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The Use of *Leptospira biflexa* Patoc Antigen in Field Investigations of Leptospirosis *

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Hitherto the laboriousness of serological procedures for the laboratory diagnosis of leptospirosis has somewhat limited their usefulness. The authors of this paper report on a simple and sensitive genus-specific serological test for this disease that is within the capabilities of ordinary diagnostic laboratories. They describe the organization and results of a trial carried out in Romania in 1962 of a complement-fixation (CF) test in leptospirosis in which an antigen derived from the Patoc I strain of Leptospira biflexa *is used. Human sera examined with this test in nine field laboratories were re-examined at the Cantacuzino Institute in Bucharest with both CF and agglutination tests.*

Of 152 sera found CF-positive in the field laboratories, 138 were found positive by the agglutination test in Bucharest—representing 90 % agreement. There was 88 % agreement between the field laboratory and central laboratory results in the CF test.

The test makes possible the early detection of human leptospirosis and gives positive reactions with sera from leptospirosis patients irrespective of the causative serotype.

The development of a rapid method for the early laboratory diagnosis of human leptospirosis has for some time been one of the main concerns of the Leptospirosis Department of the Dr I. Cantacuzino Institute in Bucharest.

The fact that *Leptospira biflexa* Patoc is co-agglutinated in over 50% of cases of leptospirosis due to a variety of other pathogenic serotypes (Combiescu et al., 1957, 1958, 1959) was confirmed by Dr Nina Sturdza and one of the present authors (M.E.) when an antigen for use in the complement-fixation (CF) test was prepared with this strain. The search carried out with this antigen has shown that it has a more marked polyvalence in the CF test than in the agglutination test (Sturdza et al., 1960; Sturdza & Elian, 1961a). We also found that during the first week of the disease, the CF test was usually positive earlier than the agglutination test, although taken over-all the two tests were in agreement in approximately 90% of cases.

In the course of the three-year period 1959-61, over 2000 human sera suspected of leptospiral infection were subjected in our laboratory to CF and agglutination tests, and the results obtained led us in 1961 to send *L. biflexa* Patoc antigen to three regional laboratories for experimental trials. By 1962 the high degree of concordance in these trials between the CF and agglutination results justified our recommending performance of the CF test with *L. biflexa* Patoc antigen in all the regional laboratories in Romania. These laboratories used the new antigen for tests in all suspected cases of human leptospirosis; all sera found positive in the CF test were sent to our laboratory in Bucharest for verification by the agglutination test and for serotyping.

With a view to demonstrating the practical value of this antigen, we report in the present paper on the results obtained in field trials carried out in nine bacteriological laboratories (under the control of Regional Antiepidemic Stations) in which the CF test with Patoc antigen has been used as a routine procedure. From 1 January to 1 October 1962 these laboratories examined 900 serum samples from persons suspected of suffering from leptopsirosis and from persons in epidemic foci.

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MATERIALS AND METHODS

Antigen

The *L. biflexa* Patoc antigen was delivered to the field laboratories by the Cantacuzino Institute early in 1962 as a routine product. It was prepared from the Patoc I strain, received from Professor B. Babudieri in Italy in 1957, which develops rapidly and abundantly. Cultures 10-12 days old in 15 ml of Korthof's medium (18/18 tubes) incubated at 28°C were centrifuged for 1 ½ hours at 15 000 revolutions per minute. The sediment was resuspended in physiological saline containing 1/10 000 thiomersal (1/20 of the initial volume) and stored at 4°C for 3-5 days. Thereafter each lot of antigen was titrated as described below according to its specific power of fixation and its non-specific activity. Titration was carried out against five known negative human sera and against six positive sera (anti-*biflexa* Patoc rabbit serum and five human sera from *L. pomona*, *L. icterohaemorrhagiae*, *L. hebdomadis*, and *L. bataviae* infections).

The CF titre of the antigen is taken as the average of the specific titre against the anti-Patoc serum and of those obtained on titration of the five positive human sera, and is calculated to the last dilution giving a +++ positive result. The test dose of antigen must contain four CF units in a concentration of at least four times less than the anti-complementary or non-specific titre.

The antigen is stable for at least six months from the time of preparation. The test dilution is indicated on each sample.

Method

The CF test was carried out according to the technique of Bengston (1941), which was chosen because it is currently used for the diagnosis of rickettsial infections and is suitable for field work. In some cases the field laboratories tested paired or serial serum samples and demonstrated rises in the titres.

