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Original Article

Indicator Enteric Microbes Associated with Selected Domesticated Pets in Mvita Sub-County, Mombasa County- Kenya

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ABSTRACT

Domesticated pets provide various benefits to humans, at the same time, human animal interactions can pose health risks such as zoonosis which are associated with microbial mutations and dreadful epidemics. The purpose of this study was to establish health risks associated with exposure to domestic dogs and cats' faeces where faecal specimen were collected and enteric microorganisms identified using standard microbiological techniques. Rotavirus was the most prevalent enteric virus at 28.1% with 20.7% and 7.4% prevalence among cats and dogs respectively (RR 2.811). The prevalence of adenovirus was 4.2% with a distribution of 2.9% and 1.3% in dogs and cats respectively (RR=1.02). The prevalence of intestinal helminths was 20.3% with hookworms reporting the highest prevalence of 16.1% (RR=1.09). Coagulase negative staphylococci was isolated among 18.8% of the animals, pathogenic E. coli was isolated among 1% of the animals and streptococcus species were isolated from 8.5% of the animals (RR=1.0). The study findings indicate potential health risks posed by close association of domesticated cats and dogs as reflected by RR ≥1.00. It is envisaged that the study findings will guide the development of a policy framework to manage and control zoonotic diseases associated with animal faeces.

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INTRODUCTION

Animals play a pivotal role in transmitting and maintaining infections caused by micro-organisms. Diseases from non-human origin and can affect humans are referred to as zoonosis (WHO, 2014). Bacteria, viruses, parasites, or unconventional agents can cause zoonotic diseases. Approximately two thirds of all human-infective organisms trace their origin from extensive diversity of animal hosts (Penakalapati *et al.,* 2017). Domesticated animals such as poultry, sheep, cattle, goats, and pigs are important source of zoonotic infections. In addition, animals kept as pets such as cats, dogs, and some poultry play major roles as sources and transmitters of zoonotic diseases (Penakalapati *et al.,* 2017). The transmission, causation, and maintenance of the diseases is attributed to close interactions between the animals and the people who keep them. Zoonotic diseases are not only a major public health concern but also a social economical concern resulting in decreased production of animal origin foods and decrease in international trade in animal products (WHO, 2014). Understanding the possible origin point of transmission routes of various microorganisms emanating from these animals can help curb the menace of zoonoses.

Africa is home to a wide spectrum of zoonotic diseases with East and West Africa being hotspots for the occurrence of new and re-appearance of the old diseases from wildlife and domesticated animals. Numerous outbreaks have been reported with viral and bacterial inclined diseases taking the centre stage (Kemunto *et al.,* 2018). In Kenya, various studies have been conducted on emerging new and old diseases but the origin of some of these diseases still remaining unknown (Munyua *et al.,* 2016).

Despite modern healthcare and quantitative studies, diseases caused by micro-organisms are still ranked top as the causes of human sufferings and deaths in the various parts of the world. Sporadic outbreak of zoonotic diseases and microbial mutations in most parts of East Africa has posed a significant challenge. More so neglected zoonotic diseases and those that are thought to cause diseases of less magnitude are the understudied group that could also contribute to a major cause of sporadic illness (Fèvre *et al.,* 2017). The experiences reveal the need for quality studies that are geared to establishing the original point of transmission which would help understand the disease transmission dynamics and further inform on better mechanisms that could be considered to enable decrease in disease transmission mutations and disease outbreaks (Kemunto *et al.,* 2018).

Problem Statement

Mombasa County is among the few towns in Kenya where families live in CBD and in close proximity with domestic animals and pets due to the small space in homes. Domesticated animals can be seen roaming around in the garbage piles scavenging for food thus exposing them to microorganisms that are transmissible to humans. In the recent past, multiple sporadic outbreaks of diseases have been reported in the county with the source remaining unknown. Whether these animals act as a source of zoonoses or microorganisms that contribute to mutation is not known.

Justification

There is no organized effort by the local government to control the movement and vaccination of stray pets and those that stay at home. There is also

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paucity of data in the county on the health risks the animal faeces pose to human health.

Research Objectives

The main objective of the research was to determine the presence of indicator micro-organisms, present in faeces. The specific objectives were: to investigate for the presence of selected enteric viruses from faecal material of domesticated cats and dogs; to determine the presence of intestinal helminths from faecal material of domesticated cats and dogs; to determine the presence of indicator enteropathogenic bacteria from faecal material of domesticated cats and dogs.

