Respiratory Viruses in Gazelles at King Khalid Wildlife Research Centre: Serological Investigation and Surveillance

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Two main species of gazelles are kept at King Khalid wildlife research centre. Arabian mountain gazelle, *Gazella gazella*, locally known as Idmi and the Arabian sand gazelle, *Gazella subgutturosa marica*, locally known as Reem. Recently, lung infections of the captive gazelles have been recorded at the center. In addition, initial postmortem examination of the infected animals revealed the presence of supportive lesions and abscission in the gazelle's lung. The objective of this study was to investigate Respiratory viruse in gazelles under captive at this Research Center. A total of 200 sera were collected from Idmi and Reem gazelles, each 100 samples between 2009 and 2010, for evaluation of the humoral immune responses of Bovine Herpesvirus-1, (BHV-1), Bovine Viral Diarrhea virus , (BVDV), Bovine Respiratory Syncytial Virus,(BRSV), Bovine Adenovirus-3, (ADENO-3), Para- influenza virus-3 (PIV-3). An indirect ELISA test (Bio-X Respiratory penta kit, BIO K 028, Belgium) was used to detect antibody prevalence in these gazelles. This study has showed that these two Arabian gazelles (Idmi and Reem) were likely to be susceptible to infection by BRSV, ADENO-3 and PIV-3 as detected by Indirect ELISA. These animals could act as a source of virus infection during the infective stage of the viruses.

Key words: Idmi, Reem, Wildlife, Lung infection, Virus, Respiratory, Indirect ELISA.

Respiratory disease is a very complicated illness, extrinsic and intrinsic stressors, infectious agents and host features are important in its occurrence and prognosis. The defense and metabolic functions of the lungs are crucial during bacterial infection and exposure to cold environment temperature. Respiratory viruses are relatively common in all parts of Saudi Arabia[1]. Pathogenicity of new neurotropic equine herpesvirus 9 (gazelle herpesvirus 1) in horses has been reported by Taniguchi A. and his colleagues by using ELISA technique[2].The contagious viral diseases of small ruminants are of economic importance in Africa, the Middle East and Asia; specially wide range of host spectrum of enterovirus type 1 (BEV-1) infection including humans has been reported[3]. Epizootic hemorrhagic disease virus (EHDV) infecting in cattle and gazelle in Turkey was investigated serologically

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and reported. Epizootic hemorrhagic disease virus (EHDV) is a vector-borne disease of ruminants disseminated in the tropic and subtropical zone of the world[4]. Gur S and Albayrak H. 2010, collected serum samples from 82 goitered gazelle to test by competitive enzyme-linked immunosorbent assay (c-ELISA). Based on their test results peste des petits ruminants specific antibodies were detected in 10 and all c-ELISA-positive sera were confirmed by virus neutralization test[5] Equid herpes virus 9 (EHV-9) was isolated from a herd of Thomson's gazelles affected by encephalitis. The natural host of EHV-9 is unknown, but zebras are suspected to be the source of infection in gazelles (6). In many ways, diagnostic technologies differ from therapeutic medical technologies. Perhaps most important, diagnostic technologies do not generally directly affect long-term patient outcomes. Instead, the results of diagnostic tests can influence the care of patients; in that way, diagnostic tests may affect long-term outcomes. Because of this, the benefits associated with the use of a specific diagnostic technology will depend on the performance characteristics, sensitivity and specificity of the test[7]. Georoff TA and his coworkers investigated idiopathic hematuria and associated pathology in Grant's gazelle's herpesvirus-1, ovine herpes virus, bluetongue virus, and epizootic hemorrhagic disease virus by using Polymerase chain reaction testing on paraffin-embedded urinary tract tissue and concluded. no exposure to any toxic agent was identified. An underlying cause for vascular lesions associated with episodic hematuria in Grant's gazelles remains to be determined (8) .Naturally occurring foot-and-mouth disease (FMD) in wildlife is a relatively mild condition but occasionally it can be devastating as has been documented in impala in South Africa and in mountain gazelles in Israel(9). Two native species of gazelle in Saudi Arabia, commonly known as Idmi and Reem are kept under the protection of the government at the center located in Thummamah, Riyadh, Saudi Arabia. There was no vaccination program against the viruses under investigation. On the basis of the results of our investigation we conclude that ELISA may be more specific and efficient technique for identification antibodies. More attention is necessary during removing these gazelles from the center and only those proven to be free of Virus infection and that

might not present a risk to others should be removed. Caution, should be applied for their housing and feeding so that these positive gazelles might not be source of infection to other gazelles in the center.

MATERIAL AND METHODS

Reagents and animals
96 welled PVC microtiter plate, eppendorf Tubes, Twelve-channel pipettor 1mL adjustable pipette, ELISA plate washer ELISA plate reader pNPP (p-Nitrophenyl-phosphate) , PBS, 0.75 M NaOH, conjugate antibovine immunoglobulin peroxidase (horse radish peroxidase labeled antibovine IgG monoclonal antibody), positive serum, carbonate buffer consisted: 1.59g Na_2CO_3, 2.93g $NaHCO_3$, 2mL 10% NaN_3, DI H_2O to 1L-pH 9.5-Stored at 4°C, washing buffer composed: PBS, 0.02% Thimerosal, 0.05% Tween-20, stopping solution.

