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Prevalence of tick-borne viruses from ticks breeding on camels imported to Egypt during the period from January 2019 to April 2021

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Abstract: Ticks show a vital role in the extent of diseases such as viral infection. Tick-borne viruses (TBVs) are a broad category with various genetic features transported by ticks. Specific TBVs are well-known for producing serious infections with high fatality ratios in humans and livestock, whereas others may cause community-health dangers that we don't yet understand. So, we aimed to detect TBVs isolated from ticks breeding on camels imported to Egypt during the period from January 2019 to April 2021. Throughout our study time, 27732 hard ticks were gathered from camels imported to Egypt to detect the existence of some medically important TBVs. Ticks were divided into pools (1385) each pool containing about 20 ticks. After that, tick pools were tested for the existence of *Crimean–Congo hemorrhagic fever virus* (CCHFV), *Dugbe virus* (DUGV), *Phleboviruses*, *Alkhumra hemorrhagic fever virus* (AHFV), and *Thogoto virus* (THOV) *via* real-time reverse transcription PCR (rRT-PCR) and nested RT-PCR. Three genera, *Hyalomma* (n = 25000 ticks; 90.1%), *Rhipicephalus* (n = 1236 ticks; 4.5%) and *Amblyomma* (n = 1496 ticks; 5.4%) were detected. The molecular analysis of both RT-PCR results showed that the CCHFV and AHFV were detected in 25/ 1385 tick pools (1.8%) and 14/1385 tick pools (1.01%), respectively. Herein, we indicated the presence of CCHFV and AHFV in Egypt and demonstrated the possibility of TB-dissemination of the virus. Additionally, further analysis on ticks and human samples are now recommended in epidemiological studies within the Egyptian land.

Keywords: Crimean-Congo Hemorrhagic fever; Alkhumra hemorrhagic fever; Nested RT- PCR; Real-time reverse transcription PCR; Egypt.

1. INTRODUCTION

Ticks thrive in a wide range of environments, from the driest to the most humid [1]. Tick populations are growing, and tick-borne diseases (TBD) are considered more prevalent, drawing the attention of a broader range of public health professionals [2]. *Argasidae*, or soft ticks, and *Ixodidae*, or the hard ticks, are the two primary families [3]. The *Ixodidae* family is classified into seven genera [4]. *Amblyomma* (Am), *Haemaphysalis* (H), *Hyalomma* (Hy), *Rhipicephalus* (R), *Ixodes* (I), and *Dermacentor* (D) are the most common genera [1]. The *Ixodid* genus *Hyalomma* alone causes massive deficits in camel and other livestock products in the North Africa and Middle East region [5]. Ticks are important arthropods that convey a broad range of pathogens, including viruses, to both individuals and animals. The viruses transferred by ticks are called tick-borne viruses (TBVs) [6].

TBVs include a heterogeneous group of vertebrate viruses categorized into one DNA viral family, *Asfarviridae*, 8 RNA viral families: *Reoviridae*, *Flaviviridae*, *Orthomyxoviridae*, *Rhabdoviridae*, *Nairoviridae*, *Phenuiviridae*, *Peribunyaviridae*, and *Nyamiviridae* [7, 8]. Nearly 25% of TBVs are closely related to infection [9].

In the *Nairoviridae* family, *Orthonairovirus* is a genus containing at least 35 viruses grouped into 7 serogroups [6]. *Orthonairoviruses* are viruses that have a community health influence and are related to serious human disorders (e.g., *Crimean–Congo hemorrhagic fever virus* (CCHFV)) and a moderate human pathogen that are antigenically and

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genetically related to CCHFV (e.g., *Dugbe viruses* (DUGV)) [10]. The most well-known and investigated nairovirus is CCHFV, causing acute febrile illness with severe hemorrhage in humans recognized as Crimean–Congo hemorrhagic fever (CCHF) [11].

CCHF is a TBD that is broadly found throughout Africa, Asia, and Europe [12] while DUGV has a much smaller geographical distribution although it's one of the key prevalent TBVs in Africa [13]. CCHFV was discovered in 1944 in the earlier Soviet Union's Crimea region and named 'Crimean hemorrhagic fever'. Subsequently, in 1956, it was detected in the Belgian Congo under the name 'Congo virus,' and in 1969, both names were assembled as Crimean–Congo hemorrhagic fever (CCHF) [14]. On the other hand, DUGV was discovered in 1964 in Nigeria [4], and it was called after the Ibadan area where the prototype strain was discovered [15]. Ticks of the *Hyalomma* spp. were established as the reservoir and natural vector of CCHFV [12]. *Am. variegatum*, *Hy. truncatum* and *R. appendiculatus* were identified as vectors for DUGV. Tick bites are the most common route for DUGV [16] and CCHFV to spread, although CCHFV can be also spread by contact with contaminated organs or blood from livestock [17]. After being bitten by infested ticks, a range of wild and domestic animals, for example, camels, sheep, hares, cattle, and goats, operate as ensured hosts [14]. CCHFV has been found as an extremely infective virus for individuals revealing a death ratio of 5-30%, according to most reports [18]. CCHFV infection in humans can cause asymptomatic, mild, or severe illness [19] whereas humans infected with DUGV can suffer from a mild febrile sickness. Only one case was evaluated for high fever, encephalitis, and prolonged thrombocytopenia [10]. To verify the existence of CCHFV in *Hyalomma* ticks, viral antigen, nucleotides amplification, and/or nucleic acid amplification tests combined with proteomics could all be employed [19]. However, for the detection of DUGV, a conventional reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) have been established [20].

