A serological survey of Crimean-Congo haemorrhagic fever in animals in the Sharkia Governorate of Egypt

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Summary

A surveillance survey was conducted over a period of 12 months between September 2004 and August 2005 by the Tropical Medicine Department of Zagazig University in collaboration with Naval Medical Research Unit No. 3 (NAMRU-3), Egypt, to evaluate the role of ruminants as a reservoir host for Crimean-Congo haemorrhagic fever virus (CCHFV). A total of 1 022 serum samples from 313 cattle, 264 water buffalo (*Bubalus bubalis*), 270 sheep and 175 goats were included in the survey. All samples were collected from the Sharkia Governorate of Egypt and were examined for anti-CCHFV IgG. Of the total of 1 022 samples examined, 32 (3.13%) were positive to IgG ELISA. Out of 270 sheep examined, 17 (6.30%) were confirmed to have anti-CCHFV IgG with the highest titre recorded at 1:800. However, CCHFV-specific IgG-positive cases among the cattle, buffalo and goats were 3.83%, 0.38% and 1.14%, respectively. Positive cases in age group B (≥2 years old) were significantly higher (p<0.001) than those in age group A (<2 years old) (5.7% versus 1.6%). Belbis City was found to have the highest number of positive cases compared to all other localities (p<0.001).

Keywords

Animal, Crimean-Congo haemorrhagic fever, Egypt, Ruminant, Sera, Serology, Survey, Virus, Zoonosis.

Indagine sierologica per febbre emorragica Crimean-Congo nel Governatorato di Sharkia-Egitto

Riassunto

*E' stata condotta un'indagine di sorveglianza per un periodo di 12 mesi (settembre 2004-agosto 2005) dal Dipartimento di Medicina Tropicale dell'Università di Zagazig in collaborazione con l'Unità di Ricerca n.3 (NAMRU-3), in Egitto, per valutare il ruolo dei ruminanti come ospiti reservoir del virus della febbre emorragica Congo-Crimea (CCHFV). Nell'indagine sono stati esaminati un totale di 1.022 campioni di siero, di cui 313 bovini, 264 bufalini (*Bubalus bubalis*), 270 ovini e 175 caprini. Tutti i campioni sono stati raccolti dal Governatorato Sharkia in Egitto e sono stati esaminati per anti-CCHFV IgG. Sul totale di 1022 campioni, 32 (3,13%) erano positivi per IgG ELISA. Dei 270 campioni di pecora esaminati, 17 (6.30%) venivano confermati anti-CCHFV IgG con un titolo elevato a 1:800. Infine, CCHFV-specific, IgG positivi tra bovini, bufali e capre esaminati erano rispettivamente 3,83%, 0,38% e 1,14%. I casi positivi nel gruppo B (≥2 anni di età) erano significativamente più alti (p<0.001) di quelli del gruppo A (<2 anni d'età) (5.7% vs 1.6%). La città di Belbis è risultata quella con il più elevato numero di casi positivi se confrontata con le altre località (p<0.001).*

Parole chiave

Animale, Egitto,Febbre emorragica del Congo-Crimea, Indagine, Ruminanti, Siero, Sierologia, Virus, Zoonosi.

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Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a potentially fatal, tick-borne viral disease (6). It has the most extensive geographic range of the medically significant tick-borne viruses (9). CCHF virus (CCHFV) is generally transmitted between ticks and vertebrates by bites or by contact (5) making it a meta-zoonotic disease. An infected tick remains infected throughout its life and transmits the infection to large vertebrates. The virus causes unapparent infection or mild fever in cattle, sheep and goats with viraemia of sufficient intensity to infect adult ticks (4).

Community acquired CCHF occurs through direct contact with blood or other tissues of viraemic livestock or humans or from bites or the crushing of infected ticks (1). Nosocomial infections have been reported among hospital health workers caring for patients with haemorrhagic symptoms (14). Animal outbreaks of CCHF have been reported in Saudi Arabia (8), the United Arab Emirates (15), Kuwait (2) and Iraq (3). Anti-CCHFV immunoglobulin G (IgG) has been reported in livestock in Egypt (7).

The presence of ticks across Egypt, the importation of animals from Africa and the landing of migratory birds in Egypt create ideal conditions for the emergence of the virus in Egypt. This study was performed to evaluate the status of CCHF in the Sharkia Governorate which is considered to be a representative sample of the Egyptian environment.

