



Bats and Zoonoses: A Pilot Study to Investigate Bats as a Potential Source of Zoonotic Mycoses

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ABSTRACT

Background: Bats are mammals that are distributed everywhere in the world except in the hottest desert and polar regions. Bats are involved in many ecosystem services that are important for the survival of a human being. Despite their positive roles in the ecosystem, bats are potentially a source of zoonotic diseases. A pilot study was conducted in selected villages within Mnisi traditional authority's area, Manyeleti Game reserve and Hans Hoheisen wildlife research station, in the Republic of South Africa from July to December 2018. The main objectives of the study were: To phenotypically characterize and list the bat species, to determine positive and negative human-bats-environment interaction and to microbiologically screen faecal samples for zoonotic mycotic diseases.

Methods: A structured questionnaire composed of multiple choice and open-ended questions was employed with a total of thirty-three participants in all three focus groups, thirty bats were mist-netted and their species were identified, fifty-five faecal samples were collected from identified bat roosting sites and captured bats.

Results: About 97.0% of the respondents were aware of either the presence or absence of bats in their immediate environment. However, most of them (87.9%) weren't comfortable with the presence of bats in their buildings and nearly half of them (48.5%) did not know whether bats have a positive role or not in the environment. Some of the respondents (15.2%) mentioned bats have positive roles in the environment like pollinating plants, spreading seeds of indigenous plants, catching harmful insects, etc. Over half of the respondents (66.7%) complained bats can cause problems. About 18.2% of the respondents indicated that they have contracted fungal diseases due to cleaning bats' dropping without wearing adequate protection. The culture analysis of faecal samples revealed that bats can harbour pathogenic fungi such as *Aspergillus*. We identified the presence of three genera of bats namely: *Rhinolophus*, *Mops* and *Chaerepon*.

Conclusion: Bats can harbour similar fungal pathogens that cause disease in humans. A comprehensive research should be conducted to get a complete list of bats species in the study area and the *Aspergillus* isolates should also be subjected to further molecular characterization.

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Keywords: Bats; Republic of South Africa; Zoonotic mycotic diseases; Environment

INTRODUCTION

Background

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 make up one-fourth of all mammalian species. 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 north-west Province. 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 [1].

Despite the above-mentioned positive roles in the ecosystem, bats are potentially a source of zoonotic diseases. They serve as a source of fungal diseases such as Aspergillosis, Histoplasmosis, Cryptococcosis and Blastomycosis.

Immunocompromised individuals are at a higher risk of developing fungal infection than immunocompetent individuals. This is due to the fact that immunocompromised individuals lack the basic mechanisms of cellular defence. The immunity of individuals can be compromised due to prolonged treatment against malignant diseases, autoimmune diseases, immune suppressive therapy and immunosuppressive diseases such as leukaemia, lymphoma or Acquired Immunodeficiency Syndrome (AIDS). Those individuals with deficient immunity are characterized by increased susceptibility to opportunistic infections. A review by Armstrong-James et al, showed that opportunistic fungal infections have been a primary driver for mortality from Human Immunodeficiency Virus (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 rate of over 25% in pregnant women and human tuberculosis is a significant cause of death in the population. Secondary infections with fungal diseases are thus of major concern in this immunocompromised population of our study area.

Aspergillosis caused by *Aspergillus* species is commonly known to cause fungal pneumonia of the localized and disseminated forms in immunocompromised patients. An infection of immunocompromised persons with *Histoplasma capsulatum* can lead to respiratory and systemic diseases with high morbidity and mortality. Respiratory signs may be mild or asymptomatic in immune-competent individuals. Recently, Medina-Pinon et al, reported the detection of *Histoplasma capsulatum* in the bone marrow of an HIV-infected patient in Mexico. Similarly, Iriart et al. reported that a disseminated histoplasmosis due to *H. capsulatum* was a severe and frequent opportunistic infection in AIDS patients in South America. Another report from Ghana by de Vries et al, showed that a pilot developed acute pulmonary histoplasmosis two weeks following bathing in the water falls of Wli and the exposure was thought to be through inhalation of

an aerosol of water and guano from the large colony of fruit bats of the falls.

Cryptococcosis is caused by *Cryptococcus neoformans* and *C. gattii*. The disease may cause pneumonia and meningitis in humans with a defective cell-mediated immune response. Blastomycosis is caused by *Blastomyces dermatitidis*. The fungus is dimorphic and often found in soil or decomposing organic matter. The disease commonly affects lung or skin in immunocompromised individuals [2].