The *Phenuiviridae* family is divided into 4 genera, only the genus *Phlebovirus*, which includes a large group of virus members transmitted by ticks and was previously identified as Tick-borne *phleboviruses* (TBPVs) [6]. *Phleboviruses* are typically found in the New World's tropics and the Old World's half-dry and pleasant zones, such as the Mediterranean, North Africa, and central and western Asia [21]. Until the recent appearance of *severe fever with thrombocytopenia syndrome virus* (SFTSV) in China and the *Heartland virus* (HRTV) in the USA, TBPVs were widely ignored as causal means of human disorder [22, 23]. The HRTV is directly associated with the SFTSV and shows a 60–70% similarity with it [6, 24]. Other *phleboviruses* with genetic similarities to SFTSV and HRTV were recently separated from ticks in several world regions [25]. SFTS patients suffer from fever, neurological abnormalities, gastrointestinal symptoms, thrombocytopenia, muscle complaints, multiple organ failure, and coagulopathy, with a 10 to 30% fatality rate [24]. The SFTS, the Bhanja, and the Uukuniemi are the three genetic groups of TBPVs [26, 27]. Development of primers that target conserved regions of the L segment RNA of all TBPVs can be used to identify *phleboviruses* using RT-PCR technology [22].

Alkhumra hemorrhagic fever virus (AHFV), another TBV, is categorized under the tick-borne encephalitis group of the *Flavivirus* genus in the Flaviviridae family [28]. It was firstly separated in the Alkhumra area of Jeddah, Saudi Arabia, in 1994–1995 from six patients. It is found across Saudi Arabia, with isolated instances along the Sudan-Egypt border [29, 30]. It can be spread through tick bites from *Hy. dromedarii*, or *via* contact with animals or raw products from animals (often camels and sheep) bitten by infested ticks [31]. The virus can also be transmitted by unpasteurized or contaminated milk from camels, as well as through skin wounds infected with the blood or bodily fluids of an infected sheep [28]. According to reports, up to 25% of people who contract the virus die [31]. Patients infected with AHFV have symptoms like influenza, but the disorder can continue to serious hemorrhagic and central nervous system (CNS) presentations [32]. In the early laboratory diagnosis of AHFV, both molecular and immunological techniques have been used [28].

In the Orthomyxoviridae family, TBVs are represented by two suggested genera, *Quarjavirus* and genus *Thogotovirus* [6]. *Thogotoviruses* are distinct from other representatives of this family in that they are transferred by ticks and are found in a wide range of mammalian species, including rodents, cattle, camels, and sheep [33]. The associates of the genus *Thogotovirus* are arboviruses transferred by ticks that infect humans and livestock in Europe, Africa, and Asia [34]. *Thogoto* virus (THOV) is the classified species of genera *Thogotovirus*, and it was recognized and separated from *Rhipicephalus* spp. ticks in Kenya and Sicily [6, 13], from *Am. variegatum*

in Nigeria, and from *Hyalomma* spp. ticks in both Nigeria and Egypt [35, 36]. The patients who attracted *Thogotovirus* infections developed a febrile illness that was frequently accompanied by neurological symptoms. Both individuals' illness development was marked by severe respiratory complications and liver damage [33].

This study was performed to detect the occurrence of CCHFV, DUGV, TBPVs, AHFV, and THOV in ticks isolated from camels imported to Egypt during the period from January 2019 to April 2021.

