original article

Association between *Papio hamadryas* populations and human gastrointestinal infectious diseases in southwestern Saudi Arabia

Mohammed A. Alqumber

From the Department of Laboratory Medicine, Albaha University, Albaha, Saudi Arabia

Correspondence: Dr. Mohammed A. Alqumber · Department of Laboratory Medicine, Albaha University, PO Box 1988, Albaha, Saudi Arabia · T: +966568677142 F: +966177253185 · dr.alqumber@gmail.com

Ann Saudi Med 2014; 34(4): 297-301

DOI: 10.5144/0256-4947.2014.297

BACKGROUND AND OBJECTIVES: *Papio hamadryas* baboons, known reservoirs of several infectious diseases, roam and deposit their excreta indiscriminately on footpaths, parks, and streets of the city peripheries of Taif, Baha, and Abha in southwestern Saudi Arabia. Nonetheless, city centers of these places are free of baboons. This study aims to determine the impact of baboons on human gastrointestinal health.

DESIGN AND SETTINGS: This is a descriptive cross-sectional analytical ecological study conducted in 3 cities located in southwestern Saudi Arabia between July 2011 and July 2012.

MATERIALS AND METHODS: We investigated the impact of these baboons on the human health through a coprological survey of infectious agents of baboons and humans in these 3 cities using macroscopic and microscopic analyses, before and after parasite concentration, and culturing of bacteria on selective and differential media, which were then identified by 16S rDNA gene sequencing. Baboon fecal samples (n=823) were collected from city peripheries. Two groups of human fecal samples, each consisting of 795 samples were collected, one from city centers and the second from city peripheries where baboons intermingle with the human population.

RESULTS: Baboon fecal samples were the most contaminated with infectious agents, except for *Staphylococcus aureus*, which was more commonly present in human fecal samples collected from city peripheries. Human fecal samples collected form city peripheries showed higher rates of most infective agents than those collected from city centers.

CONCLUSION: This indicates that baboons are medically important reservoirs of infectious agents associated with higher human coproprevalence of gastrointestinal infectious agents.

Microbial infections are a worldwide problem causing millions of deaths annually. It is estimated that more than 40 million individuals are infected worldwide with helminthic or protozoan parasites alone.[1] Baboons and several other nonhuman primates can get infected and become carriers of human bacteria, and helminthic and protozoan parasites.[2-8] The southwestern regions of Saudi Arabia are known to be natural habitat for *Papio hamadryas*.[9] Fruits and vegetables can also get contaminated, during their growth, collection, or transportation, with animal feces or with soil or water contaminated with their feces.[10] Moreover, getting the hands contaminated with such soil or water before eating can infect children. *Papio hamadryas* in Saudi Arabia are know to act as reservoir for several pathogenic bacteria and parasites such as *Escherichia coli*, *Giardia intestinalis*, *Entamoeba histolytica*, *Hymenolepis nana*, *Enterobius vermicularis*, *Ascaris sp*, *Trichuris sp*, and hookworms.[3,11] Nonetheless, an accurate determination of the prevalence rates of infectious agents in this reservoir and its links to the prevalence of these same infectious agents in neighboring human populations in southwestern Saudi Arabia is not yet determined. An attempt to link the prevalence of infectious agents in the 2 populations can provide useful information about the health risks of *P hamadryas*. In this study, we aimed to calculate the prevalence of infectious diseases in human populations living in proximity to *P hamadryas* and

compare it to human population living in locations free of *P hamadryas* within the same cities.

MATERIALS AND METHODS

Study design

The study was conducted in Saudi Arabia from July 2011 to July 2012. All sampled nonhuman primates belonged to wild *P hamadryas* sp of the southwestern region of Saudi Arabia, which commonly intermingle with humans at the peripheries of cities. Three sampled populations from 3 cities, Taif, Baha, and Abha, about 250 km apart were studied. In addition, 2 groups of human fecal samples were collected: one from public toilets (inside mosques) located at city centers and the other from toilets of mosques located at peripheries of the studied cities where monkeys commonly intermingle with the human population. City centers are defined as areas surrounded with a minimum of 1-km radius of 95% fully constructed land sections, roads, and shops and located within the city center or Central Business District as defined by the local municipality. City peripheries are areas located at the boundaries of the city and surrounded by immediate neighboring native natural land uninhabited by humans. The city centers are characterized by being completely free of any baboons roaming into these highly populated human areas. By contrast, baboon herds commonly roamed the peripheries. More than one herd roamed the city peripheries that were chosen for human sample collection.

