Detection of rabies virus specific antibody in stray dogs in Palestine

Short Communication

Detection of rabies virus specific antibody in stray dogs in Palestine

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Abstract

Stray dogs are considered as a major public health risk in rabies-endemic countries such as Palestine. The objective of the present study was to assess the presence of antibody against rabies in stray dogs in order to estimate the immune status of stray dogs for the purpose of risk management. The present study reports the first investigation for the detection of specific antibodies to rabies virus (RABV) in the serum of stray dogs in Palestine. The serum samples were collected randomly from 92 stray dogs from different seven Palestinian districts and were tested for the presence of antirabies antibodies by ELISA. Only 11.95% of stray dogs (n=11) had protective immune status against rabies with antirabies antibody titer (>0.5 IU) according to World Health Organization criteria. This result suggest that there is a high potential for reintroduction of canine rabies into stray dogs, leading to rabies transmission to human. Therefore, a new strategy to enable a broader vaccination coverage in stray dogs in conjunction with control breeding of these dogs must be launched to reduce the risk for transmitting rabies to human.

Keywords: Antirabies antibody, ELISA, Rabies, Stray dogs

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Introduction

Rabies is a rapidly neuroinvasive infectious viral disease affecting humans and mammals (Jackson, 2003). The disease is caused by an RNA virus belongs to the genus Lyssavirus of the family Rhabdoviridae (Yousaf *et al.*, 2012). The virus has a high tropism to nervous system causing acute, fatal encephalitis (Yousaf *et al.*, 2012). Foxes, coyotes, skunks and dogs are considered to be the most important reservoirs for rabies virus worldwide (Rupprecht *et al*., 2004), human cases transmitted from rabid dogs constitute most of human reported cases (Fooks *et al.*, 2014).

The disease is considered as fatal disease once the clinical signs of disease occur except for rare cases (Jackson, 2003). Despite continued attempts to treat human rabies, medical treatment are unsuccessful and the disease remains with the highest case fatality ratio (Hemachudha *et al.*, 2002). It's estimated that 7 million people are exposed to rabies annually, most of them in developing countries resulting in 55,000 death cases reported in Africa and Asia annually (Hampson *et al.*, 2011).

Rabies was first reported in Palestine since during the 4thCentury (king *et al.*, 2004). In addition, rabies is practically endemic in most of Palestine and Israel (Shimshony, 1997). There is a little information available for Palestine (king *et al.*, 2004). Red foxes and golden jackals were the main vectors of rabies in Israel and Palestine. However, since 2009, canine rabies has re-emerged in the country and were considered as the main animal reservoir highlighting the risk of virus transmission between human and both domestic and wild animals (Yakobson *et al.*, 2017). Stray dogs have a direct contact with wild animals such as red foxes and golden jackals thus representing a major risk for transmitting the virus to other domestic animals and humans (Cleaveland and Hampson, 2017: Bannazadeh *et al.*, 2018). Therefore, it is important to assess the level of antirabies antibodies in animals to verify the effectiveness of rabies vaccination campaigns. However, to the best of our knowledge, no epidemiological studies have yet been conducted to assess antirabies antibodies in stray dogs in Palestine. In addition, the effectiveness of vaccination through measuring the antirabies antibodies titer has not been evaluated before. The present study aimed for the first time to test for the presence of rabies virus (RABV) sepcificantibody in serum samples from stray dogs from different Palestinian districts. The results of this study will be necessary for increasing awareness against rabies and developing a national strategy program for controlling rabies in Palestine.

Materials and Methods

Study design: A cross-sectional study on RABV specific antibody in stray dogs was conducted in the northern and southern Palestinian districts. Blood samples were randomly collected from 92 stray dogs in Nablus (n = 15), Jenin (n = 8), Tulkarm (n = 44), Qalqīlyaȟ (n = 5), Ramallah (n = 1), Bethlehem (n = 5) and Hebron (n = 14) Palestinian districts in West Bank (Fig 1).