Animals
Animals were 200 adult Idmi and Reem gazelles.

Method

Indirect ELISA
We could not find specific test kit for gazelles, so for the evaluation of the humoral immune responses, for Bovine Viral DiarrheaVirus (BVDV), Bovine Herpesvirus-1 (BHV-1), Para influenza Virus-3 (PIV-3), Bovine Adenovirus-3 (BAV-3) and Bovine Respiratory Syncytial Virus (BRSV) an indirect ELISA (Bio-X Respiratory penta kit, BIO K 028, Belgium) were applied. For serological screening, a total of 200 sera were collected from adult Idmi and Reem gazelles at a center located in Thummamah, Riyadh, Saudi Arabia. Before Sampling animals were randomly selected from the captive gazelle breeding pens. There was no vaccination program against the viruses under investigation. The sampled gazelles were not showing clinical disorder at the sampling time conducted between 2009 and 2010. Blood samples were collected into vacationer tubes and serum was centrifuged at 3000 rpm for 10 min and then serum samples were distributed in sterile tubes and were frozen at "20°C until testing. The dilution buffer was diluted to 1:5 in DI water. The lyophilized positive control was added 0.5 ml DI to dissolve. The serum samples were diluted to

1:100. The positive control was diluted 1:100 and the conjugate was diluted 1:50. After the dilution procedure was finished, 100µl diluted serum was placed in each of the antigen coated wells of the microtiter plate and was incubated for one hour at 37 and washed three times with washing buffer. Placed 100µl of diluted conjugate to each of the wells then incubated for one hour at 37 and wash three times with washing buffer. After washing and drying with plotting paper was over, 100µl of chromogen was added to each well and incubate for 10 minutes at room temperature then add 50µl of stop solution per micro well. The optical density was read by using plate reader at 450nM filter. The results were interpreted by subtracting the signal of the corresponding negative control values from the values of the recorded column according to the direction of indirect ELIS A.

RESULTS

The results of antibody prevalence for BHV-1, BVDV, BRSV, PIV-3, ADENO3 and Confidence interval were calculated @ 95% level of confidence and the result obtained was 4.81% (Table1.). 28 sera were positive out of 200 samples (table1). Idmi and Reem sera samples showed positive of BRSV, PIV3, and ADENO3, while BHV-1 and BVDV antibodies were not prevailed (Fig1.). The infection rates in 100 Idmi and 100 Reem were found to be different. ADENO3 antibody was not found in Reem sera. Although BVDV are widespread worldwide in our case we found with a seroprevalence up to 0% while BHV-1 was also found 0 % both in Idmi and Reem respectively. BRSV has been isolated in most European countries, North America, and Japan. In our study we found, highest antibody rate of BRSV in Idmi; 5% Fig1. These observed serological responses suggested that Idmi gazelles are more susceptible to viral infections than Reem gazelles. But using (chi test), the result obtained was 0.878672 which is more and not equal to   0.05 indicating the not significance of Idmi being more susceptible than Reem to respiratory virus of this study Table2.

Table 1. Indirect ILISA analysis for antibody prevalence to BHV-1, BVDV, BRSV,PIV-3 and ADNO-3 in Idmi and Reem gazelles. For the calculation of the confidence intervals, Confidence Interval for Proportion Calculator http://www.dimensionresearch.com/resources/calculators/conf_prop.html was used

Agents	Idmi	Reem	Idmi ELISA positive	Reem ELISA positive	Confidence interval
BH-1	100	100	0	0	
BVDV	100	100	0	0	
BRSV	100	100	10	3	4.81%
PIV-3	100	100	7	7	
ADENO-3	100	100	1	0	

Table 2.

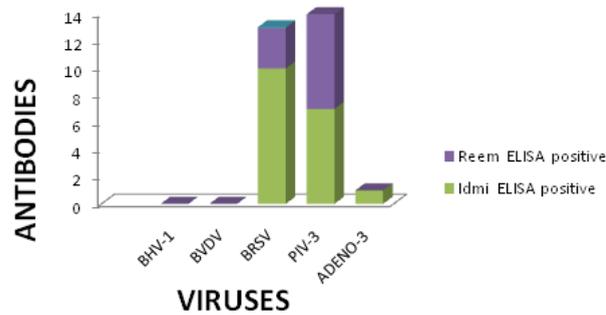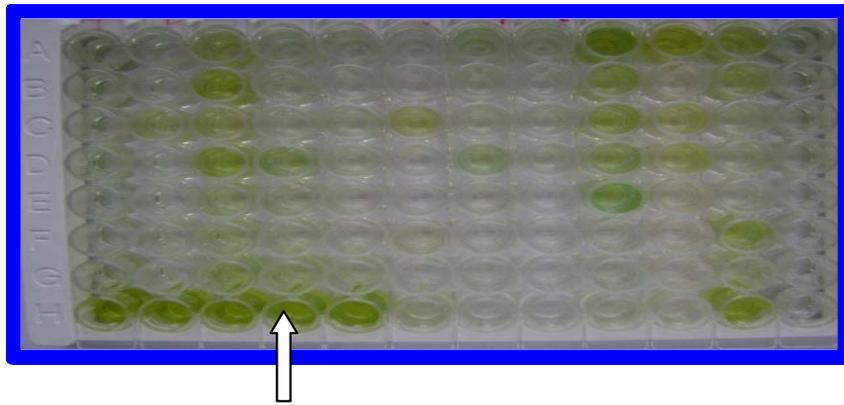| Animals	Idmi	Reem	Row total value
Observed	10	3	13
Value	7	7	14
	1	0	1
Column Total Value	18	10	28
Expected Value	8.357143	4.642857	
	9	5	
	0.642857	0.357143	
X.value	0.258687		
P. value	0.878672		
Degree of freedom	2		

