Comparison of the IFA and Other Tests for Trichinella spiralis Antibodies

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THE INDIRECT fluorescent antibody (IFA) test for detection of antibodies to *Trichinella spiralis* has been evaluated in at least four previous reports. Sadun and associates (*1*), in developing the test, used 142 serums from persons with trichinosis, 76 serums from persons with disease in other categories, and 27 serums from healthy human controls, as well as serums of 10 rabbits infected with *T. spiralis*. They compared results on five positive serums in the IFA test, bentonite flocculation (BF) test, the complement-fixation test with ethanol insoluble antigen (CF-EI), the complement-fixation test with ethanol soluble antigen (CF-ES), and the Walter Reed Army Institute of Research slide flocculation test (WRAIR-SF). They found the IFA test to have high sensitivity and specificity and described a prozone reaction with serums of high titer.

Labzoffsky and associates (*2*) reported an evaluation of an unpublished IFA test described by Baratawidjaja and associates. Serums from three patients with trichinosis and from two rabbits infected with *T. spiralis* were used. Comparing results in the IFA, precipitin, and complement-fixation (CF) tests, Labzoffsky and associates found that the IFA test showed positive results at least 2 weeks before the other two tests.

Scholtens and associates (*3*) compared results of the IFA, Suessenguth-Kline (SK), BF, latex agglutination (LA), and charcoal agglutination (CA) tests, using serums from 10 pigs that were given varying dosages of *T. spiralis*. Three pigs not dosed with *T. spiralis* were used as controls. Scholtens and co-workers found that the sensitivities of the IFA and CA tests were equal to each other and higher than those for the other tests used in their evaluation.

While developing a storage-stable antigen made from cuticles of *T. spiralis*, Sulzer (*4*) tested 68 human serums by the bentonite flocculation and the indirect fluorescent antibody tests, using both the whole larval antigen described by Sadum and associates (*1*) and the cuticular antigen. The IFA test reproduced BF test titers well. The IFA test was also found to have good internal reproducibility when the two types of IFA antigen were compared.

In our evaluation, we compared IFA and BF test results on 1,585 human serums. Of these serums, 1,302 had been submitted to the Communicable Disease Center, Public Health Service, for routine testing for *T. spiralis* antibodies, 215 were in an evaluation battery, and 68 were from residents of Uruguay. We analyzed the test results statistically for internal reproducibility of titers. We also tested serums of 48 rats (18 not infected with *T. spiralis* and 30

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The paper is the second in a projected series of research reports on indirect fluorescent antibody tests for parasitic diseases.

with heavy infections) by IFA and BF and compared results. The swine serums used were tested at lower dilutions than in the study by Scholtens and associates (*3*), whose findings we extended.

The IFA test equaled the BF test in internal reproducibility and exceeded it in sensitivity as shown by detection of antibodies in human, rat, and porcine serums. On the porcine serums the IFA test was more sensitive than the BF, SK, LA, and CA tests, and it detected antibodies earlier after infection than the other tests. No evidence of nonspecificity was found in the IFA test.

Materials and Methods

Human serums submitted to the Communicable Disease Center for the diagnosis of trichinosis were used in this study. Serums were also tested from 4 rats with no known exposure to nematode parasites, from 14 rats infected with *Nippostrongylus brasiliensis* whose serums were known to contain antibodies to that nematode, and from 30 rats infected with *T. spiralis* 1½ to 3 months before blood samples were collected. Swine serums for this evaluation were collected from 12 animals before and after experimental infection with *T. spiralis*.

Antigen was prepared from the cuticles of *T. spiralis* larvae (*4*), and anti-species conjugates were prepared from antiserums produced in rabbits and chickens.

We performed the indirect fluorescent antibody test essentially as described by Sadun and associates (*1*) except that after exposure to a test serum the antigen was washed at least four times to prevent false-negative reactions. The BF test was performed according to the modification described by Norman and associates (*5*), the latex test was performed according to Innella and Redner (*6*), the charcoal test according to Anderson and associates (*7*), and the Suessenguth-Kline test as described by Suessenguth and Kline (*8*).

All dilutions for titration began at 1 to 4 and progressed in fourfold steps. The only exception was with swine serums, which were titrated from full strength. A change from negative to positive at a 1 to 4 dilution was considered to be a fourfold change.

