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Serological Tests for Detecting Rift Valley Fever Viral Antibodies in Sheep from the Nile Delta

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To determine the accuracy of serological methods in detecting Rift Valley fever (RVF) viral antibodies, we examined serum samples obtained from 418 sheep in the Nile Delta by using five tests. The plaque reduction neutralization test (PRNT) was considered the standard serological method against which the four other tests were compared. Twenty-four serum samples had RVF viral antibodies detected by PRNT. Hemagglutination inhibition and enzyme-linked immunosorbent assay antibodies to RVF virus were also present in the same 24 serum samples. Indirect immunofluorescence was less sensitive in comparison with PRNT, and complement fixation was the least sensitive. These results extend observations made with laboratory animals to a large field-collected group of Egyptian sheep.

Rift Valley fever (RVF), a widespread viral zoonosis in sub-Saharan Africa, became epidemic in Egypt in 1977 and 1978 (7, 9). Since 1980, RVF virus transmission has not been documented in Egypt (3). To monitor the presence of RVF virus, we have carried out a serosurveillance of sentinel sheep (1).

During the RVF epidemic in Egypt as well as during subsequent serosurveys, the serological diagnosis of RVF virus infection was based on the plaque reduction neutralization test (PRNT) (4) and the hemagglutination inhibition test (HAI) (6, 9). The complement fixation test (CF) (8) and indirect immunofluorescence assay (IFA) (11) have also been used for detecting RVF viral antibodies. Recently, the enzyme-linked immunosorbent assay (ELISA) has been adapted for this purpose (10). Despite the apparent usefulness of these tests in laboratory-derived samples (12, 13), a field-based comparison of these methods has not been performed. This has prevented the selection of a single sensitive, specific, and technically feasible method for use in field situations.

Accordingly, by using five serological tests, we measured RVF viral antibodies in serum samples obtained from sheep in the Nile Delta. The serological observations were compared to determine the appropriate use for each method in screening field-collected serum samples for RVF viral antibodies.

MATERIALS AND METHODS

Serum samples. Blood was collected from sheep representing 32 flocks in the Nile Delta (1). Serum samples were separated and stored in aliquots at −70°C. All serum samples were coded, and RVF viral antibodies were measured by PRNT, HAI, IFA, ELISA, and CF.

Serological methods. PRNT was carried out by starting with a serum dilution of 1:40 in 16-mm wells containing monolayers of Vero (green monkey kidney) cells and by using an inoculum of approximately 80 PFU of strain ZH501 RVF virus (4). Serum samples neutralizing 80% of PFU at a titer of ≥80 were considered positive. HAI was performed by starting with a kaolin-extracted serum dilution of 1:40 and by using a standard, commercially obtained, beta-propiolactone-inactivated mouse liver antigen prepared from the Entebbe strain of RVF virus (Salk Institute, Swiftwater, Pa.) (14). Eight to sixteen units of antigen were used at the appropriate pH; domesticated male goose erythrocytes were used (2). Serum samples with HAI antibody titers of ≥20 were considered positive.

IFA was performed as described by Riggs (11) by starting with a serum dilution of 1:10 and by using Vero cells infected with strain ZH501 RVF virus. Serum samples with IFA antibody titers of ≥20 were considered positive. ELISA used serum at a dilution of 1:200 and a modification of the method described by Meegan et al. (J. M. Meegan, R. J. Yedloutschnig, B. A. Peleg, J. Shy, C. J. Peters, J. S. Walker, and R. E. Shope, submitted for publication) that uses the RVF antigen described above and a mixture of monoclonal antibodies directed against RVF virus (provided by J. Meegan). The monoclonal antibody mixture included antibodies reactive with epitopes on each of the two virion surface proteins, G_1 and G_2, and the internal nucleocapsid protein of RVF virus. Anti-sheep immunoglobulin G (heavy and light chains) antibody conjugated to horseradish peroxidase was used in conjunction with 2,2-azino-di-(3-ethylbenzthiazoline sulfonate) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) as the substrate. The optical density was recorded at 405 nm. Only those serum samples which had optical densities more than 2 standard deviations above the mean optical density of 20 known negative sheep sera were considered positive by ELISA. CF was carried out by the method of Casals (1a) by starting with a serum dilution of 1:4. Two full units of complement were used with four units of antigen. Serum samples with CF antibody titers of ≥8 were considered positive. All comparative serological tests were confirmed.