Some 200 human sera were received by the Leptospirosis Department of the Cantacuzino Institute from the regional laboratories. Of these, 170 (representing 153 leptospirosis cases) had been found positive in the CF test by the regional laboratories and the remaining 30 had been found negative in both the CF and the agglutination tests. We repeated the CF test on 71 of the 170 sera found positive in the regional laboratories.

We also carried out the rapid slide-agglutination test with live antigen (Sturdza & Elian, 1961b, p. 168) on all the 200 sera received. For this purpose a 1 : 50 dilution of the serum under test was made in physiological saline. A number of drops of this dilution were placed on two or three slides, the number being equal to the number of leptospirosis strains to be tested. With a fine-tipped Pasteur pipette, one drop of each strain was added to a corresponding drop of serum (all drops being of equal volume). Each double drop was then mixed with a glass rod and results were read against a dark ground after 20 minutes. The slides were kept in a humid atmosphere under Petri cover-glasses. If the result of the first examination was positive, further tests were carried out with successive twofold serum dilutions until the final titre was reached which still agglutinated the strain.

The following 12 *Leptospira* serotypes were used; they had been obtained in 1957 from the Leptospirosis Reference Laboratory in Amsterdam (Professor J. W. Wolff):

L. icterohaemorrhagiae Wijnberg AB	*L. hyos* Mitis Johnson
L. canicola Utrecht	*L. australis* A Ballico
L. grippotyphosa Bernkopf	*L. autumnalis* Akiyami AB
L. sejroe M84	*L. bataviae* Swart
L. hebdomadis Hebdomadis	*L. ballum* Mus 127
L. pomona Pomona	*L. javanica* Poi

RESULTS

As shown in the table, 152 persons were found positive by the CF test in the field laboratories, with titres ranging from 1/4 to 1/4096. Control tests carried out in our laboratory in Bucharest confirmed 138 of these as positive in the agglutination test. Of the negative CF sera received in our laboratory (with no indication of the day of illness on which the sera were taken) one was found to have anti-*hyos* agglutinins at a titre of 1 : 12 800.

The CF test was thus in concordance with the agglutination test in 90% of these samples. Closer scrutiny of the data showed that five of the 14 sera negative in the agglutination test but positive in the CF test were from patients in the third or fourth day of the disease and the remaining nine were from patients whose date of onset was not stated.

As to the 71 sera positive in the CF test in field laboratories on which that test was repeated by our laboratory, we obtained the same titre as the field laboratories with 56 specimens. With eight samples our results were negative (although the agglutination test was positive for three of these), and with the

COMPARISON OF DISTRIBUTION OF COMPLEMENT-FIXATION (CF) AND AGGLUTINATION TITRES

Agglutination	No. of sera	CF titre												Geometric mean of CF titre
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1 024	1/2 048	1/4 096	
Neg.	14		6	4	4									1/8.5
1/100	14		1	3	2	4	2	1	1					1/50
1/200	17				3	3	4	2	2	2	1			1/186
1/400	27					3	4	8	6	5		1		1/278
1/800	22					2	1	10	5	4				1/215
1/1 600	17					2		3	3	8	1			1/372
1/3 200	21					3		4	2	10		1	1	1/589
1/6 400	17						1	6	3	5	1		1	1/545
1/12 800	4	1						1		1	1			
Total	153	1	7	7	9	17	12	35	22	35	4	2	2	

remaining seven we obtained positive reactions at two or three dilutions lower than the field laboratories. These discrepancies may be accounted for by possible technical errors.

The use of the CF test by the field laboratories led to the detection of 65 sporadic cases of leptospirosis and of 79 cases in five small epidemic foci in five regions. In consequence of the preliminary CF results further epidemiological and serological investigations were pursued which made it possible to determine the full number of human cases in these foci (another nine cases were found) and to establish the existence of an animal reservoir of infection.

We may add that in one of the field laboratories the inoculation of guinea-pigs with urine from a patient with a positive CF test result led to the isolation of a leptospirosis strain.

The agglutination tests carried out in our laboratory in Bucharest showed that the predominant serotype was *pomona*, which caused a high number of cases in the first ten months of 1962. The 138 cases confirmed by the agglutination test were due to the following serotypes: *pomona*, 65 cases; *sejroe*, 34 cases; *icterohaemorrhagiae*, 13 cases; *hyos*, 6 cases; *canicola*, 3 cases; undetermined, 17 cases.

