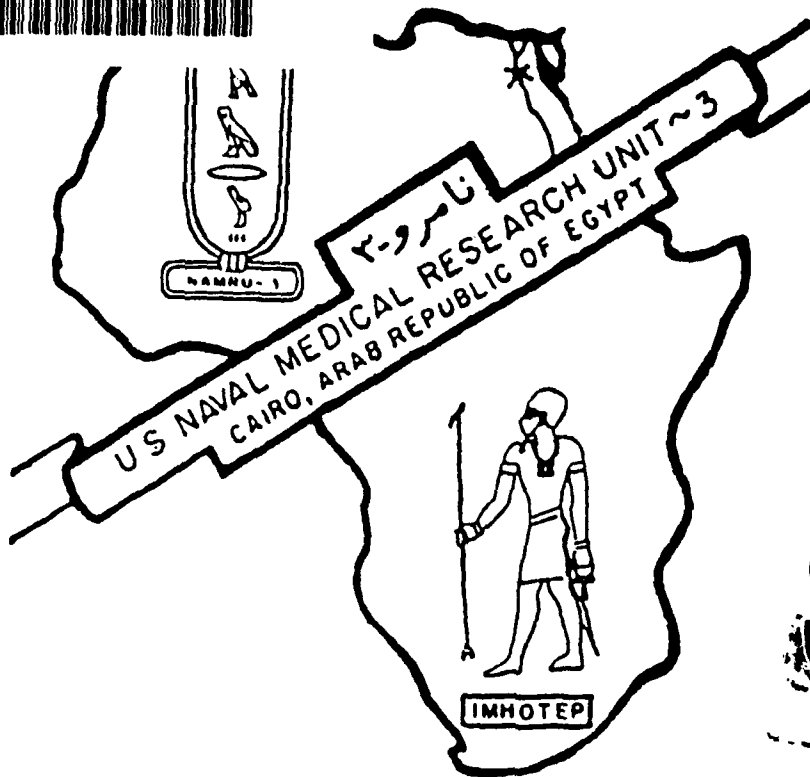


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COMMUNITY-BASED PREVALENCE PROFILE OF ARBOVIRAL, RICKETTSIAL,  
 AND HANTAAAN-LIKE VIRAL ANTIBODY IN THE NILE RIVER DELTA OF EGYPT

BY

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## COMMUNITY-BASED PREVALENCE PROFILE OF ARBOVIRAL, RICKETTSIAL, AND HANTAAN-LIKE VIRAL ANTIBODY IN THE NILE RIVER DELTA OF EGYPT

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**Abstract.** To determine the current prevalence of antibody to arboviruses, rickettsiae, and hantaan-like viruses, a survey was carried out in the Nile River Valley of Egypt, one of the principal foci of the 1977-1978 Rift Valley fever (RVF) outbreak. Blood specimens were obtained from 915 persons representing 190 study households. Enzyme immunoassay testing showed that the overall prevalence of IgG antibody was 4% to sand fly fever Sicilian (SFS), 2% to sandfly fever Naples (SFN), 15% to RVF, 20% to West Nile, and 4% to Hantaan (HTN) viruses. Antibody was demonstrated among 32% of the same study subjects to *Coxiella burnetii*, 58% to *Rickettsia typhi*, and 32% to *R. conorii*. The prevalence of agent-specific antibody tended to increase with age. Particularly notable was the low prevalence of RVF infection in children born after the height of the RVF outbreak. No detectable antibodies were found in the population less than seven years of age and in only 3% of those 7-12 years old. In contrast, 26% of the study population 13-19 years old, who were young children and infants at the time of the outbreak, were found to have RVF antibodies, suggesting that the level of intensity associated with transmission decreased considerably following the documented 1977-1978 outbreak. Geometric mean titers (GMT) ranged from 139 for *C. burnetii* to 1,305 for RVF, and did not vary significantly by age, except for high titers for RVF in the 20-49-year-old age group. A significant upward trend in GMT was also noted when antibody was detected in the specimen for more than one phlebovirus. This was observed for SFS, SFN, and RVF, i.e., 1,172 for RVF only, 1,334 for RVF and SFN, 1,828 for RVF with SFS, and 2,111 for RVF with SFN and SFS ( $P < 0.05$ ). These figures attest to the boosting phenomenon found when antibody for one phlebovirus is found in association with others. These findings show prevalences of rickettsial antibodies and warrant further study of disease incidence associated with acquired infection. Additionally, familial clustering of infected cases in households was evident only for *C. burnetii*, *R. typhi*, and *R. conorii*, and was independent of sex.