The enzyme-linked immunosorbent assay (ELISA) was a highly sensitive and specific tool for CCHF diagnosis (10).

Materials and methods

A total of 1 022 sera (313 cattle, 264 water buffalo [*Bubalus bubalis*], 270 sheep and 175 goats) were collected at random from abattoirs in Zagazig, Alkyinaiat, Alkoreen and Belbis in the Sharkia Governorate of Egypt.

Blood samples were collected from each animal in sterile test tubes after slaughter. The tubes were labelled with location, age and sex of the animal. They were allowed to clot at room temperature and then transferred immediately to the Naval Medical Research Unit No. 3 (NAMRU-3) in an ice box. The tubes were centrifuged at 1 000 rpm for 10 min to separate serum. Sera were inactivated in a water bath at 56°C for 30 min and then stored at –20°C until use.

An ELISA was performed as follows: each well of a 96-well microplate (Immunoplate, Nunc, Roskilde) was coated with 100 µl of capture antibody (anti-CCHFV hyperimmune ascitis fluid diluted 1:1 000 in phosphate buffered saline, or PBS) and incubated overnight at 4°C. The wells were then washed three times with PBS containing 0.05% Tween 20 (PBST). Thereafter, CCHFV antigen 100 µl 1:20 (inactivated supernatant from tissue culture cells infected with CCHFV) was added to each well and the plates were incubated for 1 h at 37°C. After washing the wells with PBST, diluted sera in PBST and 3% skimmed milk (PBSTM) was added to each well. The plates were then incubated for 1 h at 37°C. After the plates were washed with PBST, 100 µl of horse radish peroxidase conjugate labelled anti-species (anti-bovine, anti-sheep and anti-goat) whole molecule IgG (1 µg/ml) were then added to each well. After incubation (1 h at 37°C) and washing with PBST, 100 µl of substrate solution (0.01% of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid], Sigma in 50 mM citric acid with 0.0035 H_2O_2) were added to each well and left for colour change by incubation at room temperature in the dark for 30 min. The optical density (OD) values were measured at 405 nm using a microplate reader (Bio Rad, Tokyo). Data were analysed using version 10 of the SPSS (Statistical Package for the Social Sciences) program for an independent Chi squared test (χ^2).

Results

The results were as follows:

- 32 (3.13%) serum samples out of 1 022 were found to be positive to CCHFV IgG ELISA
- out of the 270 sheep examined, 17 (6.30%) were confirmed to have anti-CCHFV IgG with the highest titre recorded at 1:800

- the positive cases of CCHFV-specific IgG among the cattle, buffalo and goats were 3.83%, 0.38% and 1.14%, respectively
- positive cases in age group B (≥2 years old) were significantly higher (p<0.001) than those for age group A (<2 years old) (5.7% versus 1.6%)
- Belbis City was found to have the highest number of positive cases in comparison with other localities (p<0.001).

Discussion

CCHV is a zoonotic disease that affects people who come into contact with livestock and ticks (12). It causes severe and often fatal haemorrhagic disease in humans but infection in animals is asymptomatic (10). Geographically, Egypt is situated among many Eurasian and African CCHF endemic foci. Importation of food animals from the Sudan where the disease is known to be endemic, the possibility of introducing ticks to Egypt through migrating birds coming from endemic areas, make it quite possible that CCHFV is circulating in Egypt.

Since the Sharkia Governorate has a station for aquatic migratory birds that land on its lakes, it was selected for the CCHF-specific IgG survey as it is considered to be representative of the Egyptian environment.

Currently, there is no CCHF surveillance programme in Egypt that involves humans. There have been no recorded human cases of CCHF in Egypt in the past. Nevertheless, suspected cases might be confused in with other haemorrhagic cases in humans.

An examination of 1 022 sera from different animal species from the Sharkia Governorate (Table I) revealed that the overall seroprevalence rate of CCHF in ruminants was 3.31%. A similar prevalence percentage of 1.87 was reported in Egypt (7), but this study did not include the Sharkia Governorate. Seroprevalence rates of 13% and 36% had been reported previously (11, 13). The higher percentage reported by Morrill *et al.* (13) could be attributed to the fact that their samples were collected from animals that had been imported from the Sudan and quarantined at the Aswan quarantine station in southern Egypt.

