

## A survey of tick infestation of animals before Eid-al-Adha as suspected cases of Crimean-Congo Hemorrhagic Fever (CCHF) in Kabul city's streets and live animal markets

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Received: 7/4/2021

Revised: 10/5/2020

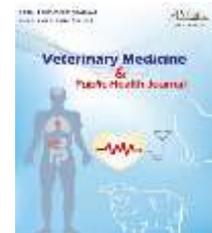
Accepted: 31/5/2021

DOI: <https://doi.org/10.31559/VMPH2021.2.2.5>

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## A survey of tick infestation of animals before Eid-al-Adha as suspected cases of Crimean-Congo Hemorrhagic Fever (CCHF) in Kabul city's streets and live animal markets

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**How to cite this article:** Samadi, A. et al., A survey of tick infestation of animals before Eid-al-Adha as suspected cases of Crimean-Congo Hemorrhagic Fever (CCHF) in Kabul city's streets and live animal markets. *Veterinary Medicine and Public Health Journal* 2(2); 2021: 57-63.

DOI: <https://doi.org/10.31559/vmph2021.2.2.5> Received Date: 7/4/2021 Revised Date: 10/5/2021 Accepted Date: 31/5/2021

### Abstract

Ticks are considered the crucial vectors of many infectious agents and considered the main vectors and reservoirs of Crimean-Congo Hemorrhagic Fever (CCHF) virus which is endemic in Afghanistan. The main objective of this study was to determine the prevalence of tick infestation in animals brought to Kabul city streets and live animal markets before Eid-al-Adha days as suspected cases of CCHF. Totally, 4,200 animals were examined in a four-day survey to estimate the prevalence of infested animals and related risk factors in Kabul city streets and live animal markets before Eid-al-Adha days in August 2019. The animals originated from 85.3% of provinces (29/34) and consisted of sheep (66.5%), goats (14.8%), cattle (14.9%), buffaloes (1.9%) and camels (0.008%). The overall prevalence of the tick infestation was 25.2% and the mean number of ticks per animal was 2.6. The prevalence was 18.2% in animals <1 year old, 23.4% in animals 1-2 years old and 33.5% in animals > 2 years old ( $p < 0.05$ ), but it was 27.1% in female and 25.1% in male respectively ( $p > 0.05$ ). From the total 2,101 ectoparasites collected, *Hyalomma* was the dominant genus (40.7%), followed by *Rhipicephalus* (6.1%), *Amblyomma* (5.4%), *Dermacentor* (1.3%), *Boophilus* (1.0%), *Ixodes* (0.05%) and *Argas* (0.1%). The overall awareness about CCHF among sellers/farmers was low, and only 5% just had heard about the disease. It has been concluded that tick infestation is very prevalent in animals brought to Kabul city before Eid-al-Adha days and low awareness about zoonotic diseases including CCHF among society greatly threaten the health of people.

**Keywords:** Tick infestation; Eid-al-Adha; CCHF; Kabul city.

## 1. Introduction

Ectoparasites that derive their nutrition through blood feeding (hematophagous) are efficient vectors of livestock and human pathogens (Walker *et al.*, 2003). Among them, ticks that are obligatory hematophagous, of reptiles, birds, livestock, companion and wild animal species and humans are considered one of the most important vectors of infectious agents including viruses, bacteria, and protozoa (Getiso and Geinoro, 2019; Greay *et al.*, 2016; Grøva, 2011). Ticks belong to the phylum of Arthropoda, class of Arachnida, order of Acari, sub-order of Ixodida, families of Ixodidae and Argasidae (Getiso and Geinoro, 2019; Walker *et al.*, 2003). From the 900 known species of the ticks (Chen *et al.*, 2014), approximately 10% of them are recognized as significant medical or veterinary importance, transmitting various types of pathogens to animals and humans (Smith *et al.*, 2011) and tick-borne diseases are currently of a great concern for animal and human health (Grøva, 2011).