Table 1. Qualitative evaluation of the indirect fluorescent antibody test for detection of antibodies to Trichinella spiralis, using human antiserums against various organisms

Antiserums	Number tested	Positive	
		Number	Percent of each category
Trichinella spiralis	31	30	96. 8
Echinococcus species	26	6	23. 1
Filaria species	23	2	8. 7
Schistosoma species	28	2	7. 1
Virus	18	1	5. 0
Ascaris species	23	1	4. 3
Toxoplasma gondii	10	0	0
Venereal disease	23	0	0
Leishamania donovani	7	0	0
Trypansoma cruzi	1	0	0
Mycobacterium tuberculosis	1	0	0
Normal	24	2	8. 3
Total	215	44	

NOTE: All serums were tested at a dilution of 1 to 4.

Results

To test for cross-reactions with other diseases, a battery of 215 human serums was assembled from persons with various infections (table 1). Thirty-one serums in this group were from patients with suspected trichinosis whose serums had reacted positively in the bentonite flocculation test. The bentonite flocculation test was 100 percent specific when the complete battery was tested. By the indirect fluorescent antibody test, 30 of the 31 trichinosis serums in this battery and 14 of the 184 serums in the other categories were positive. The 14 serums from other disease categories that were positive by IFA but negative by BF were titrated, but none reacted beyond a 1 to 4 dilution.

Since 6 of 26 (23 percent) of the *Echinococcus* antiserums in the battery were positive by the IFA test (table 1), further studies were made. In the battery of 68 serums collected from persons in Uruguay, 36 serums were positive by the hemagglutination (HA) test for echinococcosis and 32 were negative. When tested by the IFA test with *Trichinella*

Table 2. Qualitative results of examination of 1,302 human serums for antibodies to Trichinella by the indirect fluorescent antibody test and the bentonite flocculation test

Bentonite flocculation	Indirect fluorescent antibody		Total
	Positive	Negative	
Positive	240	22	262
Negative	66	974	1, 040
Total	[1] 306	996	1, 302

[1] Chi-square analysis shows that the greater number positive by IFA is statistically significant.

antigen, one of the positive *Echinococcus* antiserums, as well as one of the negative *Echinococcus* serums, was positive. These results indicate that antibodies to *Echinococcus* probably do not cross-react in the IFA test for antibodies to *Trichinella.*

A comparison of qualitative results for 1,302 human serums for antibodies to *Trichinella* is shown in table 2. By the BF test, 262 (20 percent) were positive; by the IFA test, 306 (24 percent) were positive. Of the 996 serums negative by the IFA test, 22 were positive by the BF test. Of the 1,040 which were negative by the BF test, 66 were positive by the IFA test. Chi-square analysis of the data indicated that the IFA test detected a significantly greater number of positives than the BF test.

Titers obtained when 122 human serums were tested by both the bentonite flocculation and indirect fluorescent antibody procedures were compared. For this comparison, a change from negative to positive at 1 to 4, or the reverse, was considered as a fourfold difference. In the two tests, 105 serums (86 percent) titrated within one fourfold dilution. Of the 17 serums that differed in titer in the two tests by 16 (64-fold), 9 titrated higher in the BF test and 8 had a higher titer in the IFA test. The most extreme differences were represented by one serum which titrated at 1 to 1,024 in the IFA test and at 1 to 16 in the BF test, and by another which titrated at 1 to 64 in the IFA test and 1 to 4,096 in the BF test—a titer difference of 64-fold in each case.

In an experiment designed to compare reproducibility of the IFA and BF tests, two groups of eight serums each were assembled. Each group contained both positive and negative specimens. Each serum specimen was divided into two replicates and coded. On day 1, serums in the first group were titrated by both IFA and BF tests. On day 2, serums in the second group were titrated by both tests. The two groups of serums were then recoded, and all were retitrated by both tests, the first group on day 3 and the second on day 4. Analysis of the data showed no statistically significant difference in reproducibility of results in the two tests. For some specimens, the indirect fluorescent antibody test gave higher titers than the bentonite flocculation test; the reverse was true for other specimens.

We tested a group of 48 rat serums by both the indirect fluorescent antibody and bentonite flocculation tests. Four were from animals with no known exposure to nematodes, 14 were from rats exposed to *N. brasiliensis* and known to contain antibodies to that parasite, and 30 were from animals heavily infected with *T. spiralis.* The heavily infected group had been exposed to *T. spiralis* via stomach tube. Since we had infected the animals 1½ to 3 months before collecting blood samples from them, the rats were in the chronic phase of trichinosis at the time of collection. The serums from the 18 rats with no exposure to *T. spiralis* were negative by both the BF and IFA tests. Of the 30 serums from rats infected with *T. spiralis*, 2 were negative by both the BF and IFA tests, 18 (60 percent) were positive by both tests, and 10 (33 percent) were negative by BF but positive by IFA. Chi-square analysis of these results showed a significantly greater number of positives by the IFA test than by the BF test.