Research Questions

- Are there enteric viruses of health concern that are excreted by domesticated dogs and cats?
- Which intestinal protozoa and helminths are common in faeces of domesticated dogs and cats?
- Are there indicator enteropathogenic bacteria in faeces of domesticated dogs and cats?

LITERATURE REVIEW

Domesticated pets have over the generations adapted to live alongside humans (Lear, 2012)**.** Domesticated animals provide many benefits to humans, at the same time, human animal interactions can pose health risks in terms of transfer of infectious agents such as viruses, bacteria, protozoa, fungi, and helminths from animals to humans resulting to zoonosis (CDC, 2017). Zoonoses and drug resistant microbial agents are an emerging challenge to the human and animal welfare with high risks of deadly disease outbreaks (Dafale *et al.,* 2020). The distribution of Zoonoses is global, transcending the natural boundaries. Their distribution is enhanced by international trade practices which have less restrictions during international movement of humans and importation of animals and animal products (Venkatesh *et al.,* 2016). The burden of zoonoses is eminent and effective control including global surveillance is necessary. Through close international relations among different countries, internationalization of control efforts should be intensified and should be retained as a priority among the actions of international agencies of each region (Venkatesh *et al.,* 2016).

Ecological changes occasioned by urbanization, industrialization, and population growth leading to either migration of population to urban areas has led to changes on the treads of certain zoonotic diseases globally. Most of the emerging diseases of mankind have been attributed by interspecies transmission. Most of the drug resistance strains of microbes have been linked to elements of organisms thought to have limited disease-causing ability from animals and human origin (Parkhe & Verma 2021). Despite these reports, endemic zoonoses still remain unprioritized. The poor approaches to these diseases contribute to a vicious cycle of ill-health, development of serious drug resistance strains with limited treatment options and subsequently poverty (Record, 2015).

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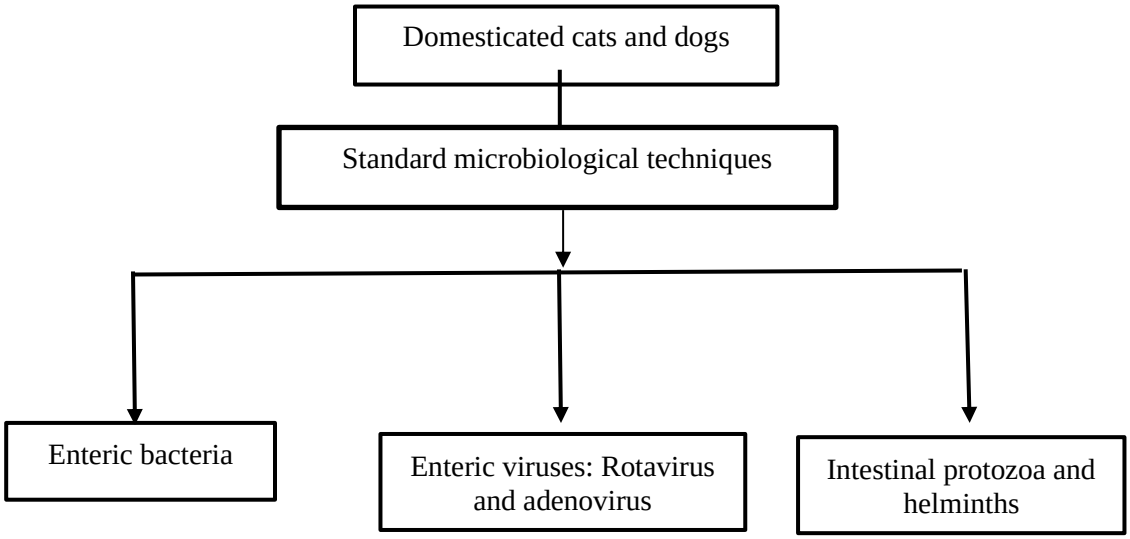
Conceptual Framework

Figure 1: Conceptual Framework



METHODOLOGY

Study Design

Cross–sectional design and snowball sampling techniques were adopted in this study. Cross sectional study design was preferred for this study because the target population was large and aimed at a snapshot data which could be a baseline for further studies. Snowball sampling technique was considered because the study subjects identification depended on referrals from the residents.

