



Prevalence of Some Pathogens in a Population of Zoo Animals

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Key Words: zoo, pathogens, bacterial, parasitological, veterinarian, exotic	ABSTRACT: Zoos unintentionally provide pathogens with a high diversity of species of different origins. Zoo practices of mixing reservoir species with other susceptible species can provide opportunities for pathogens to spread beyond normal hosts. This paper describes some pathogens of bacterial, parasitological and viral origin that were identified in some bovines (five species), caprines (two species), cervids (two species), primates (two species) and felines (two species) groups. Bacterial examination of fecal samples revealed the detection of E.coli, Salmonella spp., Pasteurella spp., Klebsiella spp., Campylobacter spp. Streptococci spp., and Staphylococci spp., with 52% overall prevalence of infection. Parasitological investigation using floatation and sedimentation technique of fecal samples indicated the occurrence of Isopora spp., Trichuris spp., Ascarids spp., Toxocara spp., Trichostrongyloid spp., and Nematodirus spp. with 19% prevalence of infection of the examined samples. Sarcoptic mange was only identified in olive baboon, <i>Papio anubis</i> through examination of skin scrapings. Antibodies against bovine viral diarrhea (BVD) and bovine herpes virus-1 (BHV-1) in antelopes and feline corona virus (FCoV) in felines were detected using specific Enzyme-linked immune assay (ELISA test). The seroprevalence of BVD and BHV-1 in the examined antelopes was 5.3% and 6.7%, respectively. Antibodies against FCoV were detected in both lions and cheetahs where cheetahs had higher seroprevalence rate (100%) than lions (50%). There is a need for zoo veterinarians to review and update the current preventive and management policies to identify sources of infection and control diseases of exotic species in future.
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1-INTRODUCTION

As natural habitats for wild animals shrink in size, the number of threatened species rises. Therefore, conservationists are obliged to seek other secured facilities to propagate species of interest before they face the blink of extinction. Zoological parks provide for this purpose essential reservoir of genetic materials for such species through captive breeding and re-introduction programs. However, zoo animals are often exposed to a variety of pathogens that pose immediate risks on the survival of threatened species, a situation exaggerated by climatic changes that leads to substantial loss in biodiversity (Harvell et al., 2002).

This article reviews some of the pathogens that were suspected and identified in a population of zoo

animals with their prevalence rate in 2014. Selection of subject animals was based on either its global status or health history. Bacterial and viral pathogens selected for detection were not part of the zoo vaccination program.

2- MATERIALS AND METHODS

2.1-Animals and housing

Animals that were selected for the study included 9 species of artiodactyles (Black buck; *Antelope cervicapra*, Water deer; *Hydropotes intermis*, Sitatunga; *Tragelaphus spikii*; Barbary sheep; *Ammotragus lervia*, Mouflon; *Ovis orientalis*, Scimitar horned Oryx; *Oryx dammah*, Dorcas gazelle; *Gazella dorcas*, Axis deer; *Axis axis*, Fallow deer; *Dama dama*), 2 species of carnivores (African lion; *Panthera leo*, Cheetah; *Acinonyx jubatus*) and 2

species of primates (Olive baboon; *Papio annubilis*, Patas monkey; *Erythrocebus patas*). All animals belonging to the artiodactyles were housed in open air single enclosures provided with sandy/ grassy or rocky substrates and protected by iron railing perimeter fences at Kuwait zoo, Kuwait. Primates were contained in wire roofed cages with concrete floors and carnivores were contained in completely protected enclosures made of interwoven steel mesh nets and provided with sandy/grassy substrates. Animal population dynamics for that year, including total population size, birth, and mortality per species was plotted.

2.2- Fecal samples for bacteriological and parasitological examination

A total of 270 fecal samples were collected for bacteriological (160 samples) and parasitological examination (110 samples). Samples were freshly collected from enclosure floors in less than 24 hrs after defecation using polyethylene sterile bags and clearly labeled by species. For bacteriological examination three grams of fecal samples by species was mixed with normal saline and centrifuged and the deposit was inoculated with 5ml of buffered peptone water (Oxoid) and incubated at 37 °c for 24 hrs. Samples were streaked on differential, selective and enrichment agars (McConkey, Manitol salt and EMG agars) and the plates were incubated overnight at 37 °c. For the identification of E.coli Brilliant green broth fitted with Durham tubes was used and incubation at 45 °c took place for 48 hrs. Suspected colonies with gas bubbles were tested for indole production. Identification of bacteria by light microscopy based on morphology, colony size and staining characteristics was carried out according to Christopher and Bruno (2003).