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TABLE 1. Antibody titers to RVF virus measured by five serological methods in 24 sheep serum samples found positive by PRNT

Serum sample	RVF viral antibodies measured by[a]:				
	PRNT	HAI	ELISA	IFA	CF
1	≥160	20	0.430	40	0
2	≥160	320	1.303	160	0
3	320	160	0.946	40	0
4	320	80	1.052	40	32
5	320	40	0.823	40	0
6	640	80	0.917	160	16
7	640	40	0.614	20	0
8	640	160	0.931	160	0
9	640	80	0.968	40	0
10	640	40	0.776	80	0
11	640	80	0.683	20	0
12	1,280	40	0.515	40	32
13	1,280	40	0.605	40	0
14	1,280	40	0.861	80	0
15	1,280	80	0.809	20	0
16	2,560	40	0.978	20	32
17	2,560	80	0.910	80	32
18	2,560	160	1.125	320	0
19	2,560	320	0.919	160	16
20	2,560	320	1.128	320	16
21	2,560	320	0.972	20	0
22	2,560	320	1.074	320	0
23	5,120	80	0.962	320	0
24	5,120	160	0.902	80	0

[a] See text for details. For ELISA, the mean ± standard deviation for 20 negative sheep serum samples was 0.061 ± 0.118.

Statistical terminology. Sensitivity, specificity, and positive predictive value (PPV) were used to assess the relative merit of the serological tests (5). Sensitivity is the number positive detected by both tests divided by the total number positive in the "standard" test (PRNT). Specificity is the number negative detected by both tests divided by the total number negative in the standard test. PPV is the number of positives by both tests divided by the total number positive in the trial test. PPV is a measurement of true-positives in the trial test.

RESULTS

The sample population in this study was composed of serum samples from 418 individual sheep. Twenty-four serum samples had PRNT antibody titers of ≥80 to RVF virus (Table 1). PRNT titers ranged from ≥160 to 5,120. The same 24 serum samples contained HAI and ELISA antibodies to RVF virus. HAI titers ranged from 20 to 320, while ELISA optical densities ranged from 0.43 to 1.128. IFA antibodies to RVF virus were present in 23 of the serum samples which were positive by PRNT (sensitivity, 96%), while CF antibodies to RVF virus were present in only 7 positive serum samples (sensitivity, 29%).

HAI and ELISA antibodies to RVF virus were present solely in the 24 serum samples positive by PRNT. Therefore, the specificity and PPV were 100% for these two tests (Table 2). IFA was less precise in distinguishing negative samples. IFA antibodies to RVF virus were present in 4 (1%) of the 394 serum samples negative by all other serological tests used. The titers for these four serum samples were 80. The specificity of IFA was 99%, while the PPV was 85%. CF antibodies to RVF virus were present solely in the seropositive standard serum samples, producing both a specificity and a PPV for this method of 100%.

DISCUSSION

These observations demonstrate that HAI and ELISA are the most precise of the four serological methods compared with PRNT for measuring RVF viral antibodies. IFA was less accurate, and CF was the least sensitive of the four methods.

Epidemiological evidence suggests that the sheep surveyed in this study were exposed to RVF virus more than 3 years prior to the sampling period (1). Therefore, the RVF viral antibodies measured in this study were long-lived, and the serological observations relate to remote infections.