Not since the outbreak of 1977-1978 has Rift Valley fever (RVF) virus been recognized in the Nile River Delta of Egypt.<sup>1</sup> A high case fatality rate was associated with the estimated 20,000-100,000 human cases, in addition to widespread viral zoonosis.<sup>2-4</sup> Clinical features described from hospital-based observations at the peak of the epidemic included encephalitic, ocular, and hemorrhagic manifestations.<sup>2</sup> Several other arboviruses have been associated with human disease in Egypt, although not in epidemic proportions. These include sand fly fever Sicilian (SFS), sandfly fever Naples (SFN), West Nile (WN), Sindbis (SIND), and Quarantil viruses.<sup>4-11</sup> Each of these viruses may cause asymptomatic infec-

tion or a mild, self-limiting acute undifferentiated febrile illness. However, sporadic cases of encephalitis among adults have been associated with WN and SIND infection.<sup>4</sup> Except for RVF, the viruses mentioned above are maintained zoonotically in Egypt by hematophagous arthropods and wild and domestic vertebrates.<sup>12-16</sup> Viral transmission to humans occurs when they serve as blood meal sources for infected arthropods.

The presence of other viral and rickettsial agents has been reported in human populations in Egypt. However, conclusive evidence of associated human disease is lacking. Isolates of Hantaan (HTN)-like virus were obtained from

humans and rodents (Hoogstraal H, unpublished data). *Coxiella burnetii*, *Rickettsia typhi*, and to a lesser extent *R. conorii* have been identified in humans and animals.<sup>17-20</sup> Humans serve as tangential hosts when bitten by infected arthropods and or from transmission via exposure to aerosolized fecal or urinary excretions.

A cross-sectional survey of households in a community-based study of arboviral, rickettsial, and hantaa-like diseases was carried out in the Nile River Delta of Egypt. The purpose of this survey was to collect baseline data for longitudinal comparisons and to determine the current prevalence of antibody to selected viruses and rickettsiae. The household served as the principal sampling unit and so ensured broad-based age representation and the analytical framework for examining familial and geographic clustering.

#### SUBJECTS AND METHODS

##### *Study population*

A survey was carried out in the village of Kafr Ayoub, located in the Bilbeis area of the Sharqiya governate, one of the principal foci identified in the 1977-1978 RVF outbreak.<sup>21</sup> Situated approximately 60 kilometers northeast of Cairo, this area is characterized by fertile agricultural lands fed by tributaries from the Nile River. Specimens and household data were collected during May, June, and July 1991 from 915 persons (356 males and 559 females) representing 190 households (mean of 4.8 persons household). Ages of the study population ranged from less than one year old to 80 years (mean  $\pm$  SD 17.23  $\pm$  15.12). The mean age of females (18.91  $\pm$  15.77 years) was significantly higher than that of the males (14.60  $\pm$  13.65) ( $P < 0.0001$ ).

The study village had previously been demographically mapped and every household and person had been assigned a unique identifier for enrollment in a longitudinal study of disease incidence. The household served as the principal sampling unit. Sample households were randomly selected using a proportional clustering strategy. Additionally, a clinical and laboratory diagnostic capability was established at the Bilbeis Epidemiology Study Unit and laboratory, clinical, and field staff had been trained to conduct applied field research.

