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Epidemiology and Risk Mapping of hard ticks (Ixodidae) infecting Small Ruminants in Khyber Pakhtunkhwa Province, Pakistan

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ABSTRACT: Ticks and tick-borne diseases (TBDs) cause negatively impact production. The development of early warning systems for TBD outbreaks requires both registration and tracking of animals, which is not possible due to the transhumant nature of the sheep and goat production system. Therefore, the present study aimed to investigate the district-wise surveillance and risk mapping to find the epidemiological profile of tick infestation in small ruminants from KP. Ticks (n=6257) were collected from six selected districts from May 2017 to April 2018. A total of 1500 animals (goats=618 and sheep=881) were screened for the presence of ticks on different host predilection sites. Epidemiological information was sought on a prescribed questionnaire depicting age, locality, breed, host predilection sites, and local climatic conditions. A geographic information system (GIS) was used to map the risk areas having varied ticks infection. In the present study, six tick species were identified namely, *Rhiphicephalus microplus, Hyalomma trancatum, H. anatolicum, H. aegyptium, H. asiaticum,* and *Haemaphysalis bispinosa.* The tick prevalence in study districts was 21.87% in Dera Ismail Khan, 18% Mansehra, 16% in Peshawar, 15.67% each for Mardan and Mingora, and 12.8% Bannu, *R. microplus* was only prevalent in Dera Ismail Khan (63.81 %) and Peshawar (36.18 %) districts, while *Haemaphysalis bispinosa* and *H. aegyptium* were only recorded in Mardan. The intensity of *Hyalomma spp.* was highest (62.92 %) followed by *Rhipicephalus* spp. (36.55%), and *Haemaphysalis spp.,* (1.15%). *Hyalomma spp.* showed the highest prevalence on all the host predilection sites viz: ears (62.2 %), groin areas (60.8%), under the tail (61.9 %), testicular areas (63.2 %), breast (64.5 %), and in hooves (64.7%) as compared to *Rhipicephalus* and *Haemaphysalis* species. Similarly, in sheep and goats, the adult ticks were more prevalent in the age group of 1-2 years (83.28% and 68.98%) followed by < 1 year of age group (82% and 68.89%). The results revealed a higher female tick infestation (61.9%) as compared to male ones (38.1%). The highest female to male tick ratio was 1.77:1 in *Haemaphysalis* species. The GIS map demonstrated that the degree of tick infestation varied within districts and villages. It was concluded that *Rhiphicephalus microplus* is the most economical tick species that was extensively distributed all over the KP province. It has been suggested that cost-effective control measures should be taken to minimize the dissemination of tick-borne diseases (TBDs).

Keyword: Hard ticks, *Rhiphicephalus, Hyalomma, Haemaphysalis,* Sheep, Goats, Pakistan.

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INTRODUCTION

Sheep and goat farming is an essential component of the animal production system in Southeast Asia, where it contributes considerably to the development of the agricultural sector (Devendra and Burns, 1983). In Pakistan, sheep and goat farming is mainly concentrated in the semi-arid, arid, humid, and sub-humid tropical zones (GOP, 2019) and constitutes a vibrant agro-economic component. The livestock sector is facing various emerging financial and health issues in sub-tropical and tropical parts of the world (De la Fuente *et al.,* 2017; Ali *et al.,* 2019). The emergence and re-emergence of tick-borne infections (TBI) can cause serious threats to public health and animal production (Paddock and Childs, 2003; Jones *et al.,* 2008; Ybanez *et al.,* 2018). Tick species are widely distributed throughout the world (Parola and Raoult, 2001; Sherrard-Smith *et al.,* 2012; Yu *et al.,* 2015; Xu *et al.,* 2016). They have different temperature and humidity requirements enabling them to feed on their animal hosts (Piesman and Eisen, 2008). Hard ticks disseminate various deadly pathogens of zoonotic importance in all vertebrate animal hosts. Identification of ticks is a prerequisite to working out the prevalence of different tick species and their role in the epidemiology of tick-borne diseases (TBDs). The epidemiological profile of hard ticks in various agro-ecological zones is generally linked with different climatic dynamics that determine the tick's dissemination patterns and their subsequent adaptation to habitats (Estrada-Pena, 2009; Ali *et al.,* 2019). The occurrence of ticks depends on many factors like the availability of their hosts, host routine life, interactions among different hosts, type of vegetation, habitat characteristics, and environmental factors that facilitate their development and survival (Gondard*et al.,* 2017). Climatic variations not only determine the diversity and distribution pattern of the ticks but also enhance the risks of pathogens› transmission to other host species (Dantas-Torres, 2015). Globally, extensive research on the surveillance of ticks has helped devise an effective control strategy to minimize the deleterious effects of ticks on their hosts.

