Serologic survey for canine distemper virus in free-ranging wild canids in the northeast of Iran

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Introduction

Canine distemper virus (CDV), a member of the genus *Morbillivirus* of *Paramyxoviridae* family, is the most important pathogen has been identified as a threat for endangered wild carnivore species [19]. Members of 8 families of the carnivores order are susceptible to the distemper virus [13] and CDV has been responsible for the significant population decline and reported in dogs, ferrets, wild dogs, foxes, jackals, coyotes, hyenas [23], lions, tigers, leopards, cheetahs [3], seals, sea lions, and dolphins [10, 24].

There are some reports about mass die-off of Caspian seal in north of Caspian Sea and subsequently its spread to south and the transmission of CDV to rural dogs in the northern region of Iran due to the same lineage [11, 14, 16]. There are three wild Canids being presented in Iran, including: the common fox (*Vulpes vulpes*), wolf (*Canis lupus*) and golden jackal (*Canis aureus*). To the best of the authors’ knowledge, unfortunately no specific surveillance programs for viral diseases such as CDV have been done in Iran for these species up to the present time.

Practical diagnosis of canine distemper is primarily based on clinical suspicion, however the neutralization test is still considered as the gold standard for measuring protection against infection and serums’ titers strongly correlating with the level of protection. Neutralizing antibodies are produced against the membrane proteins of the virus, the first signs of them appear beginning 10 to 20 days post infection, and can persist for the life of a recovered animal [3].

According to all the reports of wild life infections by CDV and also the role of domestic dogs as an important source of CDV infection for wildlife, the investigation to assess the

SUMMARY

Following reports representing wildlife infection and massive mortality of Caspian mammals by Canine Distemper Virus (CDV), this research conducted to investigate the prevalence of antibodies reactive to CDV in free-ranging wild canids in the north eastern part of Iran. For this purpose, 60 common foxes, 22 wolves and 18 golden jackals, from Semnan and Khorasan province, included in the present study. Then serum antibody titers for CDV were measured by the mean of the virus neutralization method. Overall prevalence of antibody to CDV was 42% in these three species. It was determined that the antibody titers of positive sera were ranged between 1/4 and 1/64. There were no correlations between the presence of CDV neutralizing antibodies and age, gender and the region of sampling but the statistical analysis revealed a significant difference among foxes and other groups in CDV neutralizing antibodies (P < 0.05). In conclusion, the results of this serologic study demonstrated that CDV is present in the wild canids’ populations in the northeast of Iran and there are many opportunities for CDV to be transmitted from dogs to other wild Canids and endangered Felids. These results can also be useful for further epizootiology and phylogenetic analysis of this virus in wild Canids’ populations.

Keywords: Canine distemper virus (CDV), Common fox, Golden jackal, Iran, Neutralizing antibody, Wild canids, Wolf

RÉSUMÉ

À la suite de rapports représentant l'infection de la faune sauvage et la mortalité massive des mammifères caspiens par le virus de la maladie de Carré (CDV), cette recherche a été menée pour étudier la prévalence d’anticorps réactifs au CDV dans les canidés sauvages en liberté dans le nord-est de l'Iran. A cet effet, 60 renards communs, 22 loups et 18 jackals dorés, de la province de Semnan et Khorasan ont été inclus dans la présente étude. Les titres d’anticorps sériques pour le CDV ont ensuite été mesurés par la méthode de neutralisation virale. La prévalence globale d’anticorps contre le CDV était de 42 % chez ces trois espèces. Il a été déterminé que les titres d’anticorps des sérums positifs variaient entre 1/4 et 1/64. Il n’y avait pas de corrélation entre la présence d’anticorps neutralisants de CDV et l’âge, le sexe et la région d’échantillonnage, mais l’analyse statistique a révélé une différence significative entre les renards et les autres groupes (P < 0,05). En conclusion, les résultats de cette étude sérologique ont démontré que le CDV est présent dans les populations de canidés sauvages dans le nord-est de l’Iran et qu’il existe de nombreuses possibilités de transmission du CDV entre canidés sauvages et félidés en voie de disparition. Ces résultats peuvent également être utiles pour d’autres analyses épidémiologiques et phylogénétiques de ce virus dans les populations de canidés sauvages.

Mots-clés: Renard commun, Jackal doré, Loup, Canidés sauvages, Iran, Maladie de Carré, Anticorps, serologie
presence of CDV in local wildlife is necessary as the first step for further investigations. Thus, this study has been conducted to evaluate the prevalence of antibodies reactive to CDV based on the serum neutralization test in free-ranging wild canids in the northeastern part of Iran. For this purpose, Semnan and Khorasan were selected as the regions of interest because of presence of valuable genetic reserve such as endanger Persian leopard (*Panthera pardus saxicolor*) and cheetah (*Acinonyx jubatus venaticus*) and accompanied with previous studies in northern part of Iran and Caspian border in prevalence of CDV.