A standardized questionnaire providing for demographic, social, and environmental infor-

mation was field tested for cultural acceptability and linguistic appropriateness. Trained interviewers then administered questionnaires in Arabic to all members of selected households, after obtaining informed consent (adults  $\geq$  18 years old provided representation as legal guardians for all minors).

##### *Serologic tests*

Family members were escorted to the study clinic located in the village. Follow-up was carried out in the household in the case of absentees. A blood specimen was obtained by finger stick from each study subject, and serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until tested for antibody. Enzyme immunoassays (EIA) were used to test sera for IgG class antibody to *R. typhi*, *R. conorii*, *C. burnetii*, and to RVF, WN, SFN, SFS, and HTN viruses. Antigens for *R. typhi* and *R. conorii* assays were supplied by Dr. G. Dasch (Naval Medical Research Institute, Bethesda, MD), and antigen for *C. burnetii* tests was provided by Dr. J. D. Chulay (U.S. Army Medical Research Institute of Infectious Diseases, [USAMRIID] Fort Detrick, Frederick, MD). Rift Valley fever antigen, provided by the Salk Institute (Swiftwater, PA), was prepared from sucrose-acetone liver extracts from infected suckling mice. Antigens for WN, SFS, and SFN were produced from supernates of infected BHK-2 cell cultures and were supplied by T. J. Kaszcek (USAMRIID). Hantaan virus antigen was obtained from Dr. J. LeDuc (USAMRIID). Negative control antigens for all rickettsiae and viruses were prepared from uninfected cells of the same cell lines that were used to prepare the test antigens.

The EIA for *R. typhi*, *R. conorii*, *C. burnetii*, and HTN virus was a four-step procedure similar to that described by Voller and others, in which the microtiter wells are directly coated with antigen.<sup>22</sup> The assays for *C. burnetii* were performed as described by Williams and others.<sup>23</sup> The assays for WN, SFN, SFS and RVF included an additional step that used virus-specific mouse hyperimmune ascitic fluid to capture the antigen on the plate. The coating buffer for both assay formats was phosphate-buffered saline (PBS), pH 7.4.

The following generalized procedure was used for the assay. Flat-bottomed, 96-well, polystyrene plates (Immulon 2; Dynatech Laboratories,

Chantilly, VA) were coated with viral antigen and negative control antigen overnight at 4°C. Wells for assays that used the capture antibody format were initially coated in the same manner with virus-specific antibody. The plates were washed five times with PBS containing 0.1% Tween 20 (PBST), serum samples (diluted 1:100 in PBST containing 5% fetal bovine serum [FBS]) were added to duplicate antigen and control wells, and the plates were incubated for 60 min at 37°C. The plates were again washed five times, goat anti-human IgG conjugated to horseradish peroxidase (Kirkegaard and Perry, Gaithersburg, MD) diluted in PBST with FBS was added to all wells, and the plates were incubated for 60 min at 37°C. The plates were washed as before, 2,2'-azino di (3-ethyl-benzthiazoline) sulfonate (ABTS) substrate (0.6 g/l) was then added to the wells, and the plates were incubated for 20 min at 37°C. Optical densities (OD) were determined at 414 nm using a Multiskan MCC 340 MK 11 multichannel spectrophotometer (Flow Laboratories, McLean, VA). A sample was considered positive if its net OD value (difference between positive and negative antigen values) exceeded the net mean plus three standard deviations of ten negative control sera. All samples that were positive at the 1:100 screening dilution were titered by two-fold endpoint dilution. Box titrations were performed to optimize the concentrations of antigens and the anti-human IgG conjugates used in the tests. Antibody-positive and -negative control sera were included in each test.

#### Statistical tests

The proportional hypothesis test method was used to determine statistical differences between two proportions from one group (overlapping categories from a single sample). In addition, 95% confidence intervals (CI) were calculated for proportions from a single sample by the exact and normal method. Chi-square tests with Yates' correction were performed when comparing multiple proportions from mutually exclusive sample groups. Finally, the Student's *t*-test and analysis of variance were used to determine the significance of differences between mean values.

