Water Strains of *Leptospira* in the Serodiagnosis of Human and Animal Leptospirosis

L. ADDAMIANO ¹ & B. BABUDIERI ²

The most widely used serological reaction for the diagnosis of leptospirosis is the agglutination test. This test, however, cannot be carried out in many laboratories because special equipment and special experience are required. It is also necessary to maintain live *Leptospira* cultures belonging to all the serotypes present in the country where the test is made. Consequently, it would be extremely useful to be able to diagnose leptospirosis by means of a single antigen, regardless of the serotype to which the *Leptospira* responsible for the infection belonged. This is particularly important for countries in which the antigenic pattern of the local *leptospirae* is not well known and in which it would thus be necessary to use a large number of *Leptospira* serotypes for each test.

Observations made in the last 11 years have suggested that the problem may be solved with the use of some non-pathogenic, water strains of *Leptospira* which seem to be agglutinated by human sera containing antibodies against pathogenic *leptospirae*. This paper reports the results of studies from 1960 to 1968 on the possibility of using water strains for serodiagnosis.

The results over the 8-year period show that the non-pathogenic strain Patoc 1 is agglutinated by a high percentage of human sera positive for pathogenic *leptospirae*: these results indicate that Patoc 1 would be useful for serodiagnosis. However, a high percentage of animal sera positive for pathogenic *leptospirae* gave negative results with the strains Patoc 1 and Sao Paulo, and thus these strains cannot be used for serodiagnosis in animals.

**REVIEW OF LITERATURE**

It is known from the literature that certain strains of water *leptospirae* are sometimes agglutinated by the sera of subjects who are or have been previously infected with *Leptospira*. This was first noted by Uhlenhuth & Zülzer (1922), who assumed that saprophytic, non-pathogenic *leptospirae* could sometimes be transformed into pathogenic *leptospirae*. Similar observations were subsequently made by Baermann & Zülzer (1928), Pettit (1928), Stefanopoulo & Hosoya (1929), Coppola & De Lorenzo (1940), and more recently by Kmety (1957-58), who noted that the water strain Patoc 1 often became agglutinated at very high titres by sera from patients affected by leptospirosis, sometimes more quickly than the pathogenic strain which caused the sickness. Combiescu et al. (1958a, 1958b) in a study in Romania during the years 1950-57, also found agglutinins for the strain Patoc 1 in leptospirosis patient’s sera.

Apart from these occasional observations, there have also been a few cases in which water strains of *Leptospira* were wrongly considered to be pathogenic strains and when used for serodiagnosis gave positive results in the agglutination test. The best-known case concerns the so-called strain “pomona Prague”. Approximately 20 years ago, an institute in Prague distributed to a number of laboratories situated in Eastern Europe a *Leptospira* strain classified as belonging to the serotype *pomona*. This strain was frequently used for diagnostic purposes and was repeatedly agglutinated by patients’ sera. Voiculescu et al. (1957) have, furthermore, described an epidemic episode in Romania which was sustained by such a serotype and Topciu et al. (1954, 1957) repeatedly found antibodies for “pomona Prague” in sera of drovers and, especially, in sera of pig-breeders.

In 1956 one of us (B. B.) demonstrated that this strain “pomona Prague” did not belong to the

---

¹ Laboratory of Microbiology, Istituto Superiore di Sanità, Rome, Italy.  
² Chief Research Worker, Laboratory of Microbiology, Istituto Superiore di Sanità, Rome, Italy; and Acting Director, Istituto di Microbiologia, Università di Trieste, Italy.
serotype *pomona* and in the following year Combiescu et al. (1957) found it to be antigenically close to the type *semaranga*, then still considered to be pathogenic, and to the water strain Madida, isolated in the USSR by Terskikh in 1931.

Clarification of the systematics of "pomona Prague" followed the studies of Füzi & Csoka (1960). They demonstrated that the strain Veldrat Semarang 173 (belonging to the serotype *Semaranga*) which was isolated in Java by Sardjito et al. (1937) and which repeatedly gave positive results in agglutination tests with leptospirosis sera (Hoekstra & Sardjito, 1938; Das Gupta, 1939; Gispen, 1939; Collier, 1948), must be considered a non-pathogenic water strain of *Leptospira*. The studies of Füzi & Csoka have recently been confirmed by one of us (Babudieri, 1961) and by Cacchione et al. (1962): the strain "pomona Prague" is identical to the strain Patoc 1, isolated in 1942 by one of us from a brook near Trieste (Babudieri & Archetti, 1948). It is probably the same strain; perhaps the culture was mislabelled and thus it was attributed to serotype *pomona*.

Another water strain of *Leptospira*, which was erroneously considered pathogenic and which was repeatedly agglutinated by sera from leptospirosis patients and convalescent patients in India as well as in Finland, was the strain CH 11, belonging to serotype *andamana*, isolated by Taylor & Goyle (1931) during a leptospirosis epidemic in the Andaman Islands. This strain, which was also held responsible for a few cases of infection in Finland (Koulumies & Salminen, 1953) and for other cases described by Corrêa et al. (1964) in Brazil, is also at times agglutinated by leptospirosis patients' sera.