Fig. 1. Shows Reem and Idmi antibody prevalence

96 wells ELISA plate show dark green color which indicate the positive samples

Fig. 2.

DISCUSSION

The role of bovine coronavirus (BCV) in clinical respiratory diseases is not clear and only few cases were reported to be associated with BCV. Some other viruses like rhinoviruses, enteroviruses and reoviruses may also play a secondary role in the etiology of respiratory diseases in gazelles management and environmental conditions are highly related to respiratory diseases, moreover the other infectious agents like bacteria *Pasteurella multocida*, *Haemophilus somnus* and *Salmonella dublin* and *Mycoplasma* spp are frequently contribute to respiratory viruses for generation of gazelle respiratory disease. Naturally occurring foot-and-mouth disease in wildlife is also a relatively mild condition but occasionally it can be devastating as has been documented in impala in South Africa and in mountain gazelles in Israel (9). Clinical signs of a disease locally referred to as "unsteady gait disease" for the Tibetan gazelle (Procapra picticaudata) were observed in the Qinghai Lake watershed area, China. The objective of their study was to determine if there was a relationship between the disease and copper (Cu) deficiency and have found that Copper concentrations in samples of blood, hair, and liver from the affected gazelles were significantly lower than those in unaffected animals.(10). Monoclonal antibody-based competitive ELISA (C-ELISA) has been used for the specific measurement of antibodies to peste des petits ruminants (PPR) viruses in sheep, goats, cattle and buffalo. Cattle and buffalo sera showed a high prevalence of antibody against PPR virus which may explain the difficulty experienced in achieving high post-vaccination immunity levels against rinderpest. Because antibodies against PPR virus are both cross-neutralizing and cross-protective against rinderpest virus, further vaccination in the presence

of antibodies against PPR virus may be a waste of national resources[11]. Equine herpesvirus 1 was isolated from an onager, zebra and a Thomson's gazelle in USA. The genetic relatedness and pathogenicity of these three viruses were investigated based on the nucleotide sequences of the glycoprotein G (gG) gene, experimental infection in hamsters, and comparison with horse isolates. The gG gene sequences of EHV-1 from onager and zebra were found to be identical. The histopathological findings were related to the virulence of each isolate. Their results indicated that EHV-1 isolates from onager, zebra and gazelle differ from horse EHV-1 and were much more virulent in hamsters[12]. Respiratory tract infections are often treated empirically without investigation to detect the aetiological agent, which may be a virus or a bacterium, including atypical pathogens such as Chlamydophila pneumoniae or Mycoplasma pneumoniae. Recently, several types Chlamydia-like intracellular bacteria have been detected in environmental samples and clinical specimens. Little is known of their geographical distribution and potential pathogen city[13]. Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere that we have only just begun to explore and understand[14]. Since neither virology nor serologic study has been made in these gazelles at the King Khalid wildlife research centre, our purpose of this study was to investigate the prominent viral risk in gazelles at this center. For this purpose, indirect ELISA was used to assess and define seroprevalence and distribution of these respiratory viruses. In many ways, diagnostic technologies differ from therapeutic medical technologies. Perhaps most important, diagnostic technologies do not generally directly affect long-term patient outcomes. Instead, the results of diagnostic tests can influence the care of patients; in that way, diagnostic tests may affect long-term outcomes. Because of this, the benefits associated with the use of a specific diagnostic technology will depend on the performance characteristics (eg, sensitivity and specificity) of the test, as well as other factors, such as prevalence of disease and effectiveness of available treatments for the disease in question. This study traced the antibody prevalence of five viruses in 200 gazelles and the results obtained were promising. The technology assessment applied in this study addresses that ELISA is cost-effectiveness and leads to decision analysis in health technology assessment. In conclusion, Viruses specific antibodies were detected for the first time in gazelles at this center and the ELISA test was found more sensitive. The obtained data showed that viruses are in circulation in gazelles of this center even though the infections were not latent and seropositivity rates were not very high. Knowing the serological status of the animals is important in order to be able to determine potential health risks and annual research is necessary in order to accomplish this aim.

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