samples of bats, our current result (12.77%, n=7/55) was in agreement with a preliminary survey conducted by Seelan (2006) who isolated fungi of the genus *Aspergillus* from anal swabs of bats (56.5%) at Kubah National Park, Matang, Sarawak in Malaysia. In our study, we confirmed that bats harbour pathogenic fungi such as *A. flavus* and *A. niger*. This showed that bats can contaminate the environment with their infected faecal material and can serve as a potential source of infection to humans. Moreover, bats dropping can serve as an ideal substrate for the growth of fungi from other animals such as rodents as it is rich in organic matters. This can lead to the maintenance of fungi at the site where the bat droppings build up. These conditions together with a lack of adequate ventilation in public spaces will increase the potential risk of inhalation of a sufficient load of fungi in the air and can result in the establishment of infection in humans.

Moreover, close contact with bats and/or their droppings can lead to infection with the pathogenic organisms they harbour. Hayman *et al*. (2013) mentioned that human activities can increase exposure to bats and, therefore, protective clothing should be worn whenever cleaning bats dropping or handling them. In addition, close contact with these animals should be avoided as much as possible to minimize the risk of environmental contamination with their droppings and transmission of infections to humans. Isolation of pathogenic fungi such as *Aspergillus* species from bats mist-netted at public spaces (church and tourist facilities) at Share village, and Manyeleti Game Reserve could probably increase the risk of transmission of fungal pathogens to humans. The risk of infection with fungal diseases is of particular concern in resource-limited areas, such as rural villages where the human population is immunocompromised due to the high prevalence of HIV and human TB, and health care services are limited.

As this was a pilot study, it might have some shortcomings and limitations. We only captured 30 bats for the faecal sample collection. For this reason, we didn't collect a large number of samples and isolate all zoonotic fungal pathogens of bats that may exist in this area. Since there is a possibility that the environmental samples could be contaminated by faeces of other species, such as rodents, the fungal pathogens isolated from the environmental samples were not necessarily from bats. Moreover, we didn't characterize, at a molecular level, the *Aspergillus* isolates we have isolated in this study.

Conclusions

In the current pilot study, the majority of the participants were aware of either the presence or absence of bats in their immediate environment. However, most of them didn't have the know-how on fungal pathogens common to bats and humans. Even though bats play a beneficial role in the environment, some of the bats sampled in the study were known to harbour fungal pathogens. The isolation of pathogenic fungi like *Aspergillus* from both environmental and bat samples indicates that these animals could serve as an important source of zoonotic fungal pathogens. The presence of zoonotic pathogens, combined with a lack of appropriate care when handling bats and/or their dropping, could expose the public to bat zoonotic fungal pathogens, resulting in serious health problems. Therefore, comprehensive research should be conducted to get a complete list of fungi pathogens in the study area, a poster should be prepared as part of an awareness creation program on the ecological roles of bats for the people of the study area, and a workshop should be organized on the risk of contact and infection with zoonotic fungal diseases from bats such as aspergillosis.

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Conflict of Interest

The author has declared that no conflict of interests exists.

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