In regard to the presence of anti-CCHFV IgG among different animal species, it was evident that CCHFV-specific IgG was detected with comparatively higher percentages in sheep (6.3%) compared to the other species (Table I). The seroprevalence rates in cattle, buffalo and goats were 3.38%, 0.38% and 1.14%, respectively. During the 1977 CCHFV serological/epidemiological survey conducted by the Ain Shams University, Egyptian Organisation for Biological and Vaccine Production and NAMRU-3, the seroprevalence rate of CCHFV-specific IgG among cattle, buffalo and sheep was 13.88%. 3.1% and 18.2%, respectively. However, in another study conducted in Egypt between 1986 and 1987, no antibodies were detected in 400 sheep and 200 cows sampled among native animals (13). Higher results were found by Williams *et al.* (16), in cattle (3%), goats (27%) and sheep (23%). Vorou *et al.* (17) reported that climate and environmental changes may affect the epidemiology of CCHF and could trigger community outbreaks.

Table I
Seroprevalence of Crimean Congo haemorrhagic fever in ruminants examined in Egypt

Source	Total no. of samples	Total positive		Age group					
				A (<2 years)			B (≥2 years)		
		No.	Percentage	No. examined	Positive No.	Positive Percentage	No. examined	Positive No.	Positive Percentage
Cattle	313	12	3.83	205	5	2.4	108	7	6.5
Buffalo	264	1	0.38	209	0	0.0	55	1	1.8
Sheep	270	17	6.30	122	5	4.1	148	12	8.1
Goats	175	2	1.14	101	0	0.0	74	2	2.7
Total	1 022	32	3.13	637	10	1.6	385	22	5.7
			p	<0.001					

In regard to the seroprevalence of CCHFV-specific IgG among the animals with respect to age, Table I clarifies that the percentage of seroprevalence was significantly higher (p<0.001) in age group B (≥ 2 years) than in age group A (< 2 years) (5.7% versus 1.6%). This suggests that the age factor may influence the rate of infection in ruminants.

The location of the animals had an impact on the results of this work. Table II illustrates that there was a highly significant difference (p<0.001) between Belbis and other districts, where the percentage of occurrence in the Belbis abattoir was 10.4 (26 out of 250). This may be related to the fact that Belbis is one of the largest collection sites of camels in the Delta and these animals act as a potential reservoir for hard ticks that may attack other animal species.

Table II
Presence of antibodies against Crimean Congo haemorrhagic fever in sera of ruminants examined by location

Source	Total no. of samples	Total positive	
		No.	Percentage
Zagazig	352	2	0.57
Belbis	250	26	10.40
Alkoreen	200	2	1.00
Alkinyat	220	2	0.91
Total	1 022	32	3.13
		p <0.001	

As shown in Table III, the highest titre of 1:800 was recorded in sheep that gave positive results to anti-CCHFV IgG. This may indicate that sheep are the most important reservoirs of CCHFV in Egypt.

Table III
Crimean Congo haemorrhagic fever immunoglobulin G titres in the positive sera of ruminants

Titre	Cattle	Buffalo	Sheep	Goats
1:100	12[a]	1[b]	4[a]	2[c]
1:200	–	–	1[b]	–
1:300	–	–	2[d]	–
1:400	–	–	7[a]	–
1:800	–	–	3[a]	–
Total	12	1	17	2

a) Belbis
b) Alkoreen
c) Zagazig
d) Alkinyat

Conclusions

Results obtained in this study show that CCHF does exist in the Sharkia Governorate and that human beings are at risk of being infected. Livestock workers should be cautious and observe strict hygiene whilst handling animals, especially when they are heavily infested with ticks. Moreover, physicians and other health workers must be aware of the risks involved when dealing with cases of haemorrhagic fever.