2. MATERIALS AND METHODS

2.1. Collection and Processing of Ticks

A total of 27732 hard ticks were gathered from imported camels to Egyptian land from January 2019 to April 2021. Ticks were derived from various body parts of camels slaughtered in the Toukh slaughterhouse (Qalyubia). Fine curved-tip forceps were used for the collection of samples very carefully to prevent their destruction. Ticks then had been collected in a glass container with pores for Oxygen entrance. The collection date and country were typed on labels for their corresponding containers. Then ticks were transported to the laboratory and morphologically categorized into species level. Consequently, ticks were divided into pools each containing about 20 hard ticks. In order to minimize additional cross-contamination from animal pelts, each individual pool was washed double times with sterilized H_2O, flushed once with ethanol (70%, v/v), and then rinsed a couple of times with Minimum Essential Medium (MEM; Sigma-Aldrich, St. Louis, MO) that contains antibiotic-antifungal mixture (GIBCO-BRL; New York, USA). The tick pools then were grinded by a sterilized mortar and pestle, with 2 mL MEM including 2% antibiotic-antifungal mix, 15% fetal bovine serum, and 2% l-glutamine (Biochrome KG; Berlin, Germany). The homogenates were clarified *via* centrifugation at a low-speed rate (5,000 rpm) for 10 minutes using a swing-out rotor (Hermle, Germany) and supernatants were separated then preserved at -70 °C up to RNA extraction step [13, 37, 38].

2.2. RNA Extraction

An aliquot of supernatant (140 µL) was extracted for viral RNA using the QIAamp® Viral RNA Mini, Cat No. 52904 (Qiagen, Germany) according to the producer's guidelines. The extracted RNA was then stored at -70 °C until used for virus detection.

2.3. Amplification of Viral Genetic Material

Qualitative detection of DUGV and THOV were performed using the conventional PCR and real-time reverse transcription PCR (rRT-PCR), while recognition of *Phlebovirus*, CCHFV, and AHFV were performed by the conventional PCR followed by nested PCR and rRT-PCR. The primers used in this study were synthesized from Invitrogen by Thermo Fisher Scientific. These primers were previously mentioned in other studies as shown in **Table 1**. Positive and negative control were included in all reactions. The sensitivity of the non-nested RT-PCR test was 100 copies per reaction while nested RT- PCR was 10 copies per reaction [43]. The specificity of each assay was 100 % as no cross-reactivity occur as positive control give positive signs while no signs were observed with negative control.

2.3.1. Conventional and Nested PCR

We used Qiagen One-Step RT-PCR kit (Qiagen, Germany) Cat No. (210212) for PCR reaction using the manufacturer's instruction. An aliquot of 10µL extracted RNA was pipetted into each singular PCR tube. The total reaction volume was 50µL and the thermal cycling settings were mentioned in **Supplementary Table 1**.

After that, amplification of the first PCR product of *Phlebovirus*, CCHFV, and AHFV by means of nested RT-PCR using Promega Go Taq G2 master mix (Cat No. M7833, Promega, Germany) using the cycle conditions described in **Supplementary Table 2** was done. Nested PCR was performed in a 50µL reaction with 2-µL of first PCR product using the primers previously described in **Table 1**. The PCR product was detected by electrophoresis (Biometre, Germany) in 2% agarose gel to visualize specific amplified products by comparing them with standard molecular Weight marker of 100 base pairs (DNA ladder). CCHFV positive samples show bands opposite to 260 bp, while AHFV positive samples yielded a product of 208 bp.

2.3.2. Real-Time Reverse Transcription PCR

Total extracted RNA of CCHFV, DUGV, AHFV, Phleboviruses, and THOV were tested using rRT-PCR assay. The primers used in this study were mentioned in other studies as shown in **Table 1**. This assay was done in triplicate using 48-well plates. The GoTaq Probe 1-Step RT-qPCR System (Cat No.

A6120, Promega, Germany) was used for CCHFV, AHFV and DUGV, while GoTaq® 2-Step RT-qPCR System (Cat No. A6010, Promega, Germany) was chosen for THOV and Phleboviruses.

Table 1. Primer used in this study for detection of *Dugbe virus* (DUGV), *Thogoto virus* (THOV), *Phleboviruses*, *Crimean–Congo hemorrhagic fever virus* (CCHFV), and *Alkhumra hemorrhagic fever virus* (AHFV).