For parasitological examination sedimentation and floatation technique was applied to the samples according to Soulsby (1992). Portions of the samples used for the identification of coccidian oocytes were mixed with 2.5% potassium dichromate solution at room temperature for 24 hrs to enhance their growth. All processed samples for parasites, eggs and larvae were inspected microscopically for identification. Skin scrapings were collected from visible skin lesions in some animals and spared for ectoparasite investigation.

2.3- Blood samples for serological tests

A total of 75 blood samples were collected using sterile vacutainer tubes and centrifuged at 2000 rpm for 20 minutes for serum collection and froze at -20 °c

for assay (Mahmoud and Ahmed, 2009). Serum samples were used to measure antibody titers for bovine viral diarrhea (BVD) using commercial ELISA test (IDEXX laboratories, West brook, Maine, Bottcher et al., 1993), Bovine herpesvirus 1 (BHV-1) using blocking ELISA test, searching for glycoprotein B (IDEXX, Switzerland, Ezzi et al., 2013) and feline corona virus (FCoV) using ELISA test (FIP Ag test, Biotech co, Ltd, Shanghai, China, Mosallanejad et al., 2012). Procedures for testing all the samples followed the manufacturer's instructions. These viral diseases are not considered part of our vaccination program.

3- RESULTS

3.1- Animals

Population dynamics, including total population size, birth and mortality in 2014 is plotted in Fig.1.

3.2- Bacterial and Parasitological findings

Out of 160 fecal samples examined for bacterial pathogens, only 83 samples proved to be positive with 52% prevalence rate of infection. The types of bacteria isolated were *Escherichia coli*, *salmonella* spp., *Pasteurella* spp., *Klebsiella* spp., *Campylobacter* spp., *Staphylococcus* spp. and *Streptococcus* spp.

As shown in Table (1) the prevalence of *Escherichia* was high in Axis deer (63%) and low in Barbary sheep (7%). The highest prevalence rate of *Salmonella* spp. was recorded in fallow deer (15%) while the highest prevalence rate of *pasteurella* spp. was recorded in Scimitar (33%). *Klebsiella* spp. pathogens were isolated from only two species, Dorcas and Mouflon with 42% and 9% prevalence rates of infection, respectively. Patas monkey, lions and cheetah showed positive results for *Campylobacter* spp. with prevalence rate of 33%, 28% and 33%, respectively. *Streptococci* spp. were isolated from only Barbary sheep while *staphylococci* spp. were recorded in Scimitar, Mouflon and Patas monkey with higher prevalence rate in the later (33%).

For parasitological examination 21 fecal samples out of 110 samples were positive for endo and exo-parasites with 19% overall prevalence rate of infection. As shown in table (2) sarcoptic mange was only isolated from Olive baboon while *Isopora* protozoan was isolated from lions and cheetah. With regard to round worms *Trichuris* spp., *Ascarids* spp., *Trichostrongyloid* spp., *Nematodirus* spp. and *Toxocara* spp. were identified in Sitatunga, Water deer, Dorcas, Barbary sheep, Mouflon, Scimitar,

Black buck, Patas monkey, Lions and Cheetah (Table 2).

3.3- Seroprevalence of some pathogenic viruses

The types of viruses identified and species involved are shown in Table (3). The seroprevalence for BVD was 5.3% in the selected species (higher in Sitatunga, 60%, low in Scimitar, 11%) except for Axis deer, Barbary sheep, Water deer, Dorcas and Fallow deer that showed no evidence of antibodies detection for the virus in blood sera. The seroprevalence for BHV-1 was 6.7% in the selected species (Axis, 27% and Fallow, 25%) except for Sitatunga, Barbary sheep, Scimitar, Dorcas, Water deer and Black buck that showed no evidence of antibodies detection for the virus in blood sera. With feline specific virus the seroprevalence of FCoV was 50% in lions and 100% in cheetahs.