#### RESULTS

Table 1 shows age-specific prevalence of IgG antibody to selected agents. The overall preva-

lence of IgG antibody among study subjects was 4% (95% CI 2.6–5.3%) for SFS, 2% (95% CI 0.8–2.7%) for SFN, 15% (95% CI 12.5–17.3%) for RVF, 20% (95% CI 17.7–23.1%) for WN, and 4% (95% CI 2.4–4.9%) for HTN viruses. Antibody was demonstrated among 32% (95% CI 29.2–35.4%) of the same population for *C. burnetii*, 58% (95% CI 54.8–61.4%) for *R. typhi*, and 32% (95% CI 28.2–34.9%) for *R. conorii*. The proportion of males exhibiting antibody titers did not differ significantly ( $P > 0.05$ ) from that of females, even when controlling for age, regardless of the agent. The prevalence of agent-specific antibody tended to increase with age, except for RVF, for which prevalence peaked (47%) in the 20–29-year old age category. The low prevalence of infection in children born after the height of the outbreak was notable. Antibodies were not detected in members of the population who were less than seven years of age and were found in only 3% of those 7–12 years old. In contrast, 26% of the 13–19-year-old age group of the population, who were children and infants at the time of the outbreak, were found to have RVF antibodies.

Table 1 also shows the agent prevalence estimates of phleboviral infection, with and without controlling for the presence of the two other phleboviral agents for which testing was done. The differences among prevalence values at each age varied significantly, except in the 0–9-year-old age category for RVF, SFS, and SFN, and the 10–19-year-old age group for SFN only. These differences were noted to increase with age in the case of all three phleboviruses.

The agent-specific geometric mean titers (GMT) presented in Table 2 for selected arboviruses ranged from 195 for SFN to 1,305 for RVF, and did not vary significantly with age ( $P \geq 0.05$ ), except for comparatively high GMTs of 1,451 ( $n = 57$ ), 2,015 ( $n = 39$ ), 2,487 ( $n = 11$ ), and 1,198 ( $n = 12$ ) for RVF in the 20–29-, 30–39-, 40–49-, and 50–59-year-old age categories, respectively. A GMT of 1,212 was demonstrated among the population  $\geq 60$  years of age ( $n = 5$ ) for SFN. The GMT values were generally lower for the rickettsiae, ranging from 139 for *C. burnetii* to 292 for *R. typhi*. There were no significant differences among age groups ( $P \geq 0.05$ ).

A significant upward trend in the GMT was noted when antibody was detected in the same specimen for more than one phlebovirus (Table

TABLE 1  
Serologic evidence of arboviral, Hantaan-like, and rickettsial infections among villagers, by age, in Bilbeis, Egypt

		Age group (years)						
		0-9	10-19	20-29	30-39	40-49	50-59	>60
<b>Arboviruses</b>								
West Nile	(No. tested)	(321)	(268)	(104)	(82)	(38)	(22)	(13)
	Prevalence*	0.062	0.127	0.404	0.476	0.368	0.682	0.204
Rift Valley fever	(No. tested)	(318)	(267)	(105)	(83)	(38)	(26)	(15)
	Prevalence*	0.009	0.154†	0.571‡	0.506‡	0.368§	0.500‡	0.466†
	Prevalence#	0.009	0.135	0.470	0.330	0.079	0.270	0.133
Sand fly fever Sicilian	(No. tested)	(317)	(266)	(104)	(82)	(38)	(24)	(12)
	Prevalence*	0.012	0.049†	0.164‡	0.256§	0.605§	0.375‡	0.666‡
	Prevalence#	0.012	0.030	0.050	0.073	0.132	0.083	0.167
Sand fly fever Naples	(No. tested)	(318)	(267)	(105)	(83)	(38)	(26)	(15)
	Prevalence*	0.000	0.018	0.048†	0.120†	0.368§	0.625‡	0.466‡
	Prevalence#	0.000	0.012	0.019	0.036	0.053	0.115	0.067
Hantaan-like virus	(No. tested)	(336)	(271)	(111)	(85)	(38)	(27)	(16)
	Prevalence	0.038	0.033	0.018	0.058	0.000	0.074	0.000
<b>Rickettsiae</b>								
<i>Rickettsia typhi</i>	(No. tested)	(335)	(271)	(111)	(85)	(38)	(27)	(16)
	Prevalence	0.364	0.649	0.784	0.764	0.842	0.777	0.937
<i>Rickettsia conorii</i>	(No. tested)	(287)	(217)	(96)	(66)	(34)	(23)	(12)
	Prevalence	0.209	0.345	0.406	0.439	0.323	0.478	0.583
<i>Coxiella burnetii</i>	(No. tested)	(335)	(271)	(111)	(85)	(38)	(27)	(16)
	Prevalence	0.340	0.295	0.252	0.376	0.421	0.296	0.437