Primer used in conventional PCR			
Gene	**Primer (5' - 3')**	**Product size**	Ref.
Dugbe virus	Forward: TGCAACAACTGGATGTGTGA Reverse: TCTCAAAGACAAACGTGCCGCAG	600	38
Thogoto virus	Forward: AGCAGCGCCACCTTATTGCT Reverse: TCCCTCTGCAGTCATGTACA	500	39
Alkhumra Hemorrhagic Fever Virus	Conventional and nested PCR primer outer forward: TGGAACCCCACACGGGTGACT outer reverse: ATGCCCACTGTCGGTTGGCG inner forward: CCCACAGCAATCGAAAAACGGCATC inner reverse: GCCCACATCACAGGTGACATGACC	400 208	40
Crimean-Congo Haemorrhagic Fever Virus	*CCHF-F2: 5′-TGGACACCTTCACAAACTC-3′, *CCHF-F2C: 5′-TGGATACTTTCACAAACTC-3′, and *CCHF-R3: 5′-GACAAATTCCCTGCACCA-3′. *CCHFF3: 5′-GAATGTGCATGGGTTAGCTC-3′, *CCHF-F3C: 5′-GAGTGTGCCTGGGTTAGCTC-3′, *CCHF-R2a: 5′-GACATCACAATTTCACCAGG-3′, and *CCHF-R2b: 5′-GACATTACAATTTCGCCAGG-3′.	536 260	41
Phlebovirus	*NPhlebo1+: 5′$_{2047}$ATGGARGGITTTGTIWSICIICC$_{2069}$3′ *NPhlebo1-: 5′$_{2600}$AARTTRCTIGWIGCYTTIARIGTIGC$_{2575}$3 ′ *NPhlebo2+: 5′ $_{2074}$WTICCIAAICCIYMSAARATG$_{2094}$ 3′, *NPhlebo2-: 5′ $_{2318}$TCYTCYTTRTTYTTRARRTARCC$_{2296}$3′	550 250	42, 43
	Primer used in rRT-PCR		
	Primer & probe	**Ref.**	
	Crimean-Congo Haemorrhagic Fever Virus		
Forward **Reverse** **probe**	RWCF: 5′CAAGGGGTACCAAGAAAATGAAGAAGGC3′ RWCR: 5′GCCACAGGGATTGTTCCAAAGCAGAC3′ SE01: 5′FAM-ATCTACATGCACCCTGCTGTGTTGACA-TAMRA3′ SE03: 5′FAM-ATTTACATGCACCCTGCCGTGCTTACA-TAMRA3′ SE0A: 5′FAM-AGCTTCTTCCCCCACTTCATTGGAGT -TAMRA3′	12	
	Dugbe virus		
Forward **Reverse** **probe**	F-DUGV: 5′CTGGCTCAAGCAGTGGAACT3′ R-DUGV: 5′AGAGGAATTGAGACAAAGTGA3′ S-DUGV: 5′[b]CACAAGGAGCACAAATAGACA[c] 3′	20	
	Alkhumra Hemorrhagic Fever Virus		
Forward **Reverse** **probe**	AHFV S1: 5 ′ -GTGAGTGGCGCTTTGTTTG TA3′ AHFV R: 5 ′ -CCCCCTTTCCTTTAAGGACG3′ TBV TM: FAMACAGCTTAG1GAGAACAAGAGCTGGGGAXT-PH	44	
	Phleboviruses		
Forward **Reverse**	NPhlebo2+: 5′ $_{2074}$WTICCIAAICCIYMSAARATG$_{2094}$ 3′ NPhlebo2-: 5′ $_{2318}$TCYTCYTTRTTYTTRARRTARCC$_{2296}$3′	43, 45	
	Thogoto virus		
Forward **Reverse**	CGGATGGCAACAAGAAGCTG AATCAGCACAACATCCCGGT	46	

*CCHF-F2& CCHF-F2C: outer forward - CCHF-R3: outer reverse- CCHFF3 & CCHF-F3C: inner forward - CCHF-R2a & CCHF-R2b: inner reverse

*NPhlebo1+: outer forward - NPhlebo1-: outer reverse - NPhlebo2+: inner forward - NPhlebo2-: inner reverse

2.3.2.1. Detection of CCHFV, AHFV, and DUGV

GoTaq Probe 1-Step RT-qPCR System had been used. A small liqoute of 5µL of sample and 25µL reaction run were used on the Applied Biosystems StepOne™ Real-Time PCR System (Thermo Scientific, Waltham, MA, USA). The reaction was performed as mentioned in **Supplementary Table 3**. The fluorescence output data were managed and measured after each elongation step. The positive and negative controls were involved in all reactions.

2.3.2.2. Detection of THOV and Phleboviruses

GoTaq® 2-Step RT-qPCR System was used. Synthesize the cDNA as described by the manufacturer using the reaction conditions (**follow Supplementary Table 4**). A 10µl cDNA template was used in an optimized total reaction volume of 50µL using the Applied Biosystems StepOne™ Real-Time PCR System (Thermo Scientific, Waltham, MA, USA) program for standard or fast qPCR. Standard conditions are listed in **Supplementary Table 5.** The fluorescence data were assembled and measured after each elongation step. The fluorescence output data were analyzed as previously described. The positive and negative controls were involved in all reactions.

3. RESULTS

3.1. Recognition of Tick

Throughout this work duration, a collective count of 27732 hard ticks were collected from imported camels came from various countries in Africa (Ethiopia, Somalia, Kenya, and Sudan), which were recognized using a stereomicroscope. Three genera, *Hyalomma* (n = 25000 ticks; 90.1%), *Rhipicephalus* (n = 1236 ticks; 4.5%), and *Amblyomma* (n = 1496 ticks; 5.4%) were detected. Only *Hyalomma* spp. were found to be the most abundant genera with most dominant two species that were noted to infest camels. Amongst, the highly abundant tick species was *Hy. dromedarii* (n = 16750/27732; 60.4%), followed by *Hy. rufipes* (n = 8250/27732; 29.7%). Ticks were categorized into 1385 pools, comprising around 20 ticks, by animal source, size, species, and collection date. The count of tick species and their dissemination based on animal sources are represented in **Table 2**. Ticks collected during this study period were organized in **Table 3**.