Samples

Fecal samples were collected every Friday afternoon during the sampling period and then fixed with 70% ethanol immediately after collection. To ensure baboon fecal samples were fresh, a Dacagon Pawkitt water activity meter (Decagon Devices, Inc., 2365 NE Hopkins Ct., Pullman, WA 99163, USA) was used for *P hamadryas* fecal samples. Samples that were greater than 0.85 a_w were selected for this study and those with lower water activity were rejected. Human fecal samples were also collected on Friday afternoons from toilet seats of mosques after the weekly ritual of Friday prayers. Moist fresh fecal samples were collected directly from toilet seats. The seats were cleaned every Friday morning before the afternoon collection. Before the fixation of fecal samples, visual examination was performed and a spatula used to look for visible helminthes. Then a direct examination of fecal sample was carried out with the wet preparation of fecal samples stained with eosin, iodine, and Ziehl-Neelsen and examined microscopically. Identified helminthes were fixed with 70% ethanol, cleared with Gater solution, and inspected microscopically. On Friday night, or the next Saturday morning by the latest, the fecal samples were processed by a formalin-ether concentration method using Fecal Parasite Concentrator kit (Evergreen Scientific, Los Angeles, USA). The morphology of identified infective agents was microscopically determined and sizes were measured. The identification of infective agents (larvae, adult worms, and cysts) was according to the published standard.[12-18]

Bacterial isolation

Bacteria were inoculated on rich, selective, and differential or chromogenic media used for the identification of methicillin-resistant *Staphylococcus sp*, *Clostridium sp*, *Campylobacter sp*, *Mycobacterium sp*, *Enterobacteriaceae*, *Streptococcus sp*, *Enterococcus sp*, and *Pseudomonas aeruginosa*. The media used were Columbia Sheep Blood agar, Mannitol Salt agar, MacConkey agar, Skirrow agar, Campy-BAP agar, Salmonella–Shigella agar, Bile Esculin agar, Lowenstein-Jensen medium, and cooked meat medium (Becton Dickinson and Company, Riyadh, Saudi Arabia).

Identification of bacteria

The 16S rDNA primers used to help establish the species identity of the isolates were those described previously.[19] Multiple isolates for each colony type were subjected to 16S rDNA amplification, and then the resulting products were sequenced. DNA extraction was performed using a Qiagen DNeasy tissue kit (QIAGEN, Hilden, Germany) according to the instructions provided, using 4-hour logarithmic growth phase cells in Todd–Hewitt Broth (Difco Laboratories, Le Pont de Claix, France). The 16S primers were used to amplify 16S rDNA genes from chromosomal DNA of the test isolated using the PCR amplification conditions described previously.[19] Finally, the similarities of the obtained sequences to those of known species were determined using the BLASTN alignment searches program within the GenBank DNA database.[20]

Methicillin susceptibility testing This was performed using oxacillin disk diffusion tests on all obtained staphylococcal isolates followed by Oxacillin M.I.C.E E-test strips (Oxoid, Hampshire, UK) according to the manufacturer's instructions.

Formol ether concentration technique

Four grams of fecal material form each specimen were suspended in 7 mL of 10% formol water, mixed well, and passed to a centrifuge tube containing 3 mL diethyl ether. The mixture was then vortexed further for 1

minute, centrifuged at 1000 g for 10 minute, and then the fecal debris, ether, and formol water were decanted. The generated sediments were examined microscopically before and after staining with iodine and Ziehl-Neelsen.

RESULTS

A total of 1342 *P hamadryas* fecal samples were collected from city peripheries at the dusk of clear (not rainy) days, of which 823 showed water activity greater than 0.85 aw and were selected for further analysis. Human samples, 795 in number, were collected from mosques within city centers, and an equal number of samples were collected from mosques located at city peripheries. The fecal samples yielded a mean of 4×10^7 cfu/g on blood agar and 7×10^5 cfu/g on MacConkey agar. On MacConkey agar, 70% of the isolates were lactose non-fermenters. The exact number and percentages of occurrence of different infectious agents in the 3 different sample types are presented in **Figure 1** and **Table 1**, respectively.