\* No. of antibody-positive sera no. of sera tested

†  $P < 0.05$ ‡  $P < 0.001$ §  $P < 0.0001$ \*  $P < 0.01$ 

# No. of agent-specific antibody-positive sera only and negative for the other two phleboviral agents no. of sera tested

TABLE 2  
Frequency distribution of IgG antibody titers among the study population, by agent\*

Agent	Titers								Total	
	10x	20x	40x	80x	1.60x	3.20x	6.40x	12.80x	No. (%)†	GMT‡
<b>Arboviruses</b>										
West Nile	89	29	23	10	15	6		1	173 (20)	224
Rift Valley fever§	7	10	19	28	51	32	17	3	167 (20)	1,305
Sand fly fever Sicilian§	12	5	5	9	7	4	4		46 (5)	575
Sand fly fever Naples§	7	10	3	2	2	2	3		29 (3)	195
Hantaan-like viruses	13	9	7		1	1			31 (4)	204
<b>Rickettsiae</b>										
<i>Rickettsia typhi</i>	197	45	70	206					518 (58)	292
<i>Rickettsia conorii</i>	95	20	48	69					232 (32)	262
<i>Coxiella burnetii</i>	210	36	20	19					285 (32)	139

\* Values are the no. of individuals with specified antibody titers.

† Percentage of all sera examined for specific agent.

‡ Geometric mean titer.

§ Titers were reported for each phlebovirus only if higher or in the absence of the other two.

TABLE 3  
*Antibody (IgG) responses to single, dual, and multiple phleboviral infections in the study population*

Viruses	No.	GMT*	% tested
Rift Valley fever only†	127	1.172	15
With sand fly fever Naples‡	4	1.345	1
With sand fly fever Sicilian	26	1.828	3
With sand fly fever Naples and Sicilian	10	2.111	1
		<i>P</i> < 0.05	
Sand fly fever (Naples) only	14	200	2
With Rift Valley fever	2	282	<1
With sand fly fever Sicilian	11	1.029	1
With Rift Valley fever and sand fly fever Sicilian	2	1.131	<1
		<i>P</i> < 0.01	
Sand fly fever (Sicilian) only	32	391	4
With Rift Valley fever	6	2.262	1
With sand fly fever Naples	5	527	1
With Rift Valley fever and sand fly fever Naples	3	1.600	<1
		<i>P</i> < 0.01	

\* Geometric mean titer.

† No antibodies were detected for either of the other two phleboviruses.

‡ Only if higher antibody titers were detected for the agent.

3). This was true for SFS, SFN, and RVF, i.e., 1.172 for RVF only, 1.345 for RVF and SFN, 1.828 for RVF with SFS, and 2.111 for RVF with SFN and SFS (*P* < 0.05). The overall GMT for specimens with RVF antibody only (1.172) varied little from the value calculated (1,180) without consideration of the other two phleboviruses. The same finding was observed for SFS (391 versus 456) and SFN (200 versus 353).