Objectives

General objectives: The study aimed to investigate if bats are a potential source of zoonotic mycotic diseases at the human-wildlife interface, in particular around tourist facilities at Manyeleti Game reserve, buildings at Hans Hoheisen wildlife research station and in rural households in villages within Mnisi traditional authority.

Specific objectives: The specific objectives of this study were:

- To phenotypically characterize and list the bat species distributed around the study sites.
- To determine positive and negative human-bats-environment interaction and to formulate potential opportunities for education and awareness regarding the ecological roles and value of bats in the study area.
- Microbiological screening of faecal samples for zoonotic mycotic diseases.
- To store faecal samples at Hans Hoheisen wildlife research station biobank for further molecular screening of zoonotic diseases.

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, in the Republic of South Africa from July to December 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. Manyeleti Game reserve is situated adjacent to 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-frontier conservation area.

Study design

A cross-sectional study was conducted on three focus groups, namely: Households and public buildings in selected villages within Mnisi traditional authority's area, lodges and tourist facilities in the Manyeleti game reserve and the buildings at Hans Hoheisen wildlife research station. These areas were chosen based on a significant risk of contact, either directly or indirectly, between bats and humans [3].

Study animals

The study animals were bats that inhabit in Mnisi traditional authority's area, Manyeleti game reserve and Hans Hoheisen wildlife research station. A total of 30 bats were randomly captured with the help of a mist net for sampling and species identification at known bat roosting areas.

Questionnaire

A structured questionnaire composed of multiple choice and open-ended questions was employed with a total of thirty-three participants in all three focus groups to identify suitable sites for sample collection. The participants were also questioned on their perception about bats and their role in disease risks, be that a positive (e.g. mosquito control) or a negative (e.g. source of pathogens), as well as their current control measures, if any, of bat populations in their immediate environment. Possible risk factors related to human activity in the study site that can promote the transmission of pathogens was also investigated through the questionnaire.

Faecal sample collection

Faecal samples were collected from the environment at identified bat roosting sites and from captured bats. Then, the faecal samples were placed in solid plastic sample bottles with leak-proof lids and each were placed in zip-locked plastic bags separately. Then, marked by date, site of collection and a unique serial number and transported in a cool, dry, airtight container on ice to Hans Hoheisen wildlife research station laboratory. Upon arrival, samples were subjected to culture at 37°C.

Bat capturing and species identification

A written consent form was presented to the owners of the building intended for mist-net erection. Upon their approval of their willingness, we erected the mist-net to capture bats. We captured bats at four different areas, namely: Manyeleti game reserve, share village (household), share village (church) and Hans Hoheisen wildlife research station. As per recommendation by Adam and Hayes, the net was placed at the

exit of bat roosting sites during the time of peak bat activity. The net was opened at sunset and kept open for about 2-3 hours after sunset. The net was closely monitored to minimize unnecessary injury to captured bats. Then, the captured bats were held in cloth bags for half an hour to collect a faecal sample. Perineal swabs of captured bats were also collected for those bats that didn't defecate at the time of sample collection. The bats were restrained by hand using the cloth bag and protective gloves.

Identification of the species of captured bats was done with the aid of the keys described by Taylor and Skinner. Accordingly, captured bats were examined for their characteristic features, their body was weighed and their forearm length was measured in millimetres. Then, all animals were released back to the environment and followed up a while for their normal activities. None of the animals involved in this study were injured or died [4].

Laboratory analysis of faecal samples

After collection, the samples were transported in sealed, impervious containers to the laboratory at Hans Hoheisen wildlife research station. The samples were plated onto potato dextrose agar, one plate each with antibiotics and one plate without antibiotics, in a biosafety level two plus (BSL2+) cabinet and incubated. The plates were all sealed with parafilm. All the plates were checked for growth daily and if any growth was found, a smear was made and stained with cotton blue. The plates were then returned to further incubate for up to six weeks. The morphology of the growth on the smears and the cultures were used to identify the species fungal growth.

Data management and analysis

All data from the questionnaire, capture and laboratory result were recorded on a sheet prepared for this purpose and then entered into excel. The analysis was done by using R statistical software. A precision of 5% and a confidence interval 95% were used for analysis. Descriptive analysis was used to describe the result of proportion analysis.

RESULTS

Demographic information of participants

Thirty-three respondents from Hans Hoheisen wildlife research station, Manyeleti Game reserve, Share, Utah and Clare A villages were interviewed during the period of this pilot study (Table 1).