The rate of occurrences of infectious agents from baboon fecal samples were higher than that from human fecal samples, except for *Staphylococcus aureus* where the human samples showed a slightly higher rate of this bacterium (72.9%) than baboon fecal samples (71.5%). However, the differences were not statistically significant (P=.2451). Nonetheless, the rate of occurrence of this bacterial pathogen was significantly higher ($P=7.55\times10^{-3}$) in fecal samples obtained from city peripheries (75.6%) than in samples obtained from city centers (70.2%).

Moreover, human fecal samples obtained from city peripheries exhibited significantly higher occurrence rates for all pathogens than samples obtained from city centers, except for Schistosoma mansoni where human samples obtained from city centers displayed a slightly higher occurrence rate of this pathogen (0.75%) than human samples obtained from city peripheries (0.25%). However, this difference was not statistically significant ($P=7.78\times10^{-2}$). Nonetheless, the rate of occurrence of S. mansoni in baboon fecal material was significantly higher than that detected in human samples (z-score=17.6431, P=0). **Figure 1** depicts the percentages of different pathogens detected in the 3 different sample types, and **Table 1** depicts the exact numbers of positive samples, and percentage of prevalence per pathogen type, and the P value for the two human populations.

S aureus was the most commonly detected pathogen in this study, detected in 71.5% of baboon samples, 75.6% of human fecal samples obtained from city pe-

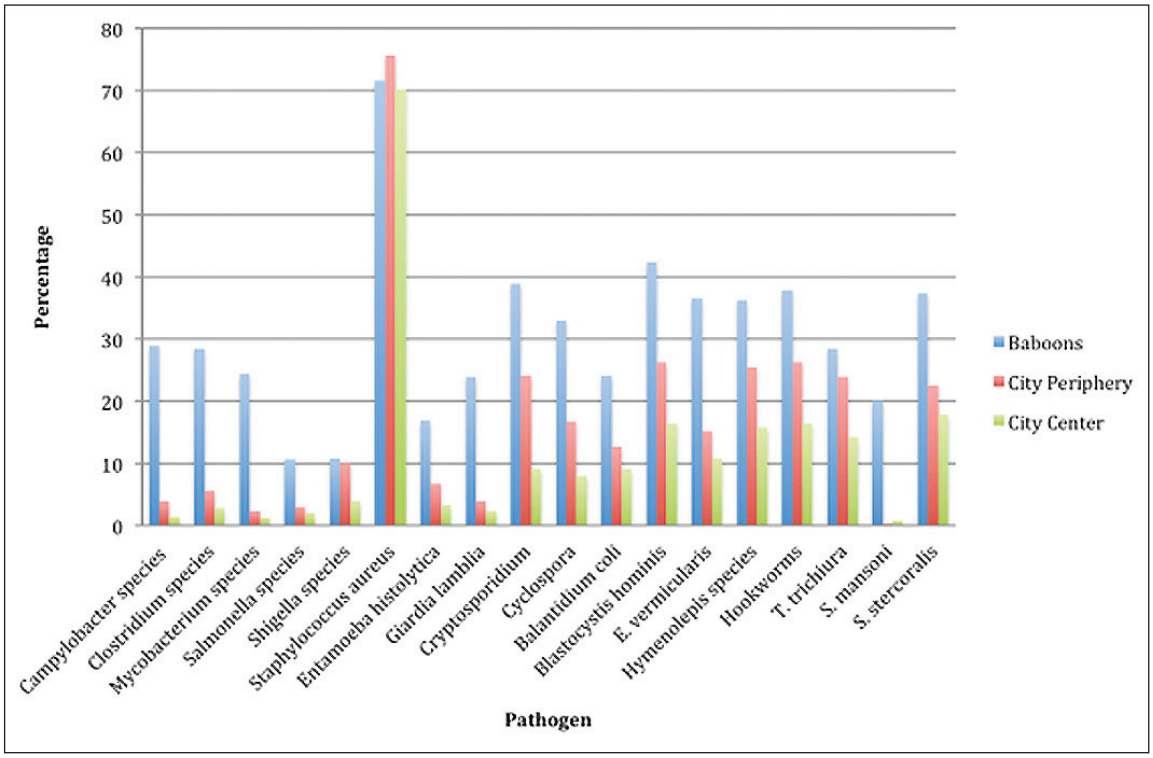

Figure 1. Parasitic presence in percentage of human and baboon fecal samples.

ripheries, and 70.2% of human samples obtained from city centers. Other pathogenic bacteria identified include *Campylobacter sp, Clostridium sp, Mycobacterium sp, Shigella sp*, and *Salmonella sp*.