Familial clustering of infected occupants in households was most evident for *C. burnetii*, *R. typhi*, and *R. conorii*, and to a lesser extent for WN and RVF, independent of sex (Table 4). Additionally, there was no apparent clustering of antibody-positive households (with one or more occupants exhibiting IgG antibody) in any of the four zones used to geographically divide the village (Table 5). This was true for all the agents.

TABLE 4  
*Household clustering (proportional distribution) of occupants with antibody to West Nile (WN), Rift Valley fever (RVF), sand fly fever Sicilian (SFS), sand fly fever Naples (SFN), and Hantaan-like (HTN) viruses, and Rickettsia typhi, R. conorii, and Coxiella burnetii, in Bilbeis Egypt*

Households with person*	Arboviruses				HTN	Rickettsiae		
	WN 106†	RVF 82	SFS 13	SFN 29		<i>R. typhi</i> 179	<i>R. conorii</i> 119	<i>C. burnetii</i> 131
1	64‡	61	90	92	84	28	49	40
2	20	26	10	8	8	23	23	28
3	8	11	0	0	8	20	18	15
4	4	2	0	0	0	10	8	8
5	4	0	0	0	0	8	1	8
6	0	0	0	0	0	7	1	1
7	0	0	0	0	0	2	1	0
8	0	0	0	0	0	1	0	0
9	0	0	0	0	0	1	0	0
10	0	0	0	0	0	1	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	1	0	0

\* Households with 1, 2, . . . 12 antibody-positive occupants.

† Values are the no. of households with at least one agent-specific, antibody-positive occupant.

‡ Values are the percentage of households (no. of households in category\* no. of households with at least one agent-specific, antibody-positive occupant).

TABLE 5

Geographic distribution (clustering) of households with at least one antibody-positive occupant for selected agents, by zone, in Bilbeis, Egypt\*

	Geographic zones								$\chi^2$ †
	1		2		3		4		
	%	No. positive no. tested	%	No. positive no. tested	%	No. positive no. tested	%	No. positive no. tested	
<b>Arboviruses</b>									
West Nile	71	(20/28)	48	(43/90)	59	(22/37)	64	(21/33)	6.1 (NS)
Rift Valley fever	39	(11/28)	60	(54/90)	62	(23/37)	45	(15/33)	5.7 (NS)
Sand fly fever Sicilian	54	(15/28)	36	(32/90)	38	(14/37)	39	(9/23)	4.7 (NS)
Sand fly fever Naples	29	(8/28)	21	(19/90)	14	(5/37)	27	(9/33)	2.9 (NS)
<b>Hantaan-like virus</b>	4	(1/28)	15	(14/91)	9	(3/34)	21	(7/34)	ND
<b>Rickettsiae</b>									
<i>Rickettsia typhi</i>	93	(26/28)	96	(87/91)	95	(35/37)	91	(31/34)	1.0 (NS)
<i>Rickettsia conorii</i>	85	(22/26)	60	(45/75)	83	(30/36)	67	(22/33)	9.4‡
<i>Coxiella burnetii</i>	68	(19/28)	73	(66/91)	62	(23/37)	68	(23/34)	1.38 (NS)

\* The village of Kafr Ayoub is divided into four distinct geographical zones. Each zone is separated from the others by natural boundaries that include roads, canals, and agricultural and playing fields. The entire village covers an area of approximately 10 km<sup>2</sup>. Values are the percentage of households (no. of households with at least one antibody-positive occupant for the select agent no. of households with occupant(s) tested for an agent-specific antibody).

† NS = not significant ( $P > 0.05$ ); ND = not done

‡  $P < 0.05$

except for *R. conorii*, for which differences in household prevalence were noted between zones 1 and 2 ( $P < 0.05$ ) and 2 and 3 ( $P < 0.01$ ).