The higher average annual growth rate of the human population in Khyber Pakhtunkhwa (KP) makes small ruminant farming a vital asset to many livestock owners in the province. Most livestock farmers are landless and have small-scale animal holdings. In addition to this, a transhumant system of sheep and goat production of KP makes it impossible to develop a reliable early warning system for any outbreak of TBDs. Therefore, regular surveillance is required to monitor the distribution pattern of ticks and their spread in different regions of KP. Presently, epidemiological knowledge of ticks in KP province is scarce and the research data available from other regions cannot be applied to control the tick population in proposed target areas because of differences in the production systems. In this investigation, a district-wise cross-sectional surveillance study was designed with the specific aim to generate baseline data on the epidemiological profile of ticks infestation in KP, Pakistan.

MATERALS AND METHODS

Study Sites

This study was carried out in six districts of the Khyber Pakhtunkhwa (KP) province of Pakistan. Site selection criteria were based on the parameters viz., security concerns in the areas, accessibility to animal herds/farms, and farmers' cooperation. Sheep and goat herds were visited once each month throughout the study period. Six districts including Peshawar, Dera Ismail Khan, Mansehra, Bannu, and Mardan with different ecological conditions were selected for the collection of ticks (Fig. 1).

Study Design and Animal Sampling Techniques

Ticks were collected from the selected districts once a month for a period of 12 months from May 2017 to April 2018. A total of 1500 animals (goats=618 and sheep=881) were screened for the presence of ticks on different host predilection sites viz., ears, under the tail and perineum, groin, breast, testes, and inter-digital area of hooves. It was assumed that if the ticks were present on a farm then 50% of animals present on that farm would be infested. Based on this criterion we selected 5 to 10 sheep and goats from each herd.

Non-probability sampling was done based on animal hosts' selection, sampling of hard ticks, and epidemiological profile. Purposive sampling was used based on the following criteria. We selected 1 male and 3 female hosts from each farm at each visit. Age of the animals was also considered as more than 50% of animals were from 1-2 year age group and 20 percent from >1 year and above 2 years.

Tick Collection and Preservation and Identification

Ticks were carefully removed by using rubber-coated forceps without any damage to mouthparts