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References

1. Acha P.N. & Szyfres B. 1991. Zoonoses and communicable diseases common to man and animals, Second Ed. Pan American Health Organization, Washington, DC, 320 pp.
2. Al-Nakib W., Lloyd G., El-Mekki A., Platt G., Beeson A. & Southee T. 1984. Preliminary report on arbovirus-antibody prevalence among patients in Kuwait: evidence of Congo/Crimean virus infection. *Trans R Soc Trop Med Hyg*, **78**, 474-476.
3. Al-Tikriti S.K., Al-Ani F., Jurji F.J., Tantawi H., Al-Moslih M., Al-Janabi N., Mahmud M.I., Al-Bana A., Habib H., Al-Munthri H., Al-Janabi S., Al-Jawahry K., Yonan M., Hassan F. & Simpson D.I. 1981. Crimean-Congo haemorrhagic fever in Iraq. *Bull World Health Organ*, **59**, 85-90.
4. Athar M.N., Baqai H.Z., Ahmad M., Khalid M.A., Bashir N. & Ahmad A.M., Balouch A.H. & Bashir K. 2003. Short report: Crimean-Congo hemorrhagic fever outbreak in Rawalpindi, Pakistan. *Am J Trop Med Hyg*, **69**, 284-287.

5. Bishop D.H. 1996. Biology and molecular biology of bunyaviruses. *In* The Bunyaviridae (R.M. Elliot, ed.). Plenum Press, New York, 19-61.
6. Chinikar S., Goya M.M., Shirzadi M.R., Ghiasi S.M., Mirahmadi R., Haeri A., Moradi M., Afzali N., Rahpeyma M., Zeinali M. & Meshkat M. 2008. Surveillance and laboratory detection system of Crimean-Congo haemorrhagic fever in Iran. *Transbound Emerg Dis*, **55**, 200-204.
7. Darwish M.A., Iman I.Z., Omer F.M. & Hoogstral H.A. 1977. Sero-epidemiological survey for CCHF virus in humans and domestic animals in Egypt. *J Egypt Public Health Assoc*, **52**,156-163.
8. El-Azazy O.M. & Scrimgeour E.M. 1997. Crimean Congo haemorrhagic fever virus infection in the western province of Saudi Arabia. *Trans R Soc Trop Med Hyg*, **91**, 175-178.
9. Ergönül O. 2006. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis*, **6** (4), 203-214.
10. Garcia S., Chinikar S., Coudrier D., Billecocq A., Hooshmand B., Crance J.M., Garin D. & Bouloy M. 2006. Evaluation of Crimean-Congo hemorrhagic fever virus recombinant antigen expressed by Semliki Forest suicide virus for IgM and IgG antibody detection in human and animal sera collected in Iran. *J Clin Virol*, **35** (2), 154-159.
11. Gonzalez J.P., LeGuenno B., Guillaud M. & Wilson M.L. 1990. A fatal case of Crimean-Congo haemorrhagic fever in Mauritania: virological and serological evidence suggesting epidemic transmission. *Trans R Soc Trop Med Hyg*, **84**, 573-576.
12. Kuljić-Kapulica N. 2004. Emerging diseases. Crimean-Congo hemorrhagic fever [in Serbian]. *Med Pregl*, **57** (9-10), 354-356.
13. Morrill J.C., Soliman A.K., Imam I.Z., Botros B.A., Moussa M.I. & Watts D.M. 1990. Serological evidence of Crimean-Congo hemorrhagic fever viral infection among camels imported into Egypt. *J Trop Med Hyg*, **93**, 201-204.
14. Shepherd A.J., Swanepoel R., Shepherd S.P., Leman P.A., Blackburn N.K. & Hallet A.F. 1985. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part V. Virological and serological observations. *S Afr Med J*, **68**, 733-736.
15. Suleiman M.N., Muscat-Baron J.M., Harries J.R., Satti A.G., Platt G.S., Bowen E.T. & Simpson D.I. 1980. Congo/Crimean haemorrhagic fever in Dubai. An outbreak at the Rashid Hospital. *Lancet*, **2**, 939-941.
16. Williams R.J., Al-Busaidy S., Mehta F.R., Maupin G.O., Wagoner K.D., Al-Awaidy S.& Suleiman A.J.M. 2000. Crimean Congo hemorrhagic fever: a sero-epidemiological and tick survey in Sultanate of Oman. *Trop Med Int Health*, **5** (2), 9-106.
17. Vorou R., Pierroutsakos I.N. & Maltezou H.C. 2007. Crimean Congo hemorrhagic fever. *Curr Opin Infect Dis*, **20** (5), 495-500.