Table 2. Distribution of tick species fitting to the animal sources.

Animal Source	**No. of Ticks (Pools)**			
	Hyalomma		***Rhipicephalus***	***Amblyomma***
	Hy. dromedarii	***Hy. rufipes***		
Ethiopia	3387 (169)	1426 (71)	309(15)	371(19)
Somalia	4141 (207)	2574 (128)	275(14)	347(17)
Kenya	3859 (193)	1718 (86)	352(18)	410(21)
Sudan	5363 (268)	2532 (126)	300(15)	368(18)
Total	16750 (837)	8250 (411)	1236 (62)	1496 (75)

Table 3. Number of ticks isolated from camels imported to Egypt during the study period.

Year	**No. of Ticks (Pools)**				**Total**
	Hy. dromedarii	***Hy. rufipes***	***Rhipicephalus***	***Amblyomma***	
2019	7046 (352)	3375 (168)	582 (29)	636 (32)	11639 (581)
2020	8843 (442)	4530 (226)	591 (30)	772 (39)	14736 (737)
2021(April)	861 (43)	345 (17)	63 (3)	88 (4)	1357 (67)
Total	**16750 (837)**	**8250 (411)**	**1236 (62)**	**1496 (75)**	**27732(1385)**

3.2. Virus Detection

Ticks' pools herein were investigated using conventional PCR and/or nested RT-PCR and rRT-PCR to identify the viral genomic material. By investing in PCR-based assays, we noticed that the DUGV, *Phlebovirus*, and THOV were not detected in any tick pools. However, the analysis of the two RT-PCR products showed that CCHFV and AHFV were identified in 25/1385 tick pools (1.8%) and 14/1385 tick pools (1.01%), respectively (**Figures 1 &2**). Positive control indicated positive amplification signs, but no signs were noticed in the negative one.

Regarding CCHFV, we found that 12 out of 581 tick pools (2.1%), 9 of 737 (1.22%), and 4 of 67 (5.97%) were detected positive during 2019, 2020, and 2021, respectively. Amongst the CCHFV-positive pools, 16/25 pools (64%) of *Hy. dromedarii*, 8/352 pools during 2019 (2.27%), 6/442 pools during 2020 (1.35%) and 2/43 pools during 2021 (4.65%). Among *Hy. rufipes* pools, 9/25 pools (36%) were detected positive for the viral genetic material, 4/168 pools during 2019 (2.38%), 3/226 pools during 2020 (1.32%), and 2/17 pools during 2021 (11.76%). The 25 positively detected tick pools were collected from camels brought from Sudan (16 pools), Somalia (7 pools), and Ethiopia (2 pools), as depicted in **Table 4**.

Table 4. Results of *Crimean-Congo hemorrhagic fever virus* detection by nested and real-time RT- PCR in tick pools.

Animal Source	No. of CCHFV Positive Pools			
	Nested RT- PCR		Real time RT- PCR	
	Hy. dromedarii	***Hy. rufipes***	***Hy. dromedarii***	***Hy. rufipes***
Ethiopia	2	0	2	0
Somalia	4	3	4	3
Kenya	0	0	0	0
Sudan	9	7	9	7
Total	**15**	**10**	**15**	**10**