**Material and Methods**

**STUDY AREA**

Kharturan and Tandoureh parks in Semnan and Khorasan province, in the extreme southeast of Caspian Sea, were chosen as the regions of interests.

Kharturan National Park is in the Semnan province and located in south of Golestan national Park with a size of 1.4 million ha. It is the second largest reserve park in Iran and contains one of the largest populations of the critically endangered Asiatic cheetahs. Tandoureh national park is also located in the northeast of Iran near the Turkmenistan border with a size of 35,540 ha and is considered as one of the best habitats of wild animal (Fig 1).

**ANIMALS AND SAMPLING**

A total of 100 foxes, wolves and golden jackals (60 Foxes, 22 wolves and 18 golden jackals) were included in the present study. They were stemming from different areas of northeast of Iran, Semnan and Khorasan. They were captured by trapping (baited cage-traps) and were anaesthetized using Ketamine 10% intramuscularly for blood sampling. Blood was collected via jugular or cephalic venipuncture into plain evacuated tubes. Samples were collected from July 2014 through February 2016. These samples were shipped on ice to diagnostic laboratory, faculty of Veterinary Medicine of University of Tehran for testing. All the samples were stored at -80˚ C pending analysis. Then serum antibody titers for CDV were measured by the use of a virus neutralization method.

**SERUM NEUTRALIZATION**

The presence of CDV-specific serum neutralizing antibodies was assessed by virus neutralization assay using Vero cells and the Onderstepoort strain of CDV (Ond-CDV) in serum samples from 100 wild canids. Vero cell suspension containing 2x10⁵ cells/ml were grown in 96-well flat bottom plates. Two subsets of sera were tested for their neutralizing activity against CDV. Serial twofold serum dilutions starting from ½ were prepared in Eppendorf tubes. A 50 µl of each serum dilution was mixed with an equal volume of 50 TCID₅₀ diluted (1:750) CDV suspension. After an overnight neutralization at 4°C, 24-well plate containing monolayer Vero cells was inoculated in duplicate with 100 µl of each serum-virus mixture. The plates were incubated at 37°C for one hour in a humidified 5% CO₂ atmosphere for adsorption of free virus particles and then wells were washed once with DMEM (Dulbecco’s modified Eagle medium by Sigma, D5523, US). The inoculated plates were further incubated under 5% CO₂ atmosphere at 37°C for 4-5 days after adding FCS-free DMEM to the wells. The results were assessed at the end of the 5-day post-inoculation (dpi). Sera resulting in CPE (Cytopathic Effect) were retested in serial dilutions in twofold step, and the highest serum dilution with a visible CPE was considered the sample titer. Currently, there are no serologic test validated about threshold of antibody titer in wild carnivores titer, thereafter titer of ≥4 was considered positive for CDV [2, 5, 9].

**STATISTICAL ANALYSIS**

The collected data has been analysed by SPSS (Version 16). Chi square have been used to evaluate of antibodies among species, age, gender and study area with 95% confidence.

**Results**

The presence of CDV-specific serum antibodies were detected in 42 (42%) of the 100 totally wild canids. All sera with a titer of ≥1/4 were considered to have virus neutralizing activity. Thirty one of 60 (51.6%) common foxes sera, six of the 22 (27.2%) wolves sera and five of the 18 (27.7%) golden Jackals by microneutralization test was positive. The seroconverted animal (n=42) had mounted neutralizing antibody titers ranged from 1/4 to 1/64. The highest ratio (33.3%, 17 of 51) of neutralizing antibody was found at 1/8 dilution. A detailed summary of antibody titer and regional distribution and prevalence values are presented in Table I and II respectively.

On a geographical basis, the highest prevalence of CDV-specific antibody was detected in Semnan with 51.1% (22 of 43) but not significantly different from Khorasan. This result obviously shows that distemper virus infection is circulated...
between wild canids in the northeast of Iran in Semnan and Khorasan regions.

Statistical analysis revealed that significant difference was observed in foxes group rather than wolves and golden jackals group (p<0.05), but there was no correlation between the presence of CDV neutralizing antibodies among age, gender and region of sampling.