DISCUSSION

The reaction almost universally used up to the present for the laboratory diagnosis of leptospirosis has been the agglutination test, which is not only relatively rapid but also provides the best indication of the causative serotype. However, the necessity of using at least 12 serotypes and the fact that live strains are often used render this method awkward for field laboratories. Moreover, the maintenance of the strains, the sensitivity of the reaction, the control tests and the nicety of interpretation required all demand the constant services of a specialist.

Our experience has shown that *in some cases* the agglutination titre of a single serum sample may vary from one titration to another. Borg-Petersen (1949) suggested that the age and density of the strain at the moment of testing would partially explain this variation. Babudieri & Castelli (1956) showed that different strains of the same antigenic serotype react very differently against the same serum and recommended the use of several strains of the same serotype or of the strains that are considered the most sensitive. Our observations have indicated that even the same strain may sometimes show a very different sensitivity from one titration to another. In 20 samples that had been CF positive in the field

laboratories' tests but had been negative on first being examined by us with the agglutination test, repetition of the agglutination test with other strains of the same serotype gave positive results, sometimes with very high titres.

In the light of our experience we may assert that a high CF titre cannot fail to correspond to an acute case of leptospirosis. In many instances, indeed, the CF result has proved a very useful indicator, obliging us to repeat our agglutination test or to introduce a new serotype into that test.

The detection of a wider range of human cases of leptospirosis has been made possible by the introduction into current laboratory practice of the *L. biflexa* Patoc antigen, which is genus-specific and seems to give positive reactions with sera from leptospirosis patients irrespective of the causative serotype. In the work described above, the CF reactions with this antigen agreed in some 90% of cases with the agglutination tests results; moreover, our tests with the Patoc antigen have indicated not only the nfections mentioned above with *pomona*, *sejroe*, *hyos* and *canicola* serotypes, but also the presence of other serotypes (*bataviae*, *grippotyphosa*, *hebdomadis* and *saxkoebing*).

As a result of our investigations with this antigen field laboratories now have available a serological reaction that is easily carried out, that makes possible the early detection of human leptospirosis—a fact of particular therapeutic and epidemiological importance—and that will reveal a wide range of leptospirosis serotypes. Specialized central laboratories can thus now be relieved of a part of their heavy work-load.

The CF test with this antigen complements the agglutination test as a reaction for the detection of recent infections. Its ability to distinguish former patients or those with long-standing infections from those with recent ones is of great epidemiological and clinical value and should, in addition, make it possible to obtain reasonably accurate information on annual incidence.

CONCLUSION

It must be acknowledged that the laboratory diagnosis of leptospirosis is still in a far from satisfactory state of perfection. However, a diagnostic procedure is now to hand that has proved itself capable of detecting about 90% of leptospirosis cases, and we feel entitled to recommend the complement-fixation test with *L. biflexa* Patoc antigen for the rapid and early detection of human leptospirosis in field investigations.

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RÉSUMÉ

La complexité et l'extrême spécificité des tests d'agglutination appliqués en laboratoire pour la recherche de la leptospirose humaine ont limité jusqu'à maintenant leur usage.

Les auteurs ont mis au point une méthode simple, sensible, et spécifique de genre, qui est à la portée des laboratoires courants. Il s'agit d'un test de fixation du complément, qui a été appliqué dans les conditions de la pratique en Roumanie, en 1962, après de minutieuses études en laboratoire. Dans ce test, l'antigène est dérivé de la souche Patoc I de *Leptospira biflexa*. Les résultats des examens sur le terrain ont été vérifiés à l'Institut Cantacuzène de Bucarest et comparés à ceux que donne e test d'agglutination.

Sur 152 cas positifs à la réaction de fixation du complément, 138 étaient positifs au test d'agglutination, ce qui représente une concordance de 90%. La comparaison des résultats du test de fixation du complément, sur le terrain et au laboratoire central, a montré une concordance de 88%. Ce test, spécifique de genre, met en évidence l'infection par de nombreuses sérotypes, tels que *pomona*, *sejroe*, *hyos*, *canicola*, *bataviae*, *grippotyphosa*, *hebdomadis*, *saxkoebing*. Il s'ensuit que ce test a une grande valeur épidémiologique et permet de déceler la présence de cas récents, une réponse d'un titre élevé correspondant certainement à un cas aigu de leptospirose. Les auteurs pensent donc pouvoir recommander ce test pour la mise en évidence rapide et précoce sur le terrain, des foyers et des cas isolés de leptospirose.

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