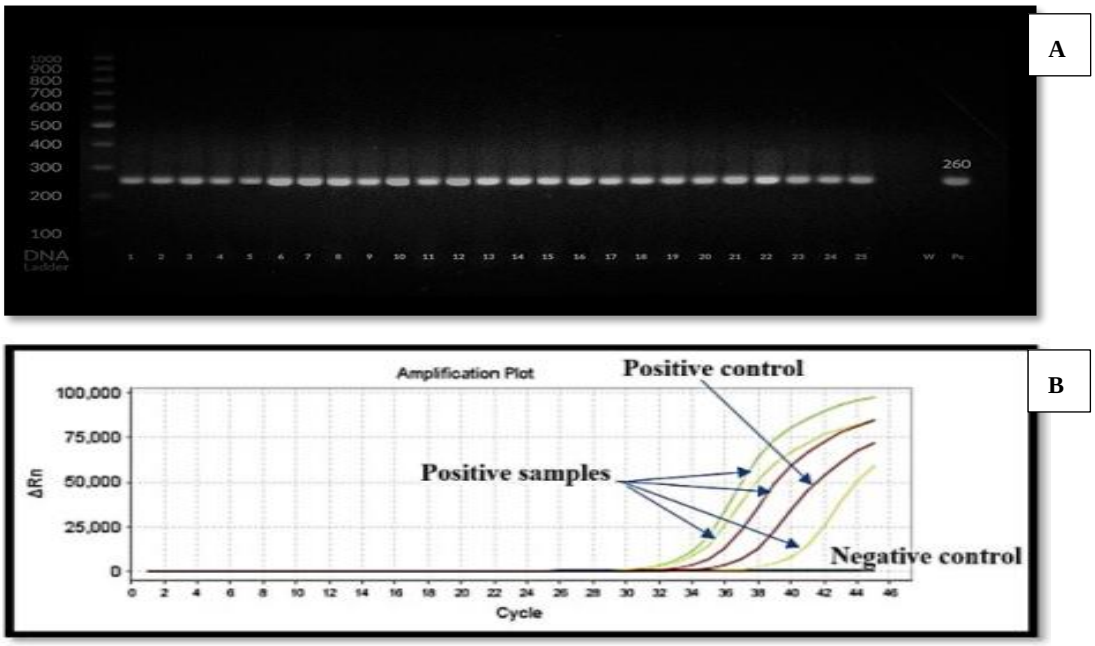

Figure 1. A) Amplification of the S segment of the *Crimean-Congo hemorrhagic fever virus* genome *via* nested RT-PCR indicating the DNA bands with 260 base pairs (bp) from infested ticks and positive control (Pc). * W negative control; Pc positive control; samples No. S1, S2, S4, S6, S10, S11, S12, S14, S16, S17, S18, S19, S20, S23, and S25 were positive *Hy. dromedarii*; S3, S5, S7, S8, S9, S13, S15, S21, S22, and S24 were positive *Hy. rufipes*. **B)** Qualitative real-time RT-PCR for *Crimean-Congo hemorrhagic fever virus* explaining Pc and W controls, and positive samples separated from infested ticks.

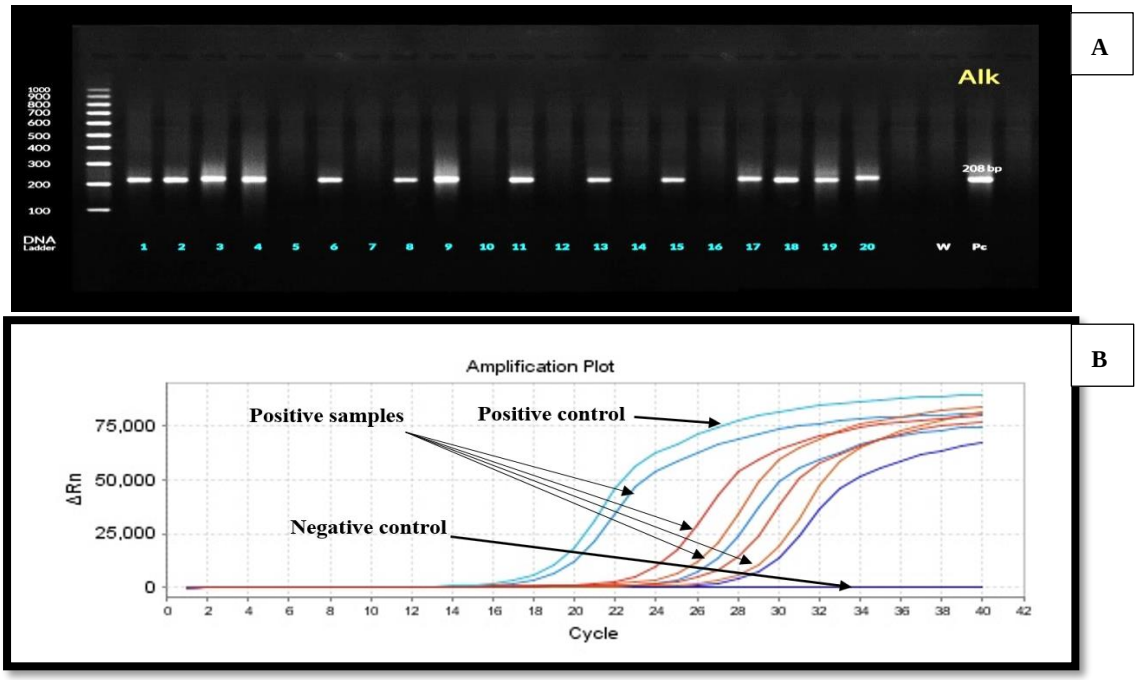

Figure 2. **A)** Amplification of the NS5 segment of the *Alkhumra hemorrhagic fever virus* genome *via* nested RT-PCR indicating the DNA bands with 208 base pairs (bp) from infested ticks and Pc. * W negative control; Pc positive control; samples No. S5, S7, S10, S12, S14 and S16 were negative **;**S3, S4, S6, S8, S9, S11, S15, S17,S18, S19 and S20 (*Hy. dromedarii*); S1, S2 and S13 (*Hy. rufipes*) were positive. **B)** Qualitative real-time RT-PCR for *Alkhumra hemorrhagic fever virus* displaying Pc and W controls, and positive samples separated from infested ticks.