**Discussion**

The prevalence survey of CDV as an aim of the present study can be considered as a fundamental research to establish an overview and future control planning due to reported transmission of this virus between wildlife and domestic canines. Cage-traps are widely used to capture carnivores for scientific investigation and dispersion of wild canids in these regions cause few alteration in sample size in foxes, wolves and golden jackals in different area. The overall prevalence of antibody to CDV was 42% in foxes, wolves and golden jackals in different area. The antibody titers of positive sera were ranged between 1/4 and 1/64. There were no correlation between the presence of CDV neutralizing antibodies and age, gender and region of sampling but statistical analysis revealed a significant difference among foxes and the other groups in CDV neutralizing antibodies.

Virus neutralizing method is considered as a gold standard method for detection of CDV; however ELISA tests have also been shown to be sensitive and specific for CDV detection [8]. We used the gold standard method which may makes our results more reliable than the surveys in other parts of the world which used ELISA for epidemiological evaluation [21].

According to geographical distribution, the highest prevalence of CDV-specific antibody was detected in Semnan with 51.1% (22 of 43) but not significantly different from Khorasan. These results may be affected by the different densities of the wild canids in evaluated areas.

The antibody titers of only 5% of sera were determined as 1/32 and 3% sera as 1/64 in this study. The titers of antibody in seropositive animals indicated that even though they had been exposed to the virus in their life, the resulting antibody response is below the efficacious protective level but has some contributory role on the etiological diagnosis of the disease.

The highest prevalence of antibody to CDV was in foxes (51.6%), which is in the upper range of the reported ones for this species in Argentina and southeastern of the United States as 2 and 78% respectively [7, 12]. A recent study from Scandinavia reported an antibody prevalence of 12.3% [2], in Spain was 17.1% [21] and in Chile was 42% [1]. The prevalence of antibody to CDV in wolves (27.2%) was higher than comparative studies from Spain 18.7% [21], Canada 24% by Nelson and 12% by Watts in Alaska Peninsula [18, 25]. CDV is short-lived in the environment [4] and the relatively high antibody prevalence in foxes and wolves in our study presumably reflects continuous circulation of the virus within the fox and wolf population or within other susceptible wild. The antibody prevalence in golden jackal (27.7%) was a little lower than the found one in another region, in Zimbabwe 50% and 63.6% of free-ranging Side-Strip jackals and Black-Backed jackals were positive respectively and Shamir reported 52.2% of golden jackals of Israel were positive against CDV [20, 22] and 71% of Black-Backed jackals in Etosha national park in Namibia were positive against CDV [6]. The CDV antibodies were only determined in surviving individuals, so the results should be interpreted as the present existence and contact of the animals with this virus.

### Table I: Neutralization antibody titers against CDV

<table>
<thead>
<tr>
<th>Total Sera no.</th>
<th>Neutralization Titer</th>
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<tbody>
<tr>
<td></td>
<td>1/4</td>
</tr>
<tr>
<td>Common Fox</td>
<td>60</td>
</tr>
<tr>
<td>Wolf</td>
<td>22</td>
</tr>
<tr>
<td>Golden Jackal</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table II: Regional distribution of neutralizing antibody**

<table>
<thead>
<tr>
<th>Total Sera no.</th>
<th>Neutralization Titer</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Common Fox Seropositive %</td>
</tr>
<tr>
<td>Semnan</td>
<td>43</td>
</tr>
<tr>
<td>Khorasan</td>
<td>57</td>
</tr>
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</table>

**Table II: Regional distribution of neutralizing antibody**
Arctic lineages of CDV were detected as responsible for the mortality of seals in the northwest Europe, Siberia and Caspian Sea. Detection of Arctic lineage in the north of Iran among rural dogs by Namroodi indicated that the transmission and spread of this lineage from Caspian Seal to the north of Iran canids population is possible and can cause distemper crisis in future in wild canids of Iran [17]. In addition, the high rate of mortality and infection is reported in wild felids, this transmission to other species shows the necessity of the epidemiological studies in Iran’s wild life.

Because of high prevalence (55.6%) of CDV among rural dogs in the north of Iran [15], and high population density of rural dogs of this region, rural dogs can be a reservoir of infection for wild carnivores. CDV deserves attention in the regions of north and northeast of Iran, especially due to the possibility of the involvement of domestic dogs in their transmission, therefore strategy establishment to prevent transmission of CDV to endanger felids and canids are very important and CDV is an important threat to the conservation of wild canids and felids.

In conclusion, results of this serologic study indicate that CDV is present in the wild fox, wolf and golden jackal population in Iran, and there are many opportunities for CDV to be transmitted from dogs to wild canids and endangered Felid, so CDV should be carefully monitored and considered as the potential threats to these wild canids. The probability of future outbreak of CDV in wild canids of Iran is very high, therefore it is necessary to continue further studies of epizootiology and phylogenetic analysis of this virus in wild canid’s population.

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References