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ORIGINAL RESEARCH

Study on bacterial flora in the Hanuman langur (*Presbytis entellus*) of the Gujarat state, India

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Abstract

Aim: To study the prevalence of bacterial flora in the Hanuman langur (*Presbytis entellus*) of the Gujarat state

Material and Methods: Thirty hanuman langur (*Presbytis entellus*) (20 male and 10 female), were screened for bacterial flora during the period of June, 2010 to March, 2011. Hanuman langur (*Presbytis entellus*) were screened by culture of nasal, oral and rectal swabs during routine health monitoring and samples incubated using appropriate media and specific selective culture methods. Bacterial organisms from the normal as well of affected with various diseases and disorders conditions viz. infected wound, electric shock, road accident, jaw injury, stomatitis, respiratory infection and diarrhea were subcultured and identified for genus and species.

Results: Significant normal pathogens of the nasal and oral swabs were found mainly *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., and *Proteus spp.* whereas from the rectal swabs of hanuman langur the significant pathogen noted were *Staphylococcus spp., Streptococcus spp., E. coli spp., Salmonella spp., Proteus vulgaris spp., Klebsiella spp.* and *Shigella spp.* The serotypes of *E.coli* isolated from rectal swabs of hanuman langur were identified as rough type, O_{138} and O_{20}. The major antigenic structure of *E. coli* revealed O138 (10), O20 (6) with rough types (2). Out of the total 10 cases of diarrhea O138 was observed from five isolates while O20 recorded from 3 cases. Out of remained two isolates one isolate of diarrhea revealed rough type and one revealed non typing antigenic structure.

Conclusion: The bacteriological prevalence in healthy and infected hanuman langurs in Gujarat reported for the first time in the current study may serve as a guideline for future studies in the same species.

Keywords: bacterial flora, Hanuman langur, nasal swab, oral swab, rectal swab

Introduction

Wild Non Human Primate population, an unexplored source of information regarding emerging infectious diseases, may hold valuable clues to the origin and evaluation of some important pathogen. Non-Human Primates are susceptible to a variety of zoonotic infectious diseases like *E.coli, Salmonellosis, Bacterial meningo-encephalitis, Streptococcossis* and *Shigellosis* also cause high morbidity and mortality [1]. They can act as reservoirs for Human pathogens. As members of biologically diverse habitats, they serve as sentinels for surveillance of emerging infectious diseases also pose serious threats to endangered and threatened primate species, studies of these diseases in Non Human Primate populations can benefit conservation efforts and may provide the missing link between laboratory studies and the well recognized needs of early disease detection, identification and surveillance. In the Gujarat state mainly two species hanuman langur and rhesus macaque of Non Human Primates are exist more frequently with a population of 100865 and 6115, respectively [2]. Transmissible pathogenic and opportunistic zoonotic bacteria comprise a recognized occupational health threat to exposed humans from Non Human Primates (NHPs).

In an effort to evaluate the occurrence of selected bacterial organisms with zoonotic and biohazard potential the prevalence study of examination of bacterial organism was planned in hanuman langur of the Gujarat state. Primary emphasis was directed specifically to detection of common bacterial organism present in hanuman langur and reported pathogens possessing recognized significant human disease potential.

Materials and Methods

Nasal, oral and rectal swabs were collected aseptically in sterile swabs with transport medium (Hi-media) after proper restraining of the total 30 (20 males and 10 females) Hanuman langur (*Prebytis entellus*), during the period of June, 2010 to March, 2011. The sample collection was carried out from Indroda park-Gandhinagar, Kamla Nehru Zoological park-Ahmedabad, Sayaji Baug Zoo- Baroda as well as from Jiv Daya Charitable Trust- Ahmedabad of the Gujarat state. The collected samples were transferred to the Department of Microbiology, Veterinary College,

Table-1. Oral bacterial isolates from hanuman langur

Name of isolates	Normal (n=7)	Infected wound (n=4)	Electric shock (n=6)	Road accident (n=6)	Jaw injury (n=4)	Stomatitis (n=3)
Staphylococcus spp.	6	4	5	6	4	3
Streptococcus spp.	5	4	5	5	4	3
Klebsiella spp.	2	3	1	1	1	3
Proteus spp.	-	1	-	1	-	-
Total	**13**	**12**	**11**	**13**	**9**	**9**

Table-2. Nasal bacterial isolates from hanuman langur

Name of isolates	Normal (n=7)	Infected wound (n=4)	Electric shock (n=6)	Road accident (n=6)	Respiratory Disorders (n=4)
Staphylococcus spp.	7	4	6	6	**7**
Streptococcus spp.	5	2	3	5	3
Klebsiella spp.	1	2	2	2	3
Proteus spp.	1	-	1	1	3
Total	**14**	**8**	**12**	**14**	**16**

Table-3. Rectal bacterial isolates from hanuman langur.