4- DISCUSSION

Animals shed many genera of bacterial pathogens in their feces with some pathogens of zoonotic importance (Brittingham et al., 1998), including *Escheishia coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Yersinia*. In the current study *E.coli* was the most prevalent and wide spread among the different animal groups in the zoo. Among bacteria of zoonotic importance *E. coli* can range in virulence from a nonpathogenic commensal pathogen to a highly virulent enteropathogenic organism, such as in O157:H7 serovar (Strauch, 1991). This

organism, therefore, can pose a real health risk for both animals and humans in case of an outbreak (Keen et al., 2007). The significance of *E.coli* infection and its spread in animal surroundings is primarily based on the organism's ability to persist in soil, water, manure and feed where other animals in the vicinity of the infected species can pick the microbe (Hancock et al., 1998). Other bacterial organisms not of less importance than *E.coli* were isolated in this study from the animals and are also of zoonotic importance, including *Salmonella* spp., *Pasteurella* spp., *Streptococci* spp., *Staphylococci* spp. and *Campylobacter* spp. These organisms, in addition of their zoonotic importance to animal keepers and visitors, provide potential microbial threats to zoo animals (Chomel et al., 2007). Oludario et al.(2013) reported 5% prevalence of salmonella spp. in captive mammals and birds and concluded that captive zoo animals can serve as asymptomatic carriers shedding the microbe in feces. Similarly, Ostrowski and Anajariya (2002) concluded that *Pasteurella* bacterium is carried freely in Oryx and cause disease when stressed, a condition that need to be prevented through vaccination. *Campylobacter* spp., on the other hand, is known as a common cause of gastroenteritis in many animals. Misawa et al.(2000) isolated 23 (22.1%) thermophilic campylobacter from seven zoo mammals and four birds and concluded that campylobacter spp. in zoo animals are highly divergent.

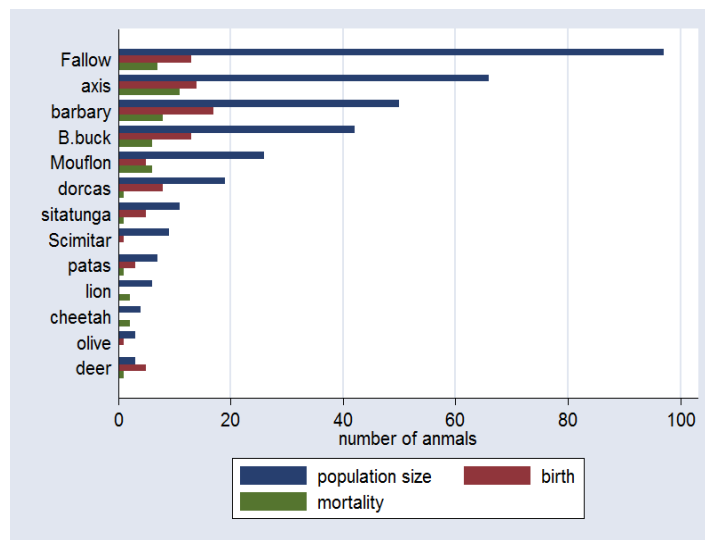


Fig 1. Population dynamics of zoo species in the study

Table 1. Prevalence of some bacterial infection isolated from fecal samples

Animal	No. of samples	Bacteria			Species			Staph. spp	Strept. spp	Sum (%)
		E.coli Spp	Salmonella spp	Pasteurlla Spp	Klebsiella Spp	Campylobacter Spp				
Sitatunga	3	1(33)								
Black buck	15	4(26)		3(20)						
Dorcas	12	3(25)			5(42)					
Water deer	7	4(57)								
Barbary sheep	29	2(7)	3(10)						3(10)	
Mouflon	32	5(16)		4(13)	3(9)			1(3)		
Axis deer	16	10(63)		2(13)						
Fallow deer	20	9(45)	3(15)							
Scimitar	9		1(11)	3(33)				3(30)		
Olive baboon	4	1(25)								
Patas monkey	3	1(33)				1(33)		1(33)		
Lion	7	3(43)				2(28)				
Cheetah	3	1(33)				1(33)				
Total # samples	160									
Total # positive		44	7	12	8	4	5	3		83(52%)

Values in parenthesis indicate percentage prevalence

Table 2. Prevalence of some parasitic infection isolated from fecal samples

Animal	No. of samples	Isopora Spp	Trichuris spp	Ascarids spp	Toxocara spp	Tristrongyl Spp	Nematodirus Spp	Sarcoptic spp	Sum
Sitatunga	2		+ (1)			+(1)			
Black buck	3					+(1)			
Dorcas	4			+(1)					
Water deer	7		+ (1)						
Barbary sheep	22		+(1)			+(2)			
Mouflon	20		+(1)			+(1)			
Axis deer	15						+(1)		
Fallow deer	15						+(1)		
Scimitar	9		+(1)						
Olive baboon	3							+(1)	
Patas monkey	2		+(1)	+(1)					
Lion	5	+(1)		+(1)	+(1)				
Cheetah	3	+(1)			+(1)				
Total # samples	110								
Total +ve		2	6	3	2	5	2	1	21 (19%)