When serums of the 30 infected rats were titrated in the BF and IFA tests, titers agreed within a fourfold limit in 20 instances, including two negative serums. Titer differences 16-fold or greater occurred with 10 serums (33 percent), nine of which were higher by IFA. Three serums showed the greatest titer differences—negative by bentonite flocculation and 1 to 256 by indirect fluorescent antibody.

Serums from 12 swine, 10 of which had been infected with *T. spiralis*, were also tested by IFA. For comparison, these serums were also tested by the BF, LA, CA, and SK tests. After blood samples were collected from all 12 animals, 10 were fed *T. spiralis* larvae. Two were kept as negative controls. Of the 10 fed *T. spiralis*, 3 received 500 larvae, 3 received 1,000, and 4 received 100,000. Following administration of the larvae, blood specimens were obtained from the 10 animals on days 7, 14, 21, 28, 43, 65, and 84. One of the pigs that had received 100,000 larvae died on the 44th day after it was infected; therefore, specimens were not available from this animal on days 65 and 84.

The serums were tested by the five procedures described. Preinfection serums and serums from the control pigs were negative by all tests except the SK, which gave weakly positive reactions with a few of these specimens. Table 3 shows the results of five test procedures performed on blood samples taken from animals infected with *T. spiralis*.

Within 7 days after the 10 swine had been infected, antibodies were detected by the indirect fluorescent antibody test in serums from 2 of the pigs which received 100,000 larvae. None of the other tests detected antibodies until the blood specimens were collected on day 14. Not until day 28 did the percent positive by any of the other tests equal the percent positive by IFA. When the last sample was taken on day 84, only the IFA and CA tests still detected antibodies in all serum specimens from pigs infected with *T. spiralis*. Serums from pigs that received the heaviest dosages of larvae were the first to show antibodies. This early production of antibodies may have been stimulated by the large number of adults which developed from the infective dose of larvae, by the massive initial number of migrating larvae produced by these adults, or by a combination of these factors.

Table 3. Comparison of sensitivity of five tests—indirect fluorescent antibody, Suessenguth-Kline, bentonite flocculation, latex agglutination, and charcoal agglutination—in the detection of antibodies in antiserums of 10 swine infected with Trichinella spiralis

Number of days after infection	Number of serums positive by each test				
	IFA	SK	BF	LA	CA
7	2	0	0	0	0
14	4	2	2	1	1
21	6	4	5	4	4
28	10	10	8	9	10
43	10	10	7	8	10
65	[1] 9	[1] 9	6	2	[1] 9
84	9	7	7	0	9

[1] 1 pig, fed 100,000 larvae of *T. spiralis*, died on day 44; therefore only 9 animals remained.

Discussion

Our data support and extend the findings of the earlier studies cited. The only exception is that we could not confirm the report of prozones with serums of high antibody titer (*1, 9*). Negative reactions at low dilutions of positive serums are probably caused by residual serum left by incomplete washing of sensitized antigen. (A detailed investigation that we made of these reactions is the subject of a report now in preparation.)

Specificity of the IFA test for antibodies to *T. spiralis* is good. None of the unexposed rat and swine serums were positive, even when the swine serums were tested full strength. The statistically greater number of positive results on human serums in the IFA test as compared to the BF test may be attributed to the IFA test's greater sensitivity.

Internal reproducibility of the IFA test was investigated in only one of the studies cited (*4*), and in that study different antigens were used. In comparing results of one test with those of another, it is valuable to compare internal reproducibilty of the two tests. This comparison is more meaningful when the same serums are used in both tests. We have shown by statistical analysis that the indirect fluorescent antibody test is at least as reproducible as the bentonite flocculation test.

The greater sensitivity of the IFA as compared to the BF test was clearly demonstrated with rat serums. The rats from which the positive serums were drawn were known to have been given 2,000 larvae; *T. spiralis* larvae had

been seen by microscopic examination of the rats' muscles, and larvae were recovered from all animals by pepsin digestion. As noted, one-third of these serums were negative by the BF but positive by the IFA test. Two serums were negative by both tests. These negative results may indicate that these rats did not produce antibodies or that neither the IFA or BF test was sensitive enough to detect the antibodies that were present.