Name of isolates	Normal (n=7)	Infected wound (n=4)	Electric shock (n=6)	Road accident (n=6)	Diarrhoea (n=7)
Staphylococcus spp.	3	1	2	3	**2**
Streptococcus spp.	1	1	2	2	1
Klebsiella spp.	-	2	-	1	1
Proteus spp.	1	-	1	-	2
E. coli spp.	3	2	3	4	6
Salmonella spp.	-	1	2	-	3
Shigella spp.	1	-	-	-	3
Total	**9**	**7**	**10**	**10**	**18**

Anand, for bacterial isolation and identification. Primary isolation of bacteria was done by culturing the samples aerobically by inoculating the swabs onto blood agar medium, Mac Conkey agar medium and nutrient agar medium simultaneously. They were then incubated at 37 ºC for 24 hours as per the method of Cruickshank [3]. As per the nature of growth and cultural characteristics of colonies smears were prepared from representative colony and stained with Gram stain to study the morphology and staining characters, on the basis of which the isolates were grouped as Gram positive or Gram negative bacteria. These isolates were further identified at genera level by primary tests *viz.*, Catalase, KOH, Oxidase and O-F tests. For further identification of gram-negative bacteria, isolated colony was innoculated on Mac Conkey agar medium and incubated at 37 ºC for 24 hours. The colony was observed as lactose-fermenting and lactose-non fermenting (pink colony) after the overnight incubation. Lactose fermented colony were further inoculated on the Eosine Methylene Blue (EMB) medium and lactose non fermented colony was inoculated on the Brilliant Green Agar (BGA) medium and colonial characteristics were noted. Secondary isolation test viz. IMVIC was performed for the identification of the organism upto genera level as per the Bergey's manual of Determinative Bacteriology [4]. Total 23 positive isolated samples of *E. coli* spp. were sent to National *Salmonella* and *Escherichia* center, Central Research Institute, Kasauli (Himachal Pradesh) for identification of antigenic structure.

Results

Total thirty swabs were collected from the oral, nasal and rectal cavity of the hanuman langur which were suffering from various diseases viz. stomatitis, respiratory infection, diarrhoea and disorders viz. infected wound, electric shock, road accident, jaw injury. From nasal and oral cavity total sixty seven and sixty four bacterial isolates respectively identified of four different genera viz. *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., and *Proteus spp.* Whereas fifty four bacterial isolates of seven different bacterial genera viz. *Staphylococcus spp., Streptococcus spp., E. coli spp., Salmonella spp., Proteus vulgaris spp., Klebsiella spp.* and *Shigella spp.* were found from rectal cavity. All the rectal swabs were harboring at least one species of bacterial organism. The serotypes of *E.coli* isolated from rectal swabs of hanuman langur were identified and result showed five samples non typing for *E. coli* while, remaining samples revealed typical *E. coli* spp. The major antigenic structure of *E. coli* revealed O138 (10), O20 (6) with rough types (2). Out of the total 10 cases of diarrhea O138 was observed from five isolates while O20 recorded from 3 cases. Out of remained two isolates one isolate of diarrhea revealed rough type and one revealed non typing antigenic structure.

Discussion

The present study revealed the higher prevalence of *Staphylococcus* spp., *Streptococcus* spp. from the

oral isolates. Similar results were recorded in some studies, like, the major streptococcal biotypes from the oral isolates of macaque reported by [5]. Whereas, Carrier *et al.* [6] observed the gram-positive cocci with predominantly of *Staphylococcus* in all samples of Non Human Primates. The prevalence of *Klebsiella spp.* in the present study was found higher in the jaw injury and stomatitis cases than the healthy animals. The high prevalence of *Klebsiella* spp. (33.00%) was recorded in stomatitis cases, are in agreement with the findings of Panaitescu *et al.* [7].

In relevance to our findings, Goswami and Chakraborty [8] noted the pathological condition in NHPs and found that the respiratory infection claimed the highest number of deaths due to above organisms. The higher prevalence of *E. coli, Salmonella* spp. and *Shigella* spp. were recorded in diarrhoea. Whereas the prevalence of *Proteus vulgaris, Staphylococcus* spp., *Klebsiella* spp. and *Streptococcus spp.* were studied in all types of cases. In accordance with our findings Arya *et al.* [9] reported *Shigella* and *Salmonella* infection isolated from enteric diseases and also from others that were free from signs. The findings supports well with Kurade *et al.* [10] study where they observed the outbreak of *Shigellosis* in Rhesus monkeys in Himachal Pradesh.

Conclusion

The bacteriological prevalence in healthy and infected hanuman langurs in Gujarat reported for the first time in the current study may serve as a guideline for future studies in the same species.

Author's contribution

RGJ planned and designed the study. SMP collected and processed the samples for bacteriological analysis in the laboratory. RAM helped in processing of the sample for bacteriological analysis. SMP drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

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