It has been estimated that more than 80% of the world domestic animals are infested with ticks (Alexander *et al.*, 2020; Getiso and Geinoro, 2019), and annually tick infestation can cause up to 18.7 billion USD loss to cattle industries (Abebe *et al.*, 2010). Tick infestation reduces the productivity of livestock in a number of ways, such as: direct effect of attachment and feeding “tick worry”; injection of toxins; damage of the skin due to their bites; reduction in weight gaining; reduction in milk production and milk quality; reduction in meat product and morbidity and mortality associated with the diseases they transmit (Alexander *et al.*, 2020; Solomon and Tanga, 2020).

Lots of studies have been conducted on ticks and tick-borne diseases, along with their negative impacts on the productivity of livestock around the world, including the neighboring countries of Afghanistan such as Iran and Pakistan, (Sofizadeh *et al.* 2014; Alam *et al.*, 2013; Barker & Walker, 2014; Karim *et al.*, 2017; Nijhof *et al.*, 2007; Rehman *et al.*, 2017; Salim Abadi *et al.*, 2010; Tavassoli, 2013; Walker *et al.*, 2003). However, to the best of our knowledge, only few studies have been conducted on Afghanistan’s ticks and tick-borne diseases (Amiri *et al.*, 2021, Samadi *et al.*, 2021; Samadi *et al.*, 2020; Mustafa *et al.*, 2011; Bulman *et al.*, 1979). A four-day survey (from August 6 to 9, 2019) was conducted at the live animal markets, Kabul city; in order to find-out the prevalence of tick infestation in animals, present at the live animal market and streets. Since majority (71%) of the total population of Afghanistan (about 30 million), live in rural areas (CSO, 2018) and raise animals mainly ruminants as the main source of income (Samadi *et al.*, 2019), the livelihood and socio-economic stability of the farmers rely on ruminants (Chakraborty *et al.*, 2014). About 26.63

million ruminants are present in Afghanistan which are reared for milk, meat and wool production and considered as ready cash for the farmers (Samadi *et al.*, 2019; Samadi *et al.*, 2020). For such purpose, thousands of sheep, goats and cattle, and few numbers of camels that might be infested by ticks or infected by infectious agents are brought to Kabul city markets especially before Eid-al-Adha (an Islamic festival). Traditionally, the animals are slaughtered by the family members themselves in their houses or nearby streets and most of the family members participate in the slaughter and carcass cutting process and meat distribution to poor families and relatives. These activities could pose high risk of zoonotic infection especially CCFH and rift valley fever (RVF) for all of those participating in such activities. It is worth to be mentioned that CCHF is endemic in Afghanistan, and on average, about 50 cases have occurred annually in Afghanistan in recent outbreaks of the disease, which were accompanied with high mortality, were probably the result of exposure with infected ticks or infected animal’s blood and tissues especially during Eid-al-Adha feast (WHO, 2018; Samadi *et al.*, 2020).

## 2. Materials and Methods

### Study area:

The study area was Kabul city streets and live animal markets. Most of the famous livestock markets are located in Kabul, and people from Kabul and other neighboring provinces sell and buy their animals in these markets. Based on the normal custom of people in Afghanistan, many people (farmers, sellers) bring their animals on the streets and live animal markets of Kabul city before Eid-al-Adha days. They select/occupy a specific location on the streets/local markets, and they stay there up to the third day of Eid. People buy the animals for Eid festival and sacrifice the animals during Eid days in their houses or streets.

### Sample size and sampling methodology:

For the survey purpose, systematic random sampling design was applied for sample selection. Since there was no any information available about the number of animals on the streets and live markets during Eid days in Kabul city to be considered for random sample selection of animals, the team leader estimated the total number of animals on the street and live markets every day. Since every team was intended to examine about 100 animals every day, the total estimated number was divided by 100 to get the interval of sampling unit for that day.

### Data collection methods:

This survey was conducted by 7 professors/lecturers, 4 technicians and 129 students of the Veterinary Science Faculty of Kabul University

(VSFKU) and 5 employees of Animal Health Directorate of Ministry of Agriculture, Irrigation and Livestock (MAIL). The total enumerators (145) were divided in small groups of three persons, and four groups were guided by a team leader (a professor, lecturer, or lab technician). In each group, two members examined the suspected animals for the presence of ticks and the third one recorded data on EpiInfo 7 in their smart phones. So, based on this approach, 11 teams containing 13 - 16 members were deployed to the field every day. The enumerators recorded the number of ticks, their morphology and locations in each animal's body surface and other related data. For such purpose, two different questionnaires were designed and applied containing questions about source of animals (from where animals were brought to Kabul city), sex, age, breed, transportation methods (by truck or foot) and other related questions (available per request). In addition, farmers/sellers knowledge and awareness about ticks and CCHF was also evaluated by specific questions.