Study Area

Multi-level sampling approach was used where Mvita sub-County, in Mombasa County was purposively selected as the study location due to its location within Mombasa municipality and its organization with a cosmopolitan population.

Study Population

Domesticated cats and dogs were considered as the study population. Only animals that lived within the homestead or sleep in the homestead were included in the study.

Sample Size Determination

Fisher (1998) formulae was used in determination of the sample size.

$$Sample\ size = \frac{Z^2 pq}{d^2}$$

Where z - The normal deviant, set at 95% confidence interval (1.96); p – The prevalence based on previous studies (this study used 36% based on Munyua *et. al*. (2016); q = 1.0 – p; d = the absolute error, set at 0.05.

$$Z^2 = \frac{3.84 \times 0.36(1-0.36)}{0.0025} = 354$$

Snow balling approach was used to pick a sample of size 354. This was drawn proportionately to the Ward population size. Table 1 presents the sample size per Ward.

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Table 1: Sample Size per ward

Ward	Population size	Sample Picked
Mji wa kale	25,875	64
Tudor	31,200	79
Tononoka	27,513	68
Shimanzi/ Ganjoni	18,806	47
Majengo	38,834	96
Total	143,128	354

Sample Collection and Analysis

Faecal samples were considered for the study. Fresh faecal samples were collected in sterile wide mouthed universal containers from one cat and dog or either present in a homestead. All samples were transported to the investigating laboratory in an ice lined cool box.

Laboratory Procedures

Processing Stool Samples for Bacteria Isolation and Detection

Animal faecal specimen for bacteriology were inoculated into ringer's solution for extraction from the faecal debris and then inoculated into MacConkey broth. Subsequently content of tubes with growth were sub cultured into the following enteric media; MacConkey with and without crystal violet and bile salts, and *Salmonella-Shigella* (SS*)* agar for isolation and identification of enteric bacteria. All cultures were incubated at 37 °C for 24 h.

Examination of Growth and Selection of Isolates

Macroscopic observation was done on the culture plates after the lapse of the incubation time for visible colonial growth. The plates without any growth were reported as negative while those with growth had the growth pattern described, noted and further investigation was carried out.

Identification of Bacteria

Gram staining was done on the selected colonies. Gram positive bacteria were subjected to catalase, coagulase, and oxidase testing. The gram-negative bacteria were subjected to an array of biochemical testing including triple Sugar Iron agar, Indole and Citrate utilization tests. All the testing panels were performed as described in the Clinical Microbiology procedures handbook (Isenberg, 2007). The biochemical reactions of *Enterobacteriaceae* in bio-typing media were interpreted as provided by the standard guidelines.

Storage of Bacterial Isolates

Identified organisms were inoculated in nutrient agar plates and incubated overnight at 37 ^{o}C. Thereafter, the bacteria were emulsified in Tryptic Soy broth containing 15% glycerol in cryotubes and stored at –70^{o}C for further analysis.

Analysis for Intestinal Protozoa and Helminths

The faecal material for parasitology analysis was processed as per the stool consistency state. Liquid, soft and formed stools were examined within 30 minutes, 1 hour and 24 hrs of passage respectively. Direct methods of stool analysis including; wet stool mount and wet iodine mounts were carried out to investigate for the motile, cystic and ova forms of the organisms. The formed stools which turned negative after direct examination were subjected to formal ether sedimentation concentration technique and processed accordingly.

Analysis for Enteric Viruses

Analysis for enteric viruses was done using the commercially available antigen rapid test kits; Rotavirus and Adenovirus combo rapid test device which is designed to detect through the immunochromatographic technique. The processing and analysis of the samples was done as per the manufacturer's instructions.

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Storage of Positive Samples for Further Analysis

All the samples that tested positive for Rotavirus and adeno virus were stored at -70ºC for molecular characterization.

Waste Disposal

All waste was segregated into the right color-coded bags and was then disposed in accordance with local clinical waste disposal policy.

Ethical Consideration

Ethical approval and clearance were sought from the TUM Ethical review committee TUM ERC no. TUM ERC EXT/004/2020. Confidentiality of all information obtained remains upheld.