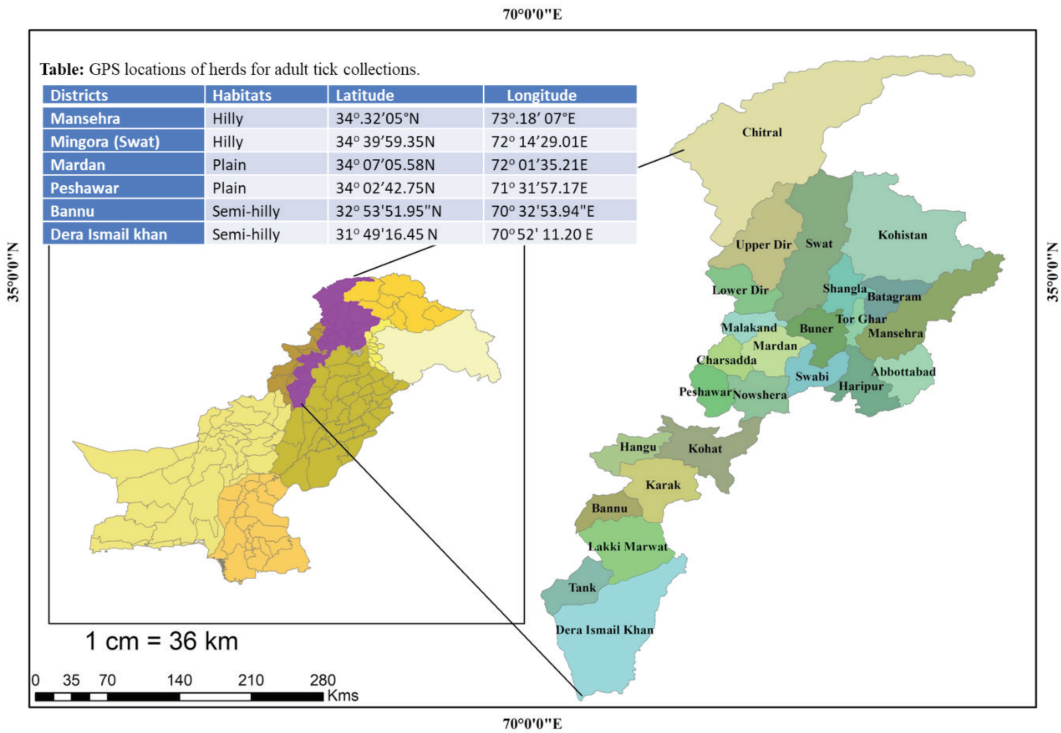

Figure 1: Map of Pakistan showing Khyber Pakhtunkhwa province, the sampling districts GPS locations and ecologies are indicated.

Figure 2: A) Number of incisor teeth present on lower jaw B) Depicting the presence of tick on ear

as suggested by Soulsby (1982). They were then transferred to McCartney sample collection bottles containing 70% ethanol and were properly labelled. Epidemiological information was sought on a prescribed questionnaire depicting age, locality, breed, host predilection sites, and local climatic conditions Tick specimens after collection were washed with distilled water, mounted on cavity glass slides, and examined under a stereomicroscope (Olympus-CH20 BIM, Japan). Ticks were identified to the species level using standard identification keys (Walker *et al.,* 2007; Walker *et al.,* 2014).

Age Estimation of the Host

Sheep and goats' age estimation was carried out by looking at the number of incisor teeth present on the lower jaw by following the description given by Whittaker *et al.* (2020) that the sheep and goats having 1 pair of incisors is roughly 1 year of age, 2 pair of incisors is 2 years of age and so forth (Fig. 2A).

Risk mapping

A tick risk map was developed based on different ecological parameters that affect its developmental stages and lifecycle. The ecological parameters included in the risk analysis include vegetation type, land topography, minimum temperature, maximum temperature, sunshine hours, precipitation, and relative air humidity. The prevalence was estimated based on the survey results from the positive herds. A herd was assumed as tick-positive if at least one positive sample was detected. A cluster analysis was performed. Point locations of all herds and tick prevalence were displayed on a map of the KP province using the geographical information system software (GIS) ArcView for Windows Version 3.2 (ESRI, Redlands, CA, USA).

Statistical Analysis

The full model of the regression equation was used to generate a GIS raster layer expressing tick prevalence based on local environmental conditions. This was done using the map-calculator function of ArcView. Data were entered and managed in a Microsoft-excel sheet and analysis was done by using a statistical package (SPSS 20 IBM, USA). Chi-square analysis and percentages were calculated for tick infestation data and the significance level was set at $p \leq 0.05$.

RESULTS

The results revealed the prevalence of three tick genera and six species namely: *Rhiphicephalus microplus*, *Hyalomma trancatum*, *H.anatolicum, H. aegyptium, H. asiaticum* and *Haemaphysalis bispinosa* from the six selected districts of KP.

District wise tick prevalence

The tick species identified showed distinctive distribution patterns in different districts of KP (Table 1). *R.microplus* was recorded in Peshawar (36.18 %) and Dera Ismail Khan (63.81 %), while *H. analoticum* was prevalent in all studied districts with the highest prevalence in Mansehra (49.82 %). *H. trancatum* prevalence was highest in Bannu 89.02%, whereas *H. asiaticum* was 76.4% in Mardan. However, *Haemaphysalis bispinosa* and *H. aegyptium* were only prevalent in the Mardan district. The highest tick diversity was recorded in Peshawar (16%) and Mardan (15.67%) districts. The distribution patterns of ticks were significantly different ($p < 0.05$) among the selected districts of KP.