Table 1: Demographic information 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 of the respondents (97.0%) were aware of the presence or absence of bats in their immediate environment. However, most of them (87.9%) were not comfortable with the presence of these animals in their buildings and nearly half of the respondents (48.5%) did not know whether bats have a

positive role or not in the environment. But some of the respondents (15.2%) mentioned bats have positive roles in the environment like pollinating plants, spreading seeds of indigenous plants, catching harmful insects (such as mosquitoes) and for study purposes. Over half of the respondents (66.7%) knew bats can cause problems such as damaging infrastructure, bad smell, sinusitis/asthma and serve as a source of infections to humans (Table 2) [5].

Table 2: Perception of participants on 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
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 on the management of bats and their droppings

Concerning the management of bats that dwell in buildings, most of the respondents (84%) did nothing to remove them. But some of the respondents (16%) indicated that they attempted to remove bats by using brooms or by closing the opening where they came in. Regarding management of bat droppings, 60% of the participants were responded that they didn't use any type of protective clothing when cleaning droppings.

The participants from villages within Mnisi traditional authority indicated that they have never worn protective clothing when cleaning bat droppings. However, the staff at Hans Hoheisen Wildlife research station and Manyeleti Game reserve use protective wears like rubber gloves and/or mask whenever they clean bat droppings (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 bats population (if any) that dwell in your building?	I do nothing	21	84
	I hire an exterminator	0	0
	I attempt to remove them	4	16
Do you regularly clean bats dropping?	Yes	19	76
	No	6	24
Do you use any protective wearing when you clean bats dropping?	Always	9	36
	Sometimes	1	4
	Never	15	60

Knowledge of participants on bats as a source of disease transmission

About 78.8% of the respondents were not familiar with diseases common to bats and humans. Only 21.2% of the respondents knew about diseases (such as rabies) shared by bats and humans. In addition, most of the respondents (75.8%) were found to have no information on the source of diseases that are common to bats and humans. Some of the respondents (18.2%) perceived to have contracted fungal diseases due to cleaning bat droppings without wearing adequate protection.

Respondents who contracted those fungal diseases were not familiar with the treatment for the cases. They also indicated, close proximity between bats and humans, lack of care when cleaning bat dropping and the nature of the pathogens common to bats and humans were major factors for disease transmission (Table 4). In addition, based on the questionnaire conducted the presence of bats in buildings, cleaning their droppings without appropriate protective wearing may serve as transmission point for fungal diseases from bats to humans (Table 5) [6].

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

Variables	Category	Frequency (n)	Percent (%)
Do you know 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
	Other animals	1	3
	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 transmission of diseases between bats and humans?	Close 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

Table 5: Possible risk factors for transmission of fungal diseases from bats to humans in Mnisi traditional authority, July to December 2018.

Variables	Fungal diseases		X ²	P-value	
	Present	Absent			
Presence of bats (n=33)	Present	6	19	2.133	0.394
	Absent	0	7		
	I don't know*	0	1		
Cleaning bat dropping regularly (n=33)	Yes	4	15	0.248	0.618
	No	2	12		
Wearing protectives when dropping (n=25)	Always	1	8	1.852	0.396
	Sometimes	0	1		
	Never	5	10		

Note: *The respondents didn't know whether bats live in their buildings or not

Bats species identification

We have identified the presence of three genera of bats namely: *Rhinolophus*, *Mops* and *Chaerepon* (Table 6).

Chaerepon pumilus and *Mops condylurus* were found to harbour fungal pathogens at Share village and Manyeleti game reserve, respectively.

Table 6: Number of bats captured for sample collection and species identification.

Site of mist-netting	Number of bats captured	Species of bat identified	Number of bats positive for fungal pathogens
Hans Hoheisen wildlife research station	3	<i>Rhinolophus</i> spp.	0
Manyeleti Game reserve	14	<i>Mops condylurus</i>	1
Share village	13	<i>Chaerepon pumilus</i>	3

Laboratory result for faecal sample analysis

The culture analysis of faecal samples from the environment and bats revealed that bats can harbour pathogenic fungi. In this study, we isolated two species of *Aspergillus* from faecal samples collected at household, church and directly from bats themselves in share village and Manyeleti Game reserve. However, none of

the samples were cultured positive for *Blastomyces*, *Cryptococcus* and *Histoplasma* species. Faecal samples collected from other localities were cultured negative for all fungal pathogens (Table 7) [7].