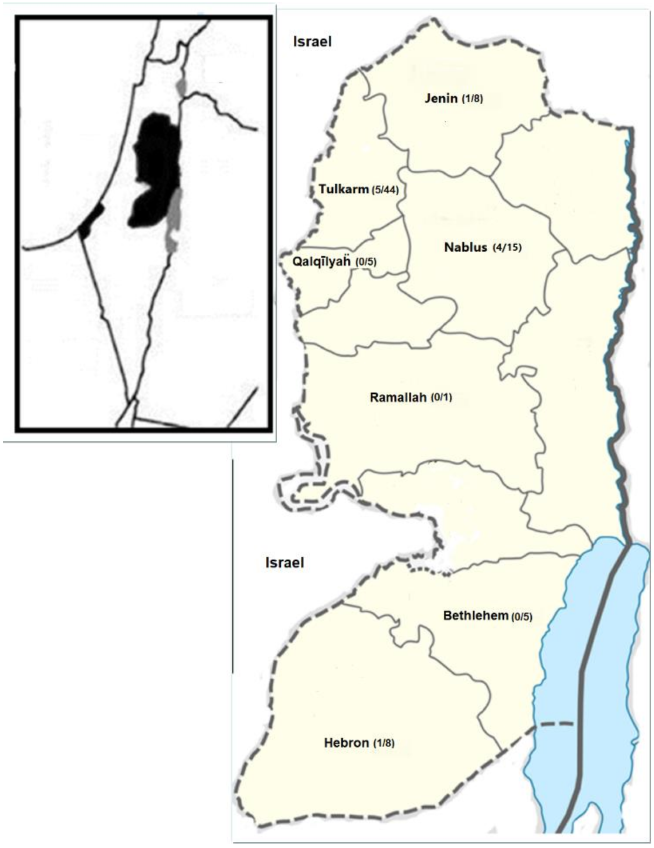

Figure 1 Map of Palestinian districts in West Bank where samples were taken. Number of dogs with protective antirabies antibodies above the protective level recommended by WHO (≥0.5 IU). First number indicates positive results and the second number represents the total number of sampled dogs.

Animals: The study was conducted for a period of one year from April 2018 to April 2019. Samples were collected from 92 stray dogs, whose history of vaccination against rabies was unknown. These dogs were captured by using an appropriate cage traps. The capturing method used in this study was humane and gentle to minimize stress on animals. For sedation, dogs were injected with Xylazine hydrochloride (Eurovet Animal Health, Bladel, Netherland) at a dosage rate of (1 mg/kg) body weight using intramuscular injection before blood collection (Cassu *et al.*, 2014). A trained veterinarian conducted sample collection after obtaining ethical approval from Palestinian Animal League Association (Palestine). Dogs were released after applying ear tags for identification.

Blood samples: Blood were obtained in plain tubes from the cephalic vein of each dog after appropriate preparation of the venipuncture site using (70% Ethanol). The samples were left to clot at room temperature for 2 hours. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes. The serum was then transferred to a new (2 ml) Eppendorf tube and stored at -20°C until analysis.

Detection of antirabies antibody: For determination of antirabies immunoglobulin G antibody (IgG) in stray dogs, a commercially available rabies ELISA kit (Demeditec Diagnostics GmbH, Germany) was used according to manufacturer's instructions. Positive and negative sera were provided in the kit. A serum titer of (>0.5 IU) was considered as protective level according to World Health Organization (WHO) criteria.

Statistical analysis: Data were analyzed with statistical software SPSS version 20th (SPSS Inc, USA). Chi-square test was used to determine the association between neutralizing antirabies antibodies and the location, sex and age of dogs. Differences were considered statistically significant at $P \leq 0.05$.

Results and Discussion

Serum samples were obtained from 92 stray dogs with unknown history of rabies vaccination collected from seven Palestinian districts. Of these 43 dogs were female (46.7%), 49 dogs were male (53.2%). Estimated ages of dogs ranged from 6 months to 4 years.

The antibody titers were calculated by comparison with antirabies antibody titer of (>0.5 IU) according to WHO reference serum (WHO, 2013). The antirabies antibody titers above (0.5 IU) threshold were considered protective (WHO, 2013). The seropositive rate was calculated as the percentage of dogs demonstrating antirabies antibody titers (>0.5 IU). Out of 92 stray dogs tested; only 11.95% (11/92) had protective antirabies antibody titers (>0.5 IU) according to WHO (WHO, 2013). Since these dogs were stray dogs, its vaccination status was unknown. From the 11 dogs harboring protective antibodies levels; 5 (number samples = 44) were from Tulkarm district, 4 (15) from Nablus district, 1 (14) from Hebron and 1 (8) from Jenin district Fig 1. Table 1 shows antirabies antibody titers in street dogs from seven different Palestinian districts.