Tick prevalence on Host Predilection Sites

It was observed that ticks showed varied preferences for their host predilection sites (Fig. 2B; Table 2). The distribution pattern of 6257adult ticks showed that the intensity of *Hyalomma* spp. was highest 3910 (62.92 %) followed by *Rhipicephalus* spp. 2275 (36.55%) and *Haemaphysalis* spp. 72 (1.15 %). *Hyalomma* spp. showed highest and equal prevalence on all host predilection sites viz: ears (62.2 %), groin

Table 1. Distribution patterns of hard ticks infesting small ruminants in different areas of Khyber Pakhtunkhwa, Pakistan

Tick's species	Localities (%)						
	Peshawar	Mardan	Bannu	Mansehra	Mingora	Dera I. Khan	Total
R. microplus	186 (36.18)	0	0	0	0	328 (63.8)	514
H. anatolicum	23 (4.24)	14 (2.58)	0	270 (49.82)	235 (43.36)	0	542
H. truncatum	18 (10.98)	0	146 (89.0)	0	0	0	164
H. asiaticum	13 (5.2)	191 (76.4)	46 (18.4)	0	0	0	250
Haemaphysalis bispinosa	0	10 (100)	0	0	0	0	10
H. aegyptium	0	20 (100)	0	0	0	0	20
Total	240 (16)	235 (15.7)	192 (12.8)	270 (18)	235 (15.67)	328 (21.9)	1500

Chi-Square (χ^2) = 4327.4 *p*-value= 0.000

Table 2. Distribution pattern of adult tick species picked from different predilection sites on host body

Adult Ticks	Predilection sites						Total
	Under tail area	Ears	Groin area	Breast	Testicular area	Hooves (Inter-digit area)	
Rhipicephalus spp.	478 (36.4)*	516 (37.8)	490 (36.7)	360 (35.4)	65 (35.5)	366 (34.9)	**2275 (36.35 %)**
Hyalomma spp.	817 (62.2)	830 (60.8)	826 (61.9)	642(63.2)	118 (64.5)	677 (64.7)	**3910 (62.92%)**
Haemaphysalis spp.	18 (1.37)	18 (1.32)	18 (1.34)	14 (1.4)	0	4 (0.38)	**72 (1.15 %)**
Total	**1313 (20.94)**	**1364 (21.79)**	**1334 (21.32)**	**1016 (16.23)**	**183(2.92)**	**1047 (16.73)**	**6257**

Values within brackets represent percentages.

areas (60.8%), under the tail (61.9 %), testicular areas (63.2 %), breast (64.5 %), and in hooves (64.7%) as compared to *Rhipicephalus* and *Haemaphysalis*species.

Age wise prevalence of ticks

The prevalence of ticks among sheep and goats is presented in Table 3, where animals are categorized into three age groups i.e., < 1, 1-2, and > 2 years of age. The results showed that the tick prevalence was significantly higher (p=0.025) in sheep from 1-2 years age group (83.28 %) than in sheep from < 1 and >2 years age groups. Similarly, in goats the adult ticks were more prevalent (69.0 %) in 1-2 years age group than in < 1 and >2 years of age groups, however, this difference was not significant (p=0.109).

Proportion and the sex-wise ratio of hard tick infestations

The results showed that animals infected with female ticks were 61.9%, while 38.1% were infested with male ticks (Table 4). The highest female to male tick infestation ratio was 1.77:1 recorded in *Haemaphysalis* spp. as compared to *Hyalomma* spp. (1.65:1) and *Rhipicephalus* spp. (1.55:1).

Risk mapping

The green pigmented clusters in some regions of Dera Ismail Khan, Peshawar, Mardan, and Mansehra districts represent areas of higher tick distribution, while the dark pigmented red, orange, and dark brown areas are those where the probability of tick infestation was lower as compared to other clusters in the study areas. These clusters were generated based on the occurrence points and prevalence of ticks infesting sheep and goat herds (Fig. 3).