#### **Tick collection and identification:**

The presence of ticks on the sampled animal were identified visually by naked eyes. After confirmation of tick presence on the animals, ticks were removed by forceps, kept in 70% ethanol and were transferred to Parasitology lab of VSFKU for further identification. Identification of ticks was carried-out to the genus level following the standard identification procedures described by Walker *et al.* (2003) and Barker and Walker (2014) using specific illustrated morphological taxonomic keys present in both; dorsal and ventral views, under stereomicroscope (SZ760-DM320, NANBEI, China).

#### **Statistical analysis:**

All necessary data were collected using EpiInfo which were installed in smart phones. The collected data were then transferred to computers using Microsoft excel, for data management, cleaning and validation and final statistical analysis was performed using SPSS software (IBM, version 20).

### **3. Results**

#### **Animal sources and their demography:**

The animals on the streets and live animal markets in Kabul city before Eid-al-Adha were originated from 85.3% of provinces (29/34) and more than 150 districts of Afghanistan that were brought by 809 farmers/sellers. Meanwhile, three sellers brought the animals from Pakistan. From the total number of animals present on the street and live animal markets before Eid day (23,687), 66.5% of them were sheep, 14.8% goats, 14.9% cattle, 1.9% buffalos and 0.008% were camels. The median number of animals per seller/farmers was 63.

The number of sellers/farmers and the total number of animals in the streets and live animal's markets of Kabul city before Eid-al-Adha dramatically increased from the days before Eid when the time closes to the Eid days. The number of farmers/sellers increased from the first day of survey to the 4<sup>th</sup> day of survey 50%, 80.2% and 308.3% respectively. Meanwhile, the number of animals also increased from the first day of survey to the 4<sup>th</sup> day of survey 61.7%, 57.5% and 266.5% respectively. The mean age of the animals was  $22.8 \pm 7.4$  months, but based on the age categories, 3.6% of surveyed animals were less than one-year-old, 78.7% were 1-2 years old and 17.7% were more than 2 years old.

#### **Prevalence of tick infestation of animals:**

The overall prevalence of the tick infestation in surveyed animals was 25.2% and the mean number of ticks per animal was 2.6 (range = 1-6). Based on the age categories, the prevalence was 18.2% in animals less than one years old, 23.4% in 1-2 years old and 33.5% in animals more than 2 years old ( $p < 0.05$ ). Based on the species, the prevalence of tick infestation was 23.9% in sheep, 26.4% in goats and 33.1% in cattle. In addition, 27.1% of female and 25.1% of male were infested by ticks ( $p > 0.05$ ) (Table1).

Tick infestation varied based on location body surface of infested animals. Abdominal region (29.8%), under the tail (29.5%) and in the ear (25.7%) were the most infested sites of animals respectively, but location of ticks in infested animals also varied based on the species, age and sex of the animals (data not shown).

**Table (1):** Prevalence of tick infestation based on sex and age of sampled animals in Kabul city, 2019

Variables	Tick infestation			$\chi^2$ value	p-value	
	Positive (%)	Negative	Total*			
Sex	Male	356 (25.05)	1065	1421	0.295	0.587
	Female	38 (27.14)	102	140		
Age categories	<1 year	10 (18.18)	45	55	13.46	0.001
	1-2 year	287 (23.42)	938	1225		
	>2 year	92 (33.45)	183	275		
Animal species*	Sheep	304 (23.93)	966	1270	6.62	0.037
	Goat	32 (26.44)	89	121		
	Cattle	54 (33.12)	109	163		