Regarding AHFV, 14 pools were infested with viral genome, 8 of 581 tick pools (1.37%) and 6 of 737 (0.81%) were detected positive during 2019 and 2020, respectively, while nothing was detected during 2021. Amongst the AHFV positive pools, 11/14 pools (78.57%) of *Hy. dromedarri*, 6/352 pools during 2019 (1.7%), and 5/442 pools during 2020 (1.13%). *Among Hy. rufipes*, 3/14 pools (21.43%) were positive, only 1/168 pools during 2019 (0.59%), and 2 /226 pools during 2020 (0.88%). The 14 positively detected pools for ticks were gathered from camels imported from Sudan (10 pools), Somalia (3 pools), and Ethiopia (1 pool) (Shown in **Table 5**).

Table 5. Results of *Alkhumra hemorrhagic fever virus* detection by nested and real-time RT-PCR in tick pools.

Animal Source	No. of AHFV Positive Pools			
	Nested RT- PCR		Real-time RT- PCR	
	Hy. dromedarii	*Hy. rufipes*	*Hy. dromedarii*	*Hy. rufipes*
Ethiopia	1	0	1	0
Somalia	3	0	3	0
Kenya	0	0	0	0
Sudan	7	3	7	3
Total	**11**	**3**	**11**	**3**

4. DISCUSSION

Ticks produce negative effects on individual and animal health *via* infecting and transmission of a broad variety of pathogens, involving viral, bacterial, and protozoal infections [4, 5]. Viruses form the main community of pathogens transferred by ticks [47]. The vast majority of TBVs of human importance are transferred by hard ticks [48]. Thus, the recognition of TBVs in vectors offers a gate to avoid disease dissemination in extreme-risk abattoir employees.

Herein, ticks were collected from camels (imported from Somalia Ethiopia, Kenya, and Sudan) and evaluated for the presence of CCHFV, DUGV, *Phleboviruses*, AHFV, and THOV. Most dromedary camels, which are traded in the Middle East, are raised up in Eastern countries in Africa, mainly in, Somalia, Ethiopia, Kenya, and Sudan [49].

Three genera, *Hyalomma*, *Rhipicephalus* and *Amblyomma* were detected in this study. We discovered *Hy. dromedarii* as the key common species infesting camels (60.4%), followed by *Hy. rufipes* (29.7%), *Amblyomma* (5.4%), and *Rhipicephalus* (4.5%), agreeing with the results of Getange *et al*. (2021) in Kenya [50], Kaaboub *et al*. (2021) in Algeria [51], Bala *et al.* (2018) in Sudan [52], Champour *et al*. (2016) in Iran [53], and Chisholm *et al*. (2012) in Egypt [54]. On the other hand, Al-Deeb and Muzaffar (2020) in the UAE, Isse *et al*. (2017) in Somalia, and Moshaverinia and Moghaddas in Iran (2015) found that *Hy. dromedarii* was the predominant tick species while no *Hy. rufipes* was detected during their study [55-57]. In contrast, Zhang *et al*. (2021) in Kenya found that *Hy. rufipes* was the most detected species [58] and Elias *et al*. (2020) in Ethiopia detect that *Rhipicephalus* was the most predominant species [59]. *Hyalomma* species are reported to be the crucial tick species attacking camels in previously mentioned countries [60]. The results emphasize the hidden impact of environment and provincial circumstances on indicate ecosystem as the dry environment outfits *Hyalomma* ticks [60]. The genera *Hyalomma*, and *Rhipicephalus* comprise the highly valuable ixodid ticks infecting animals. *Hyalomma* types were noticed on camels and cows [5].

The approach of using different methods of PCR assays to detect TBVs was adopted, making this study one of the most important attempts performed in Egypt.

Both nested PCR and rRT-PCR were utilized for the recognition of *phleboviruses* in the current study. Both methods revealed that *phleboviruses* were not detected in any tick pool. In contrast, López *et al*. (2020) in Colombia found that 5/229 pools were positive using conventional PCR and rRT-PCR techniques. Also, Souza *et al*. (2018) in Brazil analyzed six groups of *R. microplus* ticks and *Lihan tick virus* (*Phlebovirus*) was detected in 5 of 6 groups of ticks using a metagenomic approach with high-throughput sequencing [45, 61]. This finding may be attributed to the collection of ticks from different hosts (bovines, equines, and dogs) and/or inability of used PCR assays to detect all *phleboviruses*. The methodology also used by Souza and his coworker was different and it is the main reason for conflict.