DISCUSSION

In this study the six tick species identified, from different districts of KP,belonged to three genera viz., *Rhiphicephalus, Hyalomma* and *Haemaphysalis*. Tick fauna identified in the study region has previously been described in South Africa (Jongejan *et al.,* 2020);Lebanon (Dabaja*et al.,* 2017);Iran (Mirzaei and Khedri, 2014);Ethiopia (Kumsa*et al.,* 2012) and in Qazvin province of Iran (Shemshad *et al.,* 2012). This common tick fauna from different regions of the world might be due to massive transportation and movements of animals. However, the ticks reported in the present study have a distribution pattern shaped by the ecology of various agro-ecological zones of KP. Singh and Rath (2013) reported that the macro and micro-climatic conditions of an area were the key factors that determined the distribution pattern of ixodid ticks. There may be several reasons for the distribution of hard ticks in small ruminants raised in KP, Pakistan. It may be due to prevailing rainfall, average temperature, relative humidity, vegetation type, altitude, and landscape of an area that might play contributing factors leading to the higher prevalence of ticks.

In the present study, risk mapping showed some of the most likely areas expected with tick occurrence. The cluster analysis showed that the herd-based tick prevalence was higher at Dera Ismail Khan district, while ticks diversity was higher at Peshawar district. The green pigmented cluster in some areas of the dis-

Table 3. Distribution pattern of hard ticks in relation to age groups of sheep and goats in Khyber Pakhtunkhwa, Pakistan

	Sheep				Goats			
Age (Years)	**No. of animals examined**	**Infected**	**Prevalence %**	**P-Value**	**No. of animals examined**	**Infected**	**Prevalence %**	**P-Value**
<1	150	123	82.00	χ^2 =7.35	90	62	68.89	χ^2 =4.28
1-2	580	483	83.28	P=0.025	374	258	68.98	P=0.109
>2	151	111	73.51		154	92	59.74	
Total	**881**	**717**	**81.38**		**618**	**412**	**66.67**	

Table 4: Proportion and sex wise ratio of adult ticks on sheep and goats collected from Khyber Pakhtunkhwa, Pakistan

Ticks	Male n (%)	Female n (%)	Total n	Female to Male Ratio
Hyalomma spp.	895 (37.75)	1476 (62.25)	2371	1.46: 1
Rhipicephalus spp.	1119 (39.09)	1745 (60.93)	2864	1.55:1
Haemaphysalis spp.	369 (36.11)	653 (63.89)	1022	1.77:1
Total	**2383 (38.09)**	**3874 (61.91)**	**6257**	

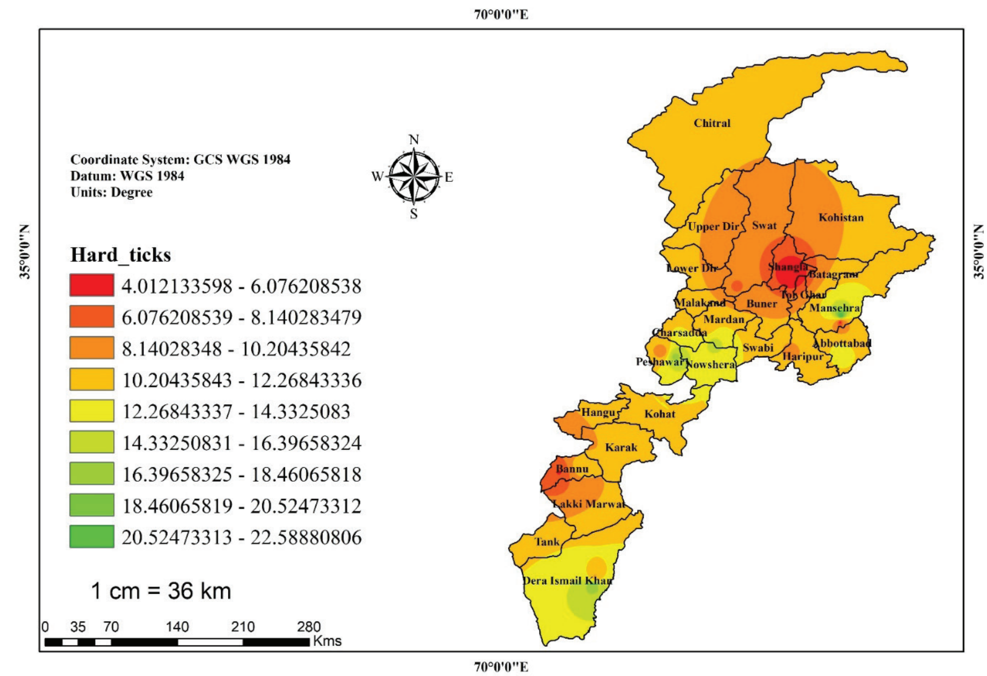

Figure 3: Risk map indicating epidemiological profile of ticks infesting sheep and goats in different districts of Khyber Pakhtunkhwa province