\*The differences in the total number of animals are due to missing values

♦ Due to very low numbers, buffalos and camels were omitted

### Microscopic identification of ticks:

Totally 1,108 tubes/samples were collected during the four days of survey. From the total of 1,108 collected tubes/samples, 1,767 (84.1%) ticks, 328 (15.6%) melophagus, 5 (0.24%) louse and 1 flea (0.047%) were recognized morphologically under stereomicroscope. Furthermore, from the total of 1,767 identified ticks; 855 (48.4%) were *Hyalomma*, 129 (7.3%) were *Rhipicephalus*, 114 (6.45%) were *Amblyomma*, 28 (1.58%) were *Dermacentor*, 21 (1.19%) were *Boophilus*, 1 (0.05%) was *Ixodes*, and 2 (0.11%) were *Argas*. But, 617 (34.9%) ticks were not possible to identify due to lysis, dryness, head removal and blood feeding (488, 27.61%); and immaturity (129; 7.3%). To consider just the identified hard tick genera, the percentage distribution of *Hyalomma*, *Rhipicephalus*, *Amblyomma*, *Dermacentor*, *Boophilus* and *Ixodes* was 74.54, 11.3, 9.8, 2.45, 1.83 and 0.08 respectively. In addition, although the sex distribution varied based on the identified genera, large proportion of identified ticks were male (72.8%) compare to female (27.2%) and male to female ratio was 2.68:1.

### Seller/farmers' perception and knowledge about ticks and CCHF:

Public awareness about zoonotic diseases is very important in prevention and control measures implementation in the country. From the total of sellers/farmers asked about CCHF, only 5% had heard about the disease. Meanwhile, sellers/farmers' knowledge and awareness about transmission methods of CCHF to humans and the role of ticks in its transmission was 2.2% and 6.0% respectively, which are considered very low. Although just 2.5% of respondent stated that they had cases of CCHF in their family members, low awareness about the disease may underestimate the real situation in society. Due to the low awareness about the role of ticks in maintenance and transmission of many zoonotic diseases including CCHF to humans, more than one third of the farmers were removing the ticks

from infested animals by their hands, which can expose them to CCHFV and other zoonotic diseases' agents.

### 4. Discussion

This was the first comprehensive survey of tick infestation on domestic animals in Afghanistan, where the surveyed animals in Kabul city's streets and live animal markets were originated from 85.3% of provinces of Afghanistan. Ticks are considered the crucial vectors of many infectious agents than any other arthropods which transmit many protozoan, bacterial and viral diseases to humans and animals (Ali *et al.*, 2020; Khalil *et al.*, 2018; Karim *et al.*, 2017). In domestic animals, they cause irritation of skins, blood loss and anemia, reduction of weight gain, dysfunction of immune system and, as a consequence, decrease significantly the production of wool, hide, meat and milk and severely affect the quality of skin (Khalil *et al.*, 2018; Karim *et al.*, 2017). Carpet, hide and animal skin are considered the key export items of Afghanistan (Samadi *et al.*, 2019), where ticks and tick-borne pathogens shortfall approximately 3 billion pieces of skin in cattle alone annually worldwide (Karim *et al.*, 2017). In addition, ticks are the main reservoirs and vectors of many endemic infectious diseases of human and animal species in Afghanistan which cause severe socio-economic and public health losses continuously (Wallace *et al.*, 2002; Mobini *et al.*, 2008; Pages *et al.*, 2010; Samadi *et al.*, 2020; Samadi *et al.*, 2021). The large movement of animals to Kabul city is accompanied by outbreaks of arthropod borne diseases such CCHF in Kabul, which could be attributed to tick bites or exposure to infected animal's blood and tissues especially during Eid-al-Adha feast (Hatami *et al.*, 2019, WHO, 2018). In addition, the peak of CCHF cases occurred between July and August, which coincides with the occurrence of Eid-al-Adha in recent years (2016 – 2019) (Samadi *et al.*, 2020).