Table 7: 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	<i>A. flavus</i>
	Share village	Church	2	6.67	<i>A. flavus</i>
	Share village	Church	1	3.33	<i>A. flavus</i> and <i>A. niger</i>
Feces collected from the environment (n=25)	Share village	Household	1	4	<i>A. niger</i>
		Church	2	8	<i>A. flavus</i>

DISCUSSION

The current result was in line with the findings of Kunz, et al. Banskar et al., and Mahandran et al., 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. In contrast, Nabhan et al., indicated that bats were considered as symbols of fortune in China although this perception in modern Chinese society is less important. 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 lack of awareness on 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. 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 due to the fact that they can serve as a potential source of zoonotic diseases. In addition, all necessary protective clothing should be worn when cleaning bat droppings, as faecal materials from these animals may contain pathogenic microorganisms [8].

Although bats were identified as sources of highly pathogenic and zoonotic diseases, some fungal diseases with zoonotic potential have lacked adequate attention in international public health efforts that lead to the lack of appropriate attention in the preventive strategies of the diseases. 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 have direct or indirect contact with bats.

A review done by Kohl and Kurth indicated that European bats harbour highly pathogenic viruses that have been implicated in human deaths. Similarly, we isolated pathogenic fungi (*A. flavus* and *A. niger*) from captured bats and their dropping. These fungi are known for their potent mycotoxins and cause infections in immunocompromised individuals [9].

Regarding fungal pathogens isolated from faecal samples of bats, our current result was in agreement with a preliminary survey conducted by Seelan who isolated fungi of the genus *Aspergillus*

from anal swabs of bats 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*. In addition, their droppings that were sampled from the environment were also confirmed to contain similar pathogenic fungi. This showed that bats can contaminate the environment with their infected faecal material and can serve as a potential source of infection to humans. 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. However, bats dropping can serve as an ideal substrate for the growth of fungi from other sources 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 lack of adequate ventilation in public spaces will increase the potential risk of inhalation of sufficient load of fungi in the air and can result in establishment of infection in humans.

Moreover, close contact with bats and/or their droppings can lead to infection with pathogenic organisms they harbour. Hayman et al., 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 tuberculosis and health care services are limited [10].

CONCLUSION

In the current pilot study, three genera of bats (*Rhinolophus*, *Mops* and *Chaerepon*) were identified from the study area. In addition, 97% of the participants were aware of either the presence or absence of bats in their immediate environment. However, 87.9% of them were not comfortable with the presence of these animals in their surroundings. This can be due to the fact that these animals are causing extensive damages to infrastructures like walls and roofs, lack of awareness on important ecological roles of bats and bad smelling associated with their urine and

faeces. The isolation of pathogenic fungi like *Aspergillus* from both environmental and bat samples obviously indicate that these animals can serve as an important source for zoonotic fungal pathogens. About 78.8% of the participants didn't have the know-how on fungal pathogens common to bats and humans. In the study area bats were found to live inside the roofs and walls of households, public buildings such as a church and tourist facilities. Furthermore, most of the individuals (60%) that clean bats dropping have never used appropriate protective clothing when handling the droppings. The presence of zoonotic pathogens coupled with lack of appropriate care when handling bats and/or their dropping can pose a risk to the public to zoonotic fungal pathogens of bats and result in significant health problems. Therefore, a workshop should be organized with the staff at public buildings and tourist facilities to create awareness on how to handle bats that live inside buildings and clean their droppings in a safe manner.

As this was a pilot study, it might have some shortcomings and limitations. We only captured 30 bats for species identification. For this reason, we might not completely list all species of bats that live in the study area and also, we didn't collect large number of samples and isolate all zoonotic fungal pathogens of bats that may exist in this area. Moreover, we didn't characterize, at molecular level, the *Aspergillus* isolates we have isolated in this study.

RECOMMENDATIONS

Therefore, based on the current understanding and findings, the following points have been noted:

- A comprehensive research should be conducted to get a complete list of bats species 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.
- A workshop should be organized on risk of contact and infection with zoonotic fungal diseases from bats such as *Aspergillosis*.
- A further molecular study should be conducted on the isolates we have isolated in this study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was obtained from the university of Botswana animal ethics committee (project number V062-18) to capture and sample bats. An approval was also obtained from university of Botswana research ethics committee (project number REC058-18) and the Mpumalanga tourism and parks agency to collect specimens from the Mnisi traditional authority's area, Manyeleti Game reserve and Hans Hoheisen wildlife research station. A verbal consent was obtained from participants for questionnaire as approved by the research ethics committee. A written consent form was also presented to the owners of the building intended for mist-net erection.

AVAILABILITY OF DATA AND MATERIAL

Data is stored electronically in the university of Botswana repository for a period of five years. A hard copy of the results is stored at Hans Hoheisen wildlife research station.

COMPETING INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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AUTHOR'S CONTRIBUTIONS

All authors have read and approved the manuscript.

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