trict Dera Ismail Khan and Peshawar represents where the tick distribution was maximum, while the red and orange pigmented shows area shaving minimum the prevalence of tick as compared to other clusters in the study area. Our risk mapping data correlate with those for 19 predominant tick species from China (Zhao *et al.,* 2021). These authors showed a relatively higher infestation of ticks in ruminants of hilly, semi hilly, and plain areas than in areas with 14-54% rate of infestation (Sajid *et al.,* 2009; 2010; Irshad *et al.,* 2010; Atif *et al.,* 2012; Iqbal *et al.,* 2013). A similar pattern of infestation was reported for hilly and semi hilly areas of Malaysia (Mariana *et al.,* 2008). The high distribution of hard ticks in the present study could be due to prevailing rainfall, average temperature, relative humidity, vegetation type, altitude, and landscape of area (Estrada-Pena, 2003).Various factors that may affect the reproduction and development of ticks in a specific region include: humidity, temperature, precipitation (Greenfield *et al.,* 2011) and vegetation (Gray, 2007), host availability, season, habitat (Teel *et al.,* 1996), altitude (Burri *et al.,* 2007), breed, age, sex, gestation period, stage of lactation, nutritional stress of host (Alonso-Díaz *et al.,* 2007; Yacob *et al.,*2008) condition of host body (Rony *et al.,* 2010), acaricides application (Bianchi *et al.,* 2003), and animal husbandry practices (Sajid *et al.,* 2010).

The distribution of *Hyalomma* spp. at different predilection host sites was highest as compared to *Rhipicephalus* spp. followed by *Haemaphysalis* spp. The predilection preferences, among tick species identified in this study were not significantly different and all distributed equally on ears, groin areas, under the tail, testicular areas, breast and in hooves. Our results agree with previous studies that most common predilection site of ticks were groin area, udder, under the tail and inter-digital area of animal hooves (Pasalary *et al.*, 2017;Akhtar*et al.*, 2019; Jongejan *et al.,* 2020). They are of the view that ticks usually prefer hairless sites on the host body viz., lower perennial, groin, genitalia, udder and under the tail sites having rich supply of blood. Nonetheless, it can be inferred from the present findings that different tick's species preferred various predilection sites on the host body. The plausible explanation of the preferences could be due to the variations in tick's mouths parts exhibited by different tick species not only for blood-sucking but also help ticks for their host location.

In this study,sheep and goats in 1-2 year age group were more infected than those older than 2 years. This agrees with previous findings that the younger animals exhibited more susceptibility to tick infestations than the adult hosts (Sajid *et al.*, 2010; Abera *et al.,* 2010). A possible explanation might be that adult hosts have developed resistance after continuous exposure to tick infections. Also, soft skin tissues of younger animals may have facilitated ticks to penetrate their mouthparts during blood-sucking (Sajid *et al.,* 2010). Furthermore, younger animals stayed at or nearer to the animal enclosure and thus got predisposed to higher tick infections.

Also, in our study female ticks were found relatively more prevalent as compared to their male counterparts as reported previously (Mustafa *et al.,* 2014). However, our findings are contrary to some reports who have recovered more male than female ticks from host bodies (Mwangi *et al.,*1985; Champour *et al.,*2013). This disagreement might be due to the time of tick sampling and because male ticks stay on the host body for a longer period than female ticks (Yakhchali and Hasanzadehzarza, 2004).

CONCLUSION

This study provided baseline epidemiological data on hard ticks associated with the small ruminants of the Khyber Pakhtunkhwa (KP) province of Pakistan. The six hard tick species identified belonged to three genera: *Rhipicephalus, Hyalomma,* and *Haemaphysalis.* The risk mapping highlighted the tick infestation areas based on ecological factors influencing the lifecycle stages of ticks. Considering the global warming and changing ecological factors like temperature, precipitation, and humidity continuous field surveillance and ecological-based studies on tick species of KP are needed for the welfare of livestock species and livelihood protection of the people of the area.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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