In our study, the prevalence of tick infestation in cattle, sheep and goats was 25.2% which is considered not very high in domestic animals compared to Iran (73.6%) the neighboring country of Afghanistan (Sofizadeh *et al.* 2014). We found higher number of female animals (27.14%) infested with the ticks than males (25.05%) which was not statistically significant ( $p > 0.05$ ) and the same result was found by Mohammed *et al.* (2017). In addition, in our study the tick infestation by species was statistically significant ( $p < 0.05$ ) and high prevalence was detected in cattle, goat and sheep respectively. Meanwhile, it was revealed that, the older the animals the higher the infestation by the ticks. Almost the same results were reported by Alexander *et al.*, (2020) where they found the heavy infestation in cattle (44.8%), sheep (33.6%) and goat (21.6%) respectively. The heavy infestation in older age group could be due to the facts that all of cattle specified for Eid sacrifice have to be  $\geq 2$  years old and sheep and goats have to be  $\geq 1$  years old (although in rare cases, sheep and goats can be 6 months too).

We found *Hyalomma*, *Rhipicephalus*, *Amblyomma*, *Dermacentor*, *Boophilus*, *Ixodes*, *Argas* tick genera in surveyed animals, and *Hyalomma* was the most prevalent genus identified. Other researchers (Amiri *et al.*, 2021; Mustafa *et al.*, 2011) also confirm the presence of *Hyalomma* spp. in animals in Afghanistan. Meanwhile, Sofizadeh *et al.* (2014) and Loui Monfared *et al.* (2015) also found *Rhipicephalus*, *Ixodes*, *Hyalomma*, *Boophilus*, *Haemaphysalis* and *Argas* in domestic ruminants (sheep, goats and cattle) in Iran, and *Rhipicephalus*, *Haemaphysalis*, *Hyalomma* and *Argas* were detected in Pakistan (Karim *et al.*, 2017), the two neighboring countries of Afghanistan. In addition, Sofizadeh *et al.* (2014) found *Rhipicephalus* as the most abundant tick in the study area in Iran, while Loui Monfared *et al.* (2015) found *Hyalomma* as the prevalent tick in domestic ruminants in the same country.

Because most of hard ticks including those identified in our survey are two to three – host ticks (Taylor *et al.*, 2016) and many tick-borne diseases including CCHF have been reported from Afghanistan (Bulman *et al.*, 1979; Wallace *et al.*, 2002; Mobini *et al.*, 2008; Pages *et al.*, 2010; Khattak, *et al.*, 2017; Samadi *et al.*, 2020; Samadi *et al.*, 2021, <https://reliefweb.int/report/afghanistan/infectious-diseases-afghanistan-report-global-disaster-information-network-gdin>), severe infestation of domestic animals by these ticks pose great threats to human and animals' health in Afghanistan.

## 5. Conclusion

With the endemic nature of many arthropod borne diseases including CCHF, low awareness about such diseases and their ecology among general population, and large movement of infested/infected

animals from all over the country to Kabul city threaten the human health more than any place in the country. Coordinated One Health approach is needed among human health, animal health and environmental professionals, municipalities, sellers/farmers and the entire society to tackle the increasing threats of zoonotic diseases in Kabul city and throughout the country. Such well-coordinated efforts must be planned well in advance before Eid-al-Adha feast and must heavily focus on the public awareness about CCHF, ticks and their infestation, how to deal with Eid-al-Adha animals, and other issues.

### Study limitations:

Data collection using smart phones by EpiInfo was the first experience in our team. Although it was successful during the survey, we faced with big challenges during transfer of data from smart phones to computers. In addition, some mobile phones were stolen during data collection on the streets and we lost the mobile and collected data in the same time. Although we examined 4,200 animals in four days of survey, only one-third of data (1,568) could be obtained from the mobile phones. Security issues are a big concern in Afghanistan. Deploying 145 staff on Kabul city streets and live animal markets was a risky action during survey. In the last day of survey, two big bomb explosions happened in Kabul city, where one of our team (Dasht –e-Barchi) just left the survey site only few seconds before the explosion in 6 district of Kabul city on August 09, 2019.

**Acknowledgments:** This survey was supported by Global Health Development | The Eastern Mediterranean Public Health Network (GHD|EMPHNET), and conducted by VSFKU staffs. VSFKU and EMPHNET are thankful from the efficient coordination and cooperation of the authorities of Kabul city municipality and security and their 16 districts during the survey time. Meanwhile, we acknowledge the MAIL for the donation of some PPE and active participation of their team during the survey.

**Compliance with Ethical Standards:** This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of Interest:** The authors declare that they have no conflict of interests.

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