Data Analysis

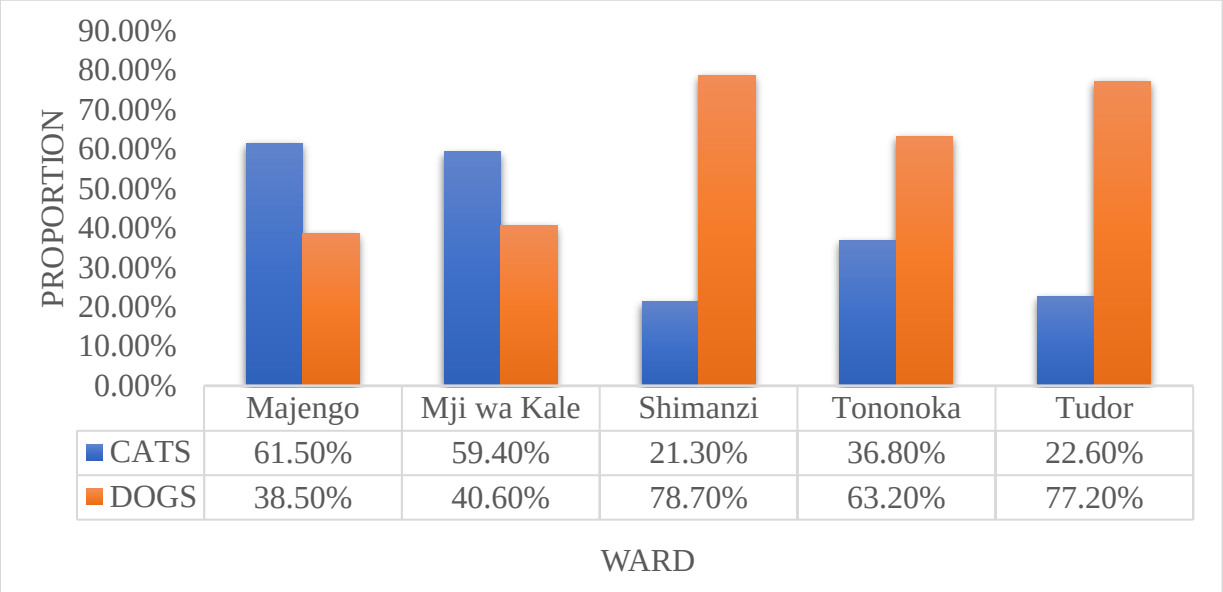
Statistical analysis will be done using SPSS version 21. Descriptive analysis was done where proportions of cases were calculated. The association between different variables was done using Chi squire ($\chi^{2)}$ and the relative risk was calculated.

RESULTS

Distribution of the Domesticate Animals Sampled Per Ward

Majengo Ward had the highest number of pets constituting 27.11% of all the pets sampled. Majengo had the highest number of cats while Tudor had the highest number of dogs (*Figure 2*).

Figure 2: Pets Distribution Per Ward



	Majengo	Mji wa Kale	Shimanzi	Tononoka	Tudor
CATS	61.50%	59.40%	21.30%	36.80%	22.60%
DOGS	38.50%	40.60%	78.70%	63.20%	77.20%

Rotavirus Distribution Among Pets

There were higher proportions of Rotavirus in cats than dogs. 20.7% cases of Rotavirus were reported among cats compared to 7.4% among the dogs (*Figure 3*).