DUGV was initially separated in 1964 at Dugbe, Nigeria, from *Am. variegatum* [38]. It's one of the crucial predominant TBVs in countries within Africa, and it is sorted often as a prevalent virus in arid areas since it is mainly isolated from ticks infecting market animals [13, 20]. In the current study, DUGV was not detected in any tick pool. In contrast, Lutomiah *et al*., 2014 [38] in Kenya, and Sang and his coworker (2006) [13] and Burt and his coworker 1996 [22] found it in their study. In previous studies, DUGV was isolated mostly from either *Rhipicephalus* spp. or *Amblyomma* spp. suggesting that they are the potential vector of DUGV. Thus, our finding may be attributed to small numbers of ticks collected from both species.

Although THOV has been detected by William and his coworker in Egypt in 1973 [62], we did not detect it in our study. Our finding is in accordance with Lutomiah *et al*. (2014) in Kenya [38]. On the other hand, Sang and his coworker (2006) also detect it in their study [13]. Detection of THOV in the previous study maybe because of the collection of ticks from different livestock (cattle).

CCHF is one of the most geographically common TBVs, with areas of endemicity including regions disseminating within the wide zone from the Middle East to Africa, southern Asia, China, and Europe [12]. Bites from virus-loaded hard ticks or direct contact with blood or tissue from viral-infected animals can cause CCHF transmission to individuals. It can also be transmitted among individuals by direct contact with blood or other body fluids [17]. A couple of RT-PCR techniques were used to identify CCHFV in the ticks. The genome of CCHFV was found in 25 tick pools (1.8%) that were collected. This result is close to that of Chisholm *et al*. (2012) in Egypt [54.

The author mentioned that five out of six positive pools were acquired from *Hy. dromedarii* and 4.3% of pools were noticed to become infested by CCHFV. This might suggest that *Hyalomma* spp. are the primary reservoir and vector for CCHF in camels. This may possibly have a greater role in the epidemiology of the virus in Africa. The higher percentage of infection may be owing to feed of ticks on several livestock before spreading to unexpected animals, and the sample size is also low compared to our study, while temporal fluctuations between the samples may also account for the discrepancies in infection rate.

However, CCHFV was not detected in recent study in the Sudan [63]. Previous serologic studies have been conducted in Egypt by Morrill *et al*. (1990) [64.] The author discovered that CCHFV antibodies represented 14% which could be explained by a collection of the samples from camels imported from Sudan and quarantined in southern Egypt. The outcomes from a number of reports showed that the infection ratio of CCHF is fluctuating and is affected by the geographical multiplicity, the existence of various species of ticks, weather, and dissimilar tick hosts.

AHFV is an emerging infectious disease that has been detected in Saudi Arabia. Sporadic cases were reported in Africa in Egypt, Djibouti, and Europe [65]. It has been suggested that AHFV originated in Africa and that, subsequently, AHFV spread to Saudi Arabia [66]. Individuals become infected with AHFV either by bites of infested ticks or direct contact with contaminated blood through a wound or consumption of raw milk [30, 65]. *Hy. dromedarii* is one of the recognized AHFV vectors. Lately, AHFV RNA was also reported in immature *Hy. rufipes* ticks. The appearance of TBDs in non-endemic zones may be associated with pathogen propagation caused by the birds and other livestock migration [65]. The initial line of AHFV infections assessment is nested RT-PCR and Taqman based real-time RT-PCR assays [30]. A couple of RT-PCR tests were utilized to identify AHFV in the ticks. AHFV genome was detected within 14 tick pools (1%) that were accumulated. Our finding is agreeing with Hoffman *et al*., 2018 [66] and Horton *et al*., 2016 [67] who reported that 0.66% and 0.36% were detected as AHFV, respectively. However, Zakham *et al*., 2021 [68] found that AHFV represent 10% of all collected samples in Saudi Arabia and these results may be attributed to the transportation of thousands of animals yearly out of Africa and other countries to Mecca, Saudi Arabia, in order to fulfill the individual need for diet and transportation during the Hajj [69]. Carletti *et al*. 2010 confirm the existence of AHFV in Egypt as it was detected in two tourists returning back to Italy from southern Egypt [40.]

5. CONCLUSIONS

Finally, this work confirmed the presence of CCHFV and AHFV in Egypt, besides demonstrating the TBVs transmission potential amongst camels and in turn directly to humans. Additional works focused on viruses from tick and human samples are epidemiologically valuable to clearly validate the epidemic state in the area. As a final point, there are no available articles about human infection from these kinds of TBVs in Egypt. This might be due to the absence of physicians' knowledge and preventive control strategies limitation; thus, attention should be given to these kinds of diseases.

Supplementary Materials:

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