Hookworms and *Strongyloides stercoralis* were the second most commonly detected pathogens (found in 18%-37.4% of tested samples). Other helminthes were also commonly detected. These commonly identified helminthes were *Hymenolepis sp* (16%-36.2%), *E vermicularis* (11%-36.6%), and *Trichuris trichiura* (14.2%-28.4%). The overall infection rate with the various gastrointestinal helminthes was 41.9%, 21.4%, and 17.8% of fecal samples obtained from baboons, and human fecal material obtained from city peripheries and city centers, respectively.

Table 1. Details of the presence of microbial pathogen in human and baboon fecal samples.

Infective agent	Baboon samples (n=823) Number (%)	Human samples		P^a
		City periphery (n=795) Number (%)	City center (n=795) Number (%)	
Campylobacter sp	238 (29)	31 (3.9)	11 (1.4)	8.7×10^{-4}
Clostridium sp	234 (28.4)	45 (5.7)	22 (2.8)	2.05×10^{-3}
Mycobacterium sp	201 (24.4)	19 (2.4)	10 (1.2)	4.551×10^{-2}
Salmonella sp	88 (10.7)	23 (2.9)	16 (2)	.1292
Shigella sp	89 (10.8)	79 (10)	31 (3.9)	0
Staphylococcus aureus	589 (71.6)	601 (75.6)	558 (70.2)	7.55×10^{-3}
Entamoeba histolytica	139 (16.9)	53 (6.7)	26 (3.3)	9.0×10^{-4}
Giardia lamblia	197 (24)	31 (3.9)	19 (2.4)	4.272×10^{-2}
Cryptosporidium	320 (38.9)	191 (24)	72 (9)	0
Cyclospora	271 (33)	133 (16.7)	63 (8)	0
Balantidium coli	198 (24)	101 (12.7)	72 (9)	9.64×10^{-3}
Blastocystis hominis	349 (42.4)	209 (26.3)	131 (16.5)	0
Enterobius vermicularis	301 (36.6)	121 (15.2)	86 (10.8)	4.53×10^{-3}
Hymenolepis sp	298 (36.2)	202 (12.7)	126 (15.8)	0
Hookworms	311 (37.8)	209 (26.3)	131 (16.5)	0
Trichuris trichiura	234 (28.4)	190 (23.9)	113 (14.2)	0
Schistosoma mansoni	165 (20)	2 (0.25)	6 (0.75)	7.78×10^{-2}
Strongyloides stercoralis	308 (37.4)	179 (22.5)	142 (17.9)	1.044×10^{-2}

[a]Calculated between the two human samples.

Bold indicates that the prevalence rates of infectious agents for the 2 human samples are significantly different.

Other microbial pathogens identified include the protozoa *Balantidium coli, Giardia lamblia, E histolytica, Blastocystis hominis, Cyclospora*, and *Cryptosporidium*. These were detected at lower rates than the above-mentioned pathogens (see **Figure 1** and **Table 1**).

DISCUSSION

In this study we investigated whether living at the peripheries of southwestern cities of Saudi Arabia, where baboons dwell, is associated with a higher infectious rate with baboon-carried diseases. The obtained results revealed a significant elevation in pathogen prevalence in baboon fecal samples than in human fecal samples, except in the case of *S aureus*, where human fecal samples bore a slightly higher prevalence rate that was not statistically significant (P=.2451). Moreover, there was a significantly higher occurrence of all tested infectious pathogen, except *S mansoni* and *Salmonella sp*, in human fecal samples collected from mosques from cities' peripheries infested with baboons than not infested cities' centers.

Schistosoma mansoni transmission requires the presence of suitable snails shedding cercariae, which have a short life, in addition to an aquatic environment from which the cercariae must penetrate the skin of potential hosts to cause an infection.[21-25] These requirements mean that simple contamination of hands or food with fecal-polluted soil material is incapable of transmitting *S mansoni*. Therefore, simply living in areas where baboon troops are dwelling is insufficient to increase the rate of infection with this trematode pathogen, even when baboons are already considered a maintenance host for *S mansoni* in Saudi Arabia.[26] That is, a shared water body between the two populations needs to exist. None of the sampled areas provided this condition.