Values in parenthesis indicate frequency of positive samples

Table 3. Prevalence of viral infection in the selected species

Species	Antibodies to BVD	Antibodies to BHV-1	Antibodies to FCoV
	+Ve	+Ve	+Ve
Sitatunga (n= 5)	(3/5)	(0/5)	
Axis deer (n=11)	(0/11)	(3/11)	
Barbary sheep (n=21)	(0/21)	(0/21)	
Scimitar (n=9)	(1/9)	(0/9)	
Dorcas (n=3)	(0/3)	(0/3)	
Water deer (n=4)	(0/4)	(0/4)	
Black buck (n=8)	(0/8)	(0/8)	
Fallow deer (n=8)	(0/8)	(2/8)	
Lion (n=4)			(2/4)
Cheetah (n=2)			(2/2)
Total (75)	4	5	4

Of all the helminthic infections recorded in the zoo collection of herbivores, Strongloids spp., Trichuris spp. and Nematodirus spp. were dominant in bovidae and certain species of Cervidae. Among carnivores, Toxocara spp., Ascarids spp. and Blantidium protozoan were the major parasitic infection in lions and cheetah. Occurrence of Ascarids and Toxocara infestations in wild animals have been reported by many workers (Lim et al., 2008, Mahmoud and Azazi, 2014), an indication of unhygienic conditions maintained in animal enclosures. Other factors, where feral cats roam in the vicinity of exotic carnivores and contaminate feed, may facilitate the occurrence of Toxocara spp. infection in these animals (Mahmoud and Azazi, 2014). Abe and Yasukawa (1996) also reported that Toxocara spp. was the most common round worms in felidae and canidae as a result of contaminated feed. This study, however reported less prevalence rate of parasitic infestation in zoo animals compared with the findings of Mahmoud and Azazi (2014) for the same surroundings. Therefore, it is of a paramount importance to survey diseases of zoo animals at a periodical interval, not only because of its zoonotic importance but due also to the detrimental effects of these diseases on the survival of endangered species. The international union of the conservation of nature (IUCN, 2004) Red List reports that in the past 500 years, 833 animal species are known to have gone extinct. Of these known extinctions, only 3.7% have been attributed, at least partly, to infectious diseases (Smith et al., 2009). Over 24% of the world's extant mammals are currently threatened with extinction, yet infectious diseases has only been listed as a major

threat for a small fraction (1.1%) (IUCN, 2007). This indicates that diseases are underrepresented as a contributing factor to wildlife extinction (Pederson et al., 2007).

The serological investigation for virus identification in this study, on the other hand, reported the occurrence of BVD and BHV-1 in some bovidae and cervidae species in the collection. Although the overall prevalence of BVD and BHV-1 in this study was low the viruses are still of high medical importance for infecting a wide range of hosts and the ability to spread beyond normal hosts (Nelson et al., 2014). Pathogens often have a limited host range, however human practices that mix reservoir species with novel, hence susceptible species can provide opportunities for pathogens to spread beyond normal host (Daszak et al., 2000). Previous studies have reported relatively high prevalence values of BVD and BHV in Arabian Oryx both under captive and free ranging conditions (Frolich et al., 2005). In addition, the existence of BVD infection in Sitatunga as reported in this study suggests that they can serve as a potential reservoir of the virus in other captive species through cross contamination (Passler et al., 2007).

The study, on the other hand, recorded a high prevalence of FCoV in both lions and cheetah with very high seroprevalence and mortality rate in cheetah (100%). Feline corona virus is a fatal immune-mediated disease that infects members of the family felidae with results ranging from seroconversion with no disease to fatal feline infectious peritonitis (FIP) (Kennedy et al., 2002). Previous studies have shown that exotic felidae, such as tigers, lions and cheetahs

are highly susceptible to FCoV infection (Kennedy et al., 2003). So there is an urgent need to adopt better management and medical protocols that enhance the immunity of susceptible species such as cheetahs which are already immune-compromised hosts as a result of homozygosity (Lin, 1992).

In conclusion, one would say that zoos unintentionally provide pathogens with a high diversity of species from different origins and habitats assembled within confined spaces that should alert authorities to take necessary preventive measures. The incidence of some diseases that are not part of the zoo vaccination protocol make our zoo veterinarians review the current vaccination and management programs to identify sources of infection and reduce rate of prevalence.

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