When Scholtens and associates (*3*) assembled the data for their report on swine serums, all serums were tested from full strength except in the IFA and BF tests. In the present study, we retested all swine serums at full strength, and the results have led us to modify those reported by Scholtens and associates (*3*).

The results with swine serums illustrate the sensitivity of the IFA test as compared to four other tests. No other test equaled the IFA test in early detection of antibodies. Only the CA test continued to detect antibodies in the serum of all the infected animals for a similar period of time. Since our study was terminated at 84 days, the experiment did not reveal how long after infection the IFA test will detect antibodies to *T. spiralis*.

Evaluation of the serologic response of prolonged infections or of infections with few larvae is especially important. These two conditions may be responsible for the greater number of positive results observed in the indirect fluorescent antibody method as compared to results observed in the bentonite flocculation tests when human serums are tested. If the IFA test is more sensitive than the BF test, as our results indicated, infections with few larvae or infections of long duration may be detected by IFA but not by BF. Detection of early infections is probably rare since human serums are not usually submitted for diagnosis until clinical symptoms appear, that is, until the disease is in its acute phase. From this standpoint, the report by Labzoffsky and associates (*2*) is especially valuable. They showed that the IFA test can detect antibodies in human serums very early after infection. Both Labzoffsky and associates (*2*) and Sadun and associates (*1*) reported that antibodies in serums of infected rabbits were detected by IFA at an early stage of infection.

High sensitivity may be a disadvantage in the diagnosis of currently acute human infections. Many old chronic infections may give positive reactions. For this reason, sensitivity of the IFA test might be reduced by considering titers only above 1 to 10 or 1 to 20 as diagnostically significant. Since, however, the bentonite flocculation test is as reproducible and as specific as the indirect fluorescent antibody test and has the further advantage of requiring less sophisticated instrumentation, we recommend it over the IFA test for the routine diagnosis of human trichinosis. When human infections of *T. spiralis* are suspected to be of recent origin, the IFA test would be preferred because it is more sensitive than any other serologic procedure.

To survey for antibodies to *T. spiralis*, the IFA test appears to be better than any other test used on animal populations in this study. Its sensitivity recommends its use when slaughter hogs are to be checked routinely by serologic methods for the presence of *T. spiralis*. Further studies of the IFA test with very light infections and infections of long duration (1 to 2 years) would go far in evaluating its limitations in this respect.

Not all the differences between the IFA and BF tests can be attributed to the greater sensitivity of the IFA test. As noted, there were a few serums in which differences in titer were extreme. If, in these cases, the IFA test gave the higher titer, this result could be attributed to greater sensitivity of the IFA procedure. There were, however, a few instances in which the BF titer was much greater than the IFA titer on the same serum. One explanation for this observation is that different antibodies may be selected in each test. Preliminary studies in our laboratory, however, in which absorption procedures were used, have not supported this hypothesis. Fractionation of serum by Sephadex G–200 indicates that the IFA test detects the 19S gamma globulin, whereas the BF test detects both 19S and 7S antibodies.

Summary

The indirect fluorescent antibody and bentonite flocculation tests for trichinosis were used to test 1,302 human serums. Four percent more positives were detected by the IFA test. Statis-

tical study showed the IFA test to have internal reproducibility equal to that of the BF test. Of 30 serums from experimentally infected rats which upon autopsy had been found positive for *Trichinella spiralis*, 28 were positive by IFA, but only 18 were positive by BF tests. On tests of serums from experimentally infected pigs, the IFA test gave positive results 1 week earlier than four other serologic tests. The IFA test detected antibodies just as late after the experimental infection of these pigs as the charcoal agglutination test and for a longer period than any of the other four tests used.

For detection of early *T. spiralis* infections in humans and animals and for testing animal serums for antibodies to *T. spiralis*, the indirect fluorescent antibody test is the method of choice.

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Students to Work with the Mentally Retarded

This summer about 700 high school and college students will have an opportunity to gain valuable experience in working with the mentally retarded through a new federally funded program, Student Work Experience and Training (SWEAT).

The purpose of the program is to interest high-caliber students in choosing careers in the field of mental retardation by exposing them to summer work experience while their career plans are still developing.

Fifty agencies providing a wide variety of services to retarded persons throughout the nation are taking part in the program. Funds to hire the students are being made available by the Public Health Service in grants to institutions that do the hiring.

SWEAT will include experience in all kinds of work with retardates, from hospital care to daycamping. Students will be assigned according to the nature of the project and their individual capabilities.

Students must have completed their junior year of high school to be considered for the program. Some projects will offer work experience in professional and technical health fields of interest to graduate students.