In this study, human carriage of *Salmonella sp* did not show any association with baboons. This pathogen is known to cause serious diseases in humans and animals. Some serovars such as *Salmonella enterica subsp enterica serovar Typhi* and *S paratyphi* are highly adapted to humans without other known natural hosts, while others such as *S typhimurium* have a broad host range and infect a wide variety of animal hosts. Typhoid fever caused by *S typhi*, self-limited enterocolitis by *S typhimurium*, and septicemic diseases with little involvement of the gastrointestinal tract by *S choleraesuis* are the most common diseases caused by these serovars.[27,28] Therefore, the lack of association between the baboon carriage rate of this pathogen and human samples may be due to the host specificity of the different serovars of *Salmonella*.

Moreover, *S aureus*, which is a common opportunistic

pathogen, is found in 20% to 55% of healthy adults.[29-31] This may explain why this pathogen was slightly more commonly recovered from human samples. Its higher rate in samples obtained from the peripheries of the studied cities, even when baboon samples showed a lower prevalence, could be associated with other unknown reasons. Nonetheless, monkeys can also carry this pathogen and get infected with it.[32,33]

In summary, we examined the infectious agent composition, prevalence, and their possible impact on human health by examining a total of 2413 fecal samples from baboons and human populations to determine the impact of the baboon population carrier rates on human infections. The results of this study showed a significantly higher occurrence of all tested infectious pathogen, except *S mansoni* and *Salmonella sp*, in human fecal samples collected from areas infested with baboons. However, there was no statistically significant difference in the rate of occurrence of *Salmonella sp, Shigella sp, Cryptosporidium, Cyclospora, B hominis*, and *S mansoni* between the two human sample types.

Acknowledgment

The author thanks the staff and students of the Department of Laboratory Medicine, Faculty of Applied Medical Science, Albaha University for their support.