Table 1 RABV specific antiraby titer among stray dogs from seven Palestinian districts according to WHO criteria.

Number of dogs	**RABV specific antibody titer**			
	(<0.125 IU)	**(0.125-0.250 IU)**	**(0.250-0.500 IU)**	**(≥0.5 IU)**
92	2 (2.17%)	51 (55.43%)	28 (30.43%)	11 (11.95%)

WHO = World Health Organization; IU = international unit; IgG = immunoglobulin G

Statistical analysis of antirabies antibody titer showed no significant differences between different Palestinian districts, sex and age of dogs ($P>0.05$).

Rabies is an important zoonotic, fatal and progressive neurological disease affecting all warm-blooded animals (Singh *et al.,* 2017). The disease remains as an important public health issue because of its prevalence through the world (Burgos-Caceres, 2011). Rabies is endemic in Israel and Palestine (king *et al.,* 2004). Despite this, there has been to date sparse information available about the disease in Palestine. According to the Palestinian Ministry of Health official data, only during the year 2018, approximately, 826 cases of dog bites were presented to the local governmental health centers and hospitals in Palestine for post-exposure prophylaxis measures (Ministry of health, personal communication, data not published). The estimated governmental cost of these measures is $ 15.000 annually. In addition, 30 animal cases of rabies were confirmed, of which 12 were reported in dogs during the year 2016 in Israeli and Palestinian territories (Israeli Ministry of Agriculture, data not published).

Stray dogs have uncontrolled rising population, representing a challenge in Palestine. Dogs were found to be the main rabies reservoir and transmitter in Israeli and Palestinian territories (Yakobson *et al.,* 2017). Moreover, almost 99% of human rabies cases are resulted from dog bites (David and Yakobson, 2011). These infected dogs move across the country borders carrying the infection to wild and domestic animals including domestic and stray dogs because of the high proximity between the populated urban and rural areas and thereafter transmitting the disease into humans (Gdalevich *et al.,* 2000). Different preventive measures have been applied to control rabies in Palestine; these include vaccination of house hold dogs, small-scale vaccination campaigns and the national oral rabies vaccination (ORV) campaign which are being performed since 2005 and covers all territories in Israel and Palestine (Yakobson *et al.,* 2006). However, monitoring of RABV specific antibodies in stray dogs to evaluate the previous control measures has never been conducted before in Palestine.

In our study, we have used an ELISA test for the detection of RABV specific antibody titer in the serum collected from stray dogs, this test provide high sensitivity, specificity as well as easy utilization for screening of the antirabies antibody titers in animal's serum (Piza *et al*., 1999). In this study, the incidence of protective immune status in stray dogs in Palestine was very low (11.95%) in comparable to other countries. For example, a higher level (49%-86%) of protective antirabies antibody in stray dogs with unknown history of rabies vaccination was reported in Thailand (Kasempimolporn *et al*., 2007). In addition, Ogawa (2009) reported that the protective seroprevalence on stray dogs in Japan was 27.7% (Ogawa *et al.,* 2009). These results indicate that the current rabies vaccination programs including the (ORV) campaign are limited. The vaccine delivery problem observed in stray dogs with ORV campaign is perhaps because these stray dogs are often live in packs, whereby one dominant dog may consume several oral vaccine baits leaving the rest of the group unvaccinated, therefore this vaccine could be a better choice for vaccinating wild animals rather than stray dogs and parenteral vaccination could be the method of choice for stray dogs (Yakobson *et al.,* 2008). The results of this study necessitate the importance for turning into a more effective vaccination strategy and the urgent need to control stray dogs by collaboration with both Public Health and the Veterinary Services, periodic surveys to test the immunization status in stray dogs, increasing the awareness about rabies and compulsory vaccination in household dogs.

Conflict of Interest: The authors declare no conflicts of interest.

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