As a standard test, PRNT is a sensitive and specific method for detecting RVF viral antibodies. In a series of neutralization tests wtih 34 phlebotomus fever viruses, RVF viral antisera obtained from hyperimmune mouse ascitic fluid neutralized only one virus (Frijoles virus, isolated in South America) at a low serum dilution (1:20) (13, 15). In Egyptian field populations, PRNT appears to retain its specificity. The disappearance of RVF antibodies in animals born after 1981 coupled with the high titer of PRNT antibodies to RVF virus among the seropositive sheep indicates specific antibodies to RVF virus rather than to cross-reacting phlebotomus fever viruses.

In comparison with PRNT, HAI was both sensitive and specific in detecting RVF viral antibodies in Egyptian sheep. Although RVF virus can cross-react in HAI with other phlebotomus fever viruses, such as Punta Toro and Saint-Floris (13), the high specificity of HAI in comparison with PRNT in this study probably reflects a relative absence of viruses in Egypt which cross-react in HAI with RVF virus.

ELISA was also sensitive and specific in detecting RVF viral antibodies in comparison with PRNT. Similarly, Niklasson et al. (10) found a close correlation between ELISA and PRNT in detecting RVF viral antibodies in serum samples from human RVF vaccinees.

In comparison with PRNT, IFA was only slightly less sensitive and specific in detecting RVF viral antibodies than either HAI or ELISA. However, the PPV for this test was low, reflecting false-positive observations which were not recorded by the other serological tests. Such false-positives would result in overdiagnosis if IFA were used independently in field surveys. In a series of serological reactions between phlebotomus fever viruses, Shope et al. (12) and

TABLE 2. Accuracy of four serological tests in detecting RVF viral antibodies as compared with PRNT

Trial test	PRNT		% Sensitivity	% Specificity	% PPV
	+	−			
HAI					
+	24[a]	0	100	100	100
−	0	394			
ELISA					
+	24	0	100	100	100
−	0	394			
IFA					
+	23	4	96	99	85
−	1	390			
CF					
+	7	0	29	100	100
−	17	394			

[a] Number of specimens.

Tesh et al. (13) found that IFA antibodies to RVF virus cross-reacted with many of the phlebotomus fever viruses which they tested. Many of these viruses have been isolated in Egypt. The present study suggests that viruses which affect sheep in the Nile Delta can produce IFA antibodies that cross-react with RVF virus.

CF is generally used for detecting RVF viral antibodies during acute infections (8). However, even during acute infections, CF lacks sensitivity in detecting RVF viral antibodies. As in this study, CF has been shown to be specific for detecting RVF viral antibodies (12).

These results indicate that PRNT, HAI, and ELISA are accurate serological methods for detecting RVF viral antibodies in Egyptian field-collected serum specimens. These methods should be cautiously extended to other geographical areas where other phlebotomus fever viruses, known to cross-react with RVF virus (15), may be active, and the results should be interpreted with caution if multiple infections with phlebotomus fever viruses may have occurred. IFA is less sensitive and contributes to false-positive observations. Although CF is quite specific, the low sensitivitiy of this method in detecting RVF viral antibodies limits its usefulness. In choosing a practical test for screening field-collected serum samples for RVF viral antibodies, reproducibility and precision as well as technical feasibility must be considered. Of the two classical tests, PRNT requires a containment facility. HAI requires a diversity of materials and reagents which must be carefully standardized and which may be difficult to obtain. Nonetheless, its ease of performance and its relative sensitivity and specificity make it a feasible test. IFA and ELISA are technically simpler than either PRNT or HAI. However, although reagents are relatively easy to obtain, IFA is very subjective, results may vary between observors, and in our experiments it resulted in false-positives. In addition, IFA requires a fluorescence microscope, which may be difficult to transport. Although ELISA reagents must be of high quality, making them difficult to obtain, ELISA results can be objectively quantitated when spectrophotometric equipment is used and when the reagents can be easily transported in field situations. Thus, if reagents are available, ELISA appears to be a precise and technically feasible method for detecting RVF viral antibodies in Egyptian field situations.

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