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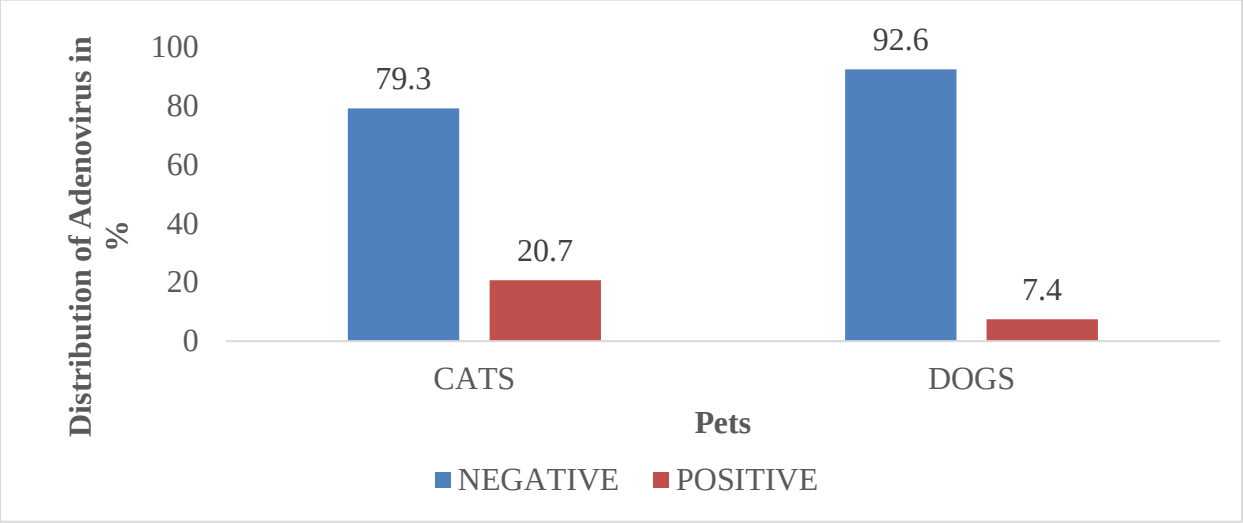
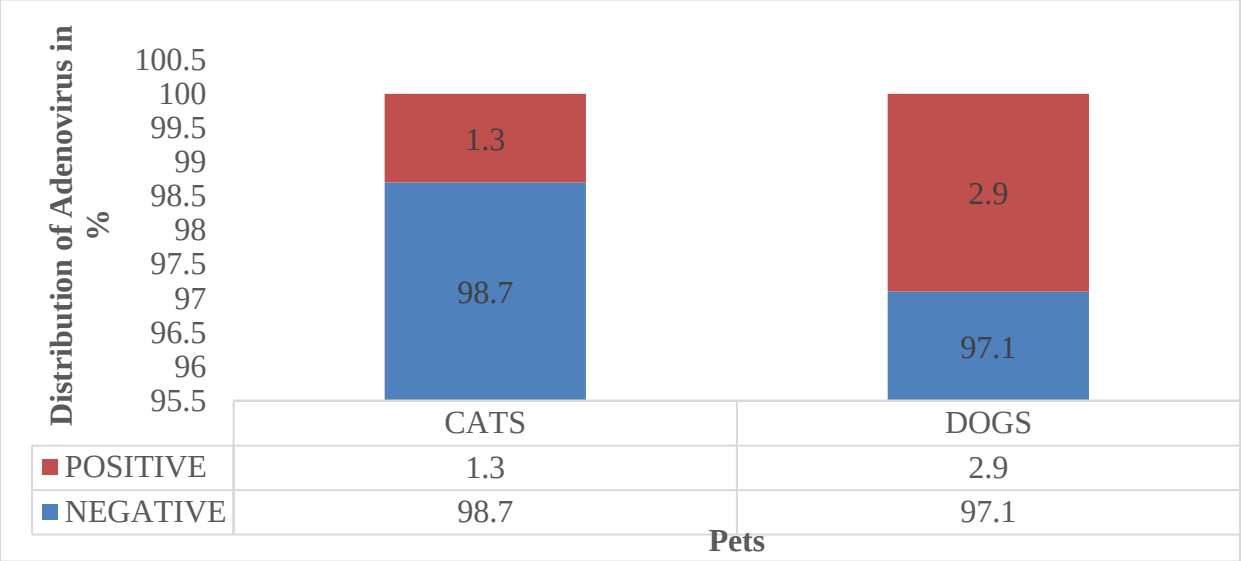
Figure 3: Rota Distribution



Adenovirus Distribution Among Pets

The proportion of cases of Adeno virus reported were almost equal between cats and dogs. The chances of a cat getting Adeno virus is equal to that of a cat (*Figure 4*).

Figure 4: Adenovirus Distribution



Helminths Distribution among Pets

The helminths ova/ larvae were reported evenly between the two pets, Larvae of *S. strongyloides*, ova of fluke and Ova of *T. trichura* were found only among the dogs. Ova of *H. worms* was more common among the cats than dogs (*Figure 5*).