#### DISCUSSION

Data reported during the 1950s showed an average prevalence of 22% for WN virus antibody and 24% or higher for SFS and SFN virus antibodies among children in the Nile River Delta, while our data indicated the overall prevalence of antibodies to WN (20%), SFS (4%) and SFN (2%) viruses to be lower.<sup>4, 8, 24</sup> In contrast, rickettsial antibodies were demonstrated in a sizable proportion of the study population, particularly for *R. typhi*, for which 512 (58%) of 883 study subjects were antibody-positive. Similarly, antibody prevalence among school children surveyed from the Nile River Delta in 1989 was 9% for SFS, 4% for RVF, 3% for WN, and 9% for HTN viruses, and 22% for *C. burnetii*, 53% for *R. typhi*, and 37% for *R. conorii*.<sup>25</sup>

Most notable was the low prevalence of RVF antibody in the population born after the peak of the 1977-1978 outbreak (< 2% of the popu-

lation less than 13 years of age). Meanwhile, the proportion of the population greater than 12 years old exhibiting antibodies (42%) was comparable to the overall prevalence found in 1978 (36%).<sup>21</sup> These data suggest that the level of intensity associated with RVF transmission decreased considerably following the documented 1977-1978 outbreak and that RVF has not re-emerged as an important agent of human infection in Egypt since the outbreak 13 years ago.

Significant differences were found in age-specific phleboviral prevalence when the analysis was performed with and without controlling for the presence or absence of the other phleboviral like-agents (Table 1). These discrepancies may be attributed to cross-reactivity or result from the natural occurrence of dual or multiple infections involving other phleboviruses. The absolute differences in proportions clearly increased with age for RVF, SFS and SFN viruses and showed the risk of acquiring more than one phleboviral infection is likely to be a function of age; as people grow older, there are more opportunities for exposure to additional viruses. Sera from patients with known exposure to a single



phleboviral agent was not available for serologic control purposes.

A possible boosting phenomenon may explain the upward trend in GMT when antibody for one phlebovirus was found in association with antibody to another. Analytical controls for possible cross-reactivity were accomplished through the selection of only the highest titers when dual or multiple infections were detected. The question raised subsequent to the outbreak as to whether sequential infection with phlebotomus group viruses broaden the antibody response and enhance protection can be partially addressed by these findings.<sup>26</sup> The evidence presented indicates that the antibody response is strengthened by the effect of boosting. Enhanced antibody response has also been reported for SFN virus in Cypriots with Toscana virus compared to when antibodies to SFN virus were found alone.<sup>27</sup>

Familial clustering of persons with antibodies to *R. typhi*, *R. conorii*, and *C. burnetii* was evident in this study. Transmission may therefore be related to environmental determinants in the household. Animal slaughter carried out as a family-oriented activity within or near the place of residence, which involves cattle, sheep, goats, and camels, may account for the clustering phenomenon associated with *C. burnetii*, while exposure to infected fleas responsible for *R. typhi* infection is likely to be linked to significant rodent infestation, which is observed both in the home and fields. The high prevalence of *R. conorii*, however, and the tendency of antibody-positive individuals to cluster by residence, requires further investigation, with emphasis on probable tick vectors. The only indication of geographic clustering of households with antibody-positive occupants was observed for *R. conorii*. Risk factors associated with zone-specific transmission are not reported in these study findings. However, significant differences in antibody prevalence of *R. typhi*, *R. conorii*, and *C. burnetii* were reported among four villages located in the Bilbeis area in the Sharqiya governate (all within 30 kilometers driving distance from each other).<sup>25</sup> Additionally, a comparatively high prevalence (29%) of antibody to SFS virus was found in only one of the villages surveyed.<sup>25</sup>

The high prevalence of rickettsial IgG antibodies among study participants warrants additional investigation. Longitudinal study of rickettsial disease incidence is currently underway. Furthermore, environmental risk deter-

minants, particularly those associated with the household, should be considered given the familial clustering evident with *R. typhi*, *R. conorii*, and *C. burnetii*.

Finally, these data show that when analytical biases are introduced to adjust for possible cross-reactivity and dual and multiple infections among phleboviruses, prevalence values may change significantly. Therefore, findings should be weighed as to the statistical controls applied in determining prevalence.

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