REFERENCES

1. Proceedings of the 2nd Seminar on Food-Borne Parasitic Zoonoses: Current Problems, Epidemiology, Food Safety and Control. Khon Kaen, Thailand, 6-9 December 1995. Southeast Asian J Trop Med Public Health 1997;28 Suppl 1:1-226.
2. Murray S, Stem C, Boudreau B, Goodall J. Intestinal parasites of baboons (Papio cynocephalus anubis) and chimpanzees (Pan troglodytes) in Gombe National Park. J Zoo Wildl Med 2000;31:176-8.
3. Ghandour AM, Zahid NZ, Banaja AA, Kamal KB, Bouq AI. Zoonotic intestinal parasites of hamadryas baboons Papio hamadryas in the western and northern regions of Saudi Arabia. J Trop Med Hyg. 1995;98:431-9.
4. Nizeyi JB, Mwebe R, Nanteza A, Cranfield MR, Kalema GR, Graczyk TK. Cryptosporidium sp. and Giardia sp. infections in mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. J Parasitol. 1999;85:1084-8.
5. Landsoud-Soukate J, Tutin CE, Fernandez M. Intestinal parasites of sympatric gorillas and chimpanzees in the Lope Reserve, Gabon. Ann Trop Med Parasitol. 1995;89:73-9.
6. Appleton CC, Boinski S. A preliminary parasitological analysis of fecal samples from a wild population of Costa Rican squirrel monkeys (Saimiri oerstedi). J Med Primatol. 1991;20:402-3.
7. Pedersen AB, Altizer S, Poss M, Cunningham AA, Nunn CL. Patterns of host specificity and transmission among parasites of wild primates. Int J Parasitol. 2005;35:647-57.
8. Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci. 2001;356:983-9.
9. Lawson Handley LJ, Hammond RL, Emaresi G, Reber A, Perrin N. Low Y chromosome variation in Saudi-Arabian hamadryas baboons (Papio hamadryas hamadryas). Heredity (Edinb). 2006;96:298-303.
10. Slifko TR, Smith HV, Rose JB. Emerging parasite zoonoses associated with water and food. Int J Parasitol. 2000;30:1379-93.
11. Nasher AK. Zoonotic parasite infections of the Arabian sacred baboon Papio hamadryas arabicus Thomas in Asir Province, Saudi Arabia. Ann Parasitol Hum Comp. 1988;63:448-54.
12. Gasbarre LC, Smith LL, Lichtenfels JR, Pilitt PA. The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. Vet Parasitol. 2009;166:281-5.
13. El-Gayar AK. Studies on some trematode parasites of stray dogs in Egypt with a key to the identification of intestinal trematodes of dogs. Vet Parasitol. 2007;144:360-5.
14. Eamsobhana P, Boranintra K. Identification of fecal parasites in the quality assessment programme for the year 1984-1987, in Thailand. J Med Assoc Thai. 1989;72:11-5.
15. Smith JW. Identification of fecal parasites in the special parasitology survey of the College of American Pathologists. Am J Clin Pathol. 1979;72:371-3.
16. Godfrey DG. Identification of economically important parasites. Nature 1978;273:600-4.
17. Scholten TH, Yang J. Evaluation of unpreserved and preserved stools for the detection and identification of intestinal parasites. Am J Clin Pathol. 1974;62:563-7.
18. Pitsch E. Laboratory identification of intestinal protozoan parasites. Am J Med Technol. 1945;11:256-62.
19. Wilson KH, Blitchington RB, Greene RC. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. J Clin Microbiol. 1990;28:1942-6.
20. Altschul SF, Madden TL, Schaffer AA, Zhang J, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389-402.
21. Ghandour AM, Zahid NZ, Banaja AA. Epidemiological study on the transmission of schistosomiasis in Saudi Arabia (western region). Ann Trop Med Parasitol. 1999;93:193-5.
22. Youssef AR, Cannon JM, Al Juburi AZ, Cockett AT. Schistosomiasis in Saudi Arabia, Egypt, and Iraq. Urology. 1998;51:170-4.
23. al-Madani AA. Dissemination of snail intermediate hosts of schistosomiasis in Assir Province, Saudi Arabia. J Egypt Soc Parasitol. 1988;18:35-45.
24. Wallace DM. Urinary schistosomiasis in Saudi Arabia. Ann R Coll Surg Engl. 1979;61:265-70.
25. Arfaa F. Studies on schistosomiasis in Saudi Arabia. Am J Trop Med Hyg. 1976;25:295-8.
26. Zahed NZ, Ghandour AM, Banaja AA, Banerjee RK, Dehlawi MS. Hamadryas baboons Papio hamadryas as maintenance hosts of Schistosoma mansoni in Saudi Arabia. Trop Med Int Health. 1996;1:449-55.
27. Medrano-Felix A, Estrada-Acosta M, Jimenez M, Gomez-Gil B, Leon-Felix J, et al. Draft Genome Sequence of Salmonella enterica subsp. enterica Serotype Oranienburg Strain S-76, Isolated from an Aquatic Environment. Genome Announc. 2013;1.
28. Eswarappa SM, Janice J, Nagarajan AG, Balasundaram SV, Karnam G, et al. Differentially evolved genes of Salmonella pathogenicity islands: insights into the mechanism of host specificity in Salmonella. PloS one. 2008;3:e3829.
29. McAnally TP, Lewis MR, Brown DR. Effect of rifampin and bacitracin on nasal carriers of Staphylococcus aureus. Antimicrob Agents Chemother. 1984;25:422-6.
30. Leedom JM, Kennedy RP, Lepper MH, Jackson GG, Dowling HF. Observations of the staphylococcal nasal carrier state. Ann N Y Acad Sci. 1965;128:381-403.
31. Hu L, Umeda A, Kondo S, Amako K. Typing of Staphylococcus aureus colonising human nasal carriers by pulsed-field gel electrophoresis. J Med Microbiol. 1995;42:127-32.
32. Trots AA, Dzhikidze EK, Stasilevich ZK. [Biological properties and ecological variants of Staphylococcus aureus strains isolated from Macaca rhesus monkeys]. Zh Mikrobiol Epidemiol Immunobiol. 1984:21-5.
33. Pollack M, Weinberg WG, Hoskins WJ, O'Brien WF, Lannini PB, et al. Toxinogenic vaginal infections due to Staphylococcus aureus in menstruating rhesus monkeys without toxic-shock syndrome. J Infect Dis. 1983;147:963-4.