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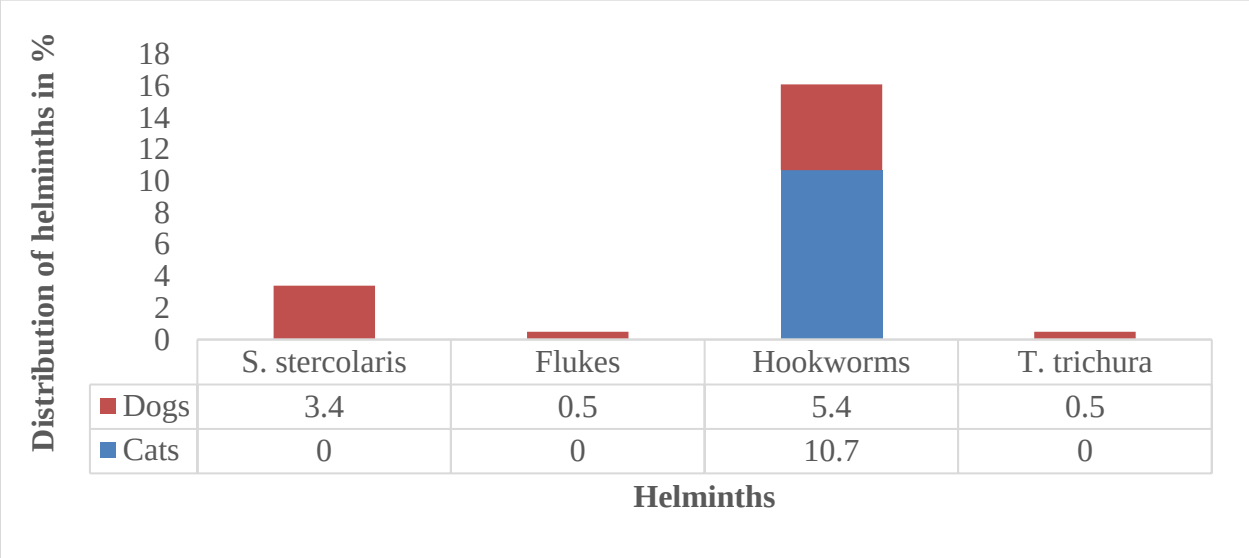
Figure 5: Parasites Distribution



Bacteria Distribution among Pets

The bacteria isolated from the animal samples were coagulase negative staphylococci species, coagulase positive staphylococci species, *E. coli* and streptococcus species (*Table 2*).

Table 2: Bacteria distribution

Bacteria distribution		Type of Animal		Total
		Cat	Dog	
coagulase negative staphylococci species	Count	9	26	35
	% within type of animal	6.0%	12.8%	18.8%
E. coli	count	0	2	2
	% within type of animal	0.0%	1.0%	1.0%
streptococcus species (catalase & coagulase negative, gram positive cocci in chains)	count	6	3	9
	% within type of animal	4.0%	2.5%	6.5%
Total	count	150	204	354
	% within type of animal	100.0%	100.0%	100.0%

Table 3: Association between Type of pet and presence of indicator micro-organism

Micro organism	Indicator	N	Cat (%)	Dog (%)	X^2 (Fisher exact)	Df	P value	Relative Risk
Rota virus	Negative	308	119 (79.3)	189 (92.6)	13.552	1	0.000	2.811
	Positive	46	31 (20.7)	15 (7.4)				
Adenovirus	Negative	346	148 (98.7)	198 (97.1)	1.012	1	0.475	1.017
	Positive	8	2 (1.3)	6 (2.9)				
Helminths	Larvae S. Stercoralis	7	0	7 (3.4)	3.894	4	0.017	1.0880
	Ova of Fluke	1	0	1 (0.5)				
	Ova of *H. worm*	27	16 (10.7)	11 (5.4)				
	Ova of T. trichura	1	0	1 (0.5)				

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Micro organism	Indicator	N	Cat (%)	Dog (%)	X^2 (Fisher exact)	Df	P value	Relative Risk
	coagulase negative staphylococci	35	9 (6)	26 (12.8)	13.74	6	0.002	1.000
	E. coli	2	0	2(1)				
	streptococcus species (catalase & coagulase negative, gram positive cocci in chains)	9	6(4)	3(2.5)				

Table 3 shows that:

- There is significant association between the type of pet and presence of rotavirus. Cats have higher risk of getting Rota virus compared to dogs. The relative risk of 2.811 implies that both animals are likely to get the Rota virus.
- There is significant association between the type of pet and presence of Adenovirus. The relative risk is 1.017 which implies that both animals are equally likely to get the Adenovirus.
- There is significant association between the type of pet and presence of helminths. The relative risk is 1.0880 which implies that both animals are equally likely to get the helminths.
- There is significant association between the type of pet and presence of bacteria. The relative risk is 1.000 which implies that both animals are equally likely to get the helminths.

DISCUSSION

Interspecies transmission of diseases between livestock, domesticated animals, and humans is a common phenomenon in society which is likely to contribute to sporadic outbreaks of diseases among the human population. Four groups of enteric micro-organisms: viruses, helminths, protozoa, and bacteria associated with domesticated cats and dogs' zoonotic diseases and excreted via faeces were investigated in this study.

Rotavirus was the most isolated enteric virus at 28.1% with 20.7% of cats and 7.4% of dogs testing positive. The findings indicated a significant association between the type of pet and presence of rotavirus. Cats had higher risk of getting Rota virus compared to dogs at a relative risk of 2.811. Past studies done by German *et al.* (2015) and De Grazia *et al.* (2007) report the detection of Rotaviruses from canines with a high probability of a gene reassortment which could result to disease outbreaks. Although feline and canine Rotaviruses are not known to cause severe diseases in animals, the same viruses have been isolated in symptomatic patients and characterized indicating animal- human transmission (German *et al.*, 2015).

On the other hand, 4.2% of the animals tested positive for Adenovirus, with 1.3% of the cats and 2.9% of the dogs testing positive. Isolation of adenovirus from dogs and cats has been reported in studies done by Alves *et al.* (2018) and Ongrádi *et al.* (2019) among others. Several studies have documented adenoviral zoonosis potential (Borkenhagen *et al.*, 2019). The possibility of adenoviruses crossing the host species barriers poses a risk of emergence of new virus strain and epidemics with negative human consequences.

Parasitic intestinal helminths and protozoa were screened from the faecal material of the study animals and no protozoa parasites were reported. Various intestinal helminths were detected from 20.3% of the animals. Larva of *S. stercolaris* was isolated from 3.4% of the study animals all being dogs. Vast studies report isolation of *S. stercolaris*

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from dogs, cats, and other animals. Some studies argue that dog associated *S. stercolaris* are zoonotic while others report host specific population phenomenon (Štrkolcová, *et al*., 2017). A study done by Jaleta *et al.* (2017), report a possible occurrence of different and overlapping populations of *S. stercolaris* among dogs and humans. *S. stercolaris* causes strongyloidiasis which is usually asymptomatic but an alteration of the immune system status from immuno-competence to immuno-suppression, which can result to fatal fulminant diseases (Forrer *et al.,* 2017).

Eggs of hookworms were detected in 16.1% of the animals, 10.7% being cats and 5.4% being dogs. Feline and canines are hosts of different Hookworm species with *Ancylostoma braziliense, A. ceylanicum, A. caninum* and *A. tubaeforme* being the most prevalent species. These species are implicated with zoonotic disease the cutaneous larva migrans in humans and severe anaemia among the affected animals (Fu *et al*., 2019, Traversa, 2012).

Three different indicator bacterial species were isolated from the animals' samples. Coagulase negative staphylococci species are considered as opportunistic organisms affecting both man and animals. They have been treated as contaminants in the past but currently they have been reported to be among the organisms with and contributing to multi- drug resistance (Lu *et al.,* 2020). The fact that they are widely spread makes the transmission of the resistance genes easy complicating the treatment approaches. Streptococci species which were catalase negative, gram positive cocci in chains were also notably isolated and these organisms were formerly classified as group D Streptococcus and reclassified as enterococcus under a family; Enterococcaceae. These organisms have been previously reported from other studies as zoonotic. They also have been associated with the multi drug resistance where co-transfer of resistance to human species has been reported (Stępień-Pyśniak *et al.,* 2021).

The study findings indicate potential health risks posed by close association of domesticated cats and dogs' excreta, with both animals harbouring different type of microorganisms of public health interest at different relative risk.

The findings call for close surveillance of domesticated animals in order to give the necessary treatment and vaccinate them regularly. There is need for further follow up molecular studies which can characterizes the isolated microbes. This will flag up the existence of gene reassortment and possible occurrence of new disease strains.

CONCLUSION AND RECOMMENDATIONS

The study findings indicate potential health risks posed by close association of domesticated cats and dogs' excreta, with both animals harboring different type of microorganisms of public health interest at different relative risk. The findings call for close surveillance of domesticated animals in order to give the necessary treatment and vaccinate them regularly. There is need for further follow up molecular studies which can characterizes the isolated microbes. This will flag up the existence of gene reassortment and possible occurrence of new disease strains.

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