The Lublin strain was isolated by Crominski from a patient on the third day of illness during a *grippotyphosa* leptospirosis epidemic in Lublin. Without any particular study of its antigenic constitution, the strain was considered to belong to the serotype *grippotyphosa* and as such was used in Poland for serodiagnosis from 1951 to 1958. It was found that this strain was agglutinated by 56% of the sera from leptospirosis patients. Babudieri & Dymowska (1961) studied this strain and were able to demonstrate that it did not belong to the *grippotyphosa* but to the *semaranga* serotype. The strain Leeds, isolated by Czkalowski & Horne (1951) from a patient's urine, also shows the characteristics of the saprophytic water strains. Two other strains, Sh, isolated by Terskikh in the USSR, and Sao Paulo isolated by one of us in Brazil (Babudieri, 1961), are also frequently agglutinated by leptospirosis patients' sera: the Sh and Sao Paulo strains are antigenically very similar to the strain Patoc 1.

Agglutinating antibodies for water leptospiroa have also been described in the sera of animals. Gardner (1928) found agglutinins for the strain Vinzent in guinea-pigs, inoculated with the serotype *icterohaemorrhagiae*; Erber (1932) found antibodies for the strains Tokio, Erlangen, and Vinzent in horses, dogs, rams, mice, rats and guinea-pigs. Acanfora (1939) found antibodies for water leptospiroa in guinea-pigs inoculated with a strain of *icterohaemorrhagiae*; Gluhovschi et al. (1956) described elevated titres for the strain "pomona Prague" in horses affected by periodic ophthalma; the same authors (1957) examined 97 specimens of *Citellus citellus* and found a high percentage of agglutinating antibodies for "pomona Prague" and furthermore were able to isolate a similar strain from one of these animals. Then Kujumgiev (1957) found agglutinating antibodies for the serotype *andamana* in chickens. Combiescu et al. (1958a, 1958b) have often found agglutinins for the strain Patoc 1 in the sera of animals with leptospirosis, and in some cases this was the only strain of *Leptospira* that did become agglutinated.

From the results presented so far it is clear that some non-pathogenic water strains of *Leptospira* are agglutinated by sera from leptospirosis patients. Among these the most important and best-known serogroup is Semaranga which includes 3 serotypes: *semaranga*, patoc, Sao Paulo. The first serotype includes the strains Veldrat Semarang 173, Madida, Sh; the second includes Patoc 1 and Lublin; and the third includes only the strain Sao Paulo.

The first attempt to utilize water leptospiroae in serodiagnosis of leptospirosis was made by Cox, Stover & Treik (1958). Using the technique perfected by Chang & McComb (1954), Cox et al. extracted from the saprophytic strain CDC a soluble antigen able to sensitize sheep red cells, which became lysed if treated with complement and human serum containing antibodies for pathogenic leptospira. Cox et al. obtained good results with human sera, but not with cattle sera. This reaction was not much used in practice, however, because the antigen is difficult to prepare, and it soon perishes: furthermore the reaction is too complicated for routine diagnostic purposes.

The first direct attempt to utilize water strains of *Leptospira* for diagnosis was by a group of Romanian research workers. These authors (Combiescu et al., 1958a, 1958b), starting from the observation made
WATER STRAINS OF LEPTOSPIRA IN SERODIAGNOSIS OF LEPTOSPIROSIS

in their laboratory that the strain Patoc 1 is agglutinated by the sera of people affected with leptospirosis in over 50% of cases, used this strain to prepare a concentrated antigen, treated with thiomersal, for the complement-fixation test. In a series of studies (Combiescu et al., 1960; Sturda, Elian & Tulpan, 1960; Sturda & Elian, 1961) they were able to show that the complement-fixation test carried out with this antigen agreed, in approximately 90% of cases, with the agglutination test carried out with the pathogenic strains that were the cause of leptospirosis in their country. Also they were able to show that at the beginning of the disease the complement-fixation test was usually positive sooner than the agglutination test and that it became negative after 2–6 months, thus allowing a diagnosis to be made only in recent cases of human leptospirosis.

In studies with other water strains of Leptospira (Madida, Veldrat Semarang 173, Peschiera, Ancona), the Romanian authors observed that the specificity and sensitivity of the strain Patoc 1 were greater than those of the other strains.

With regard to the serodiagnosis of animal leptospirosis, using the complement-fixation test with antigen Patoc 1, the Romanian authors obtained practically negative results with sera from swine, cattle and horses. With immune-rabbit sera, on the contrary, the complement-fixation test gave positive results in 85.7% of cases when the antigen Patoc 1 was used. With other antigens, such as Peschiera, Ancona, Madida and Veldrat Semarang 173, fewer positive results were obtained.

The negative results obtained in cases of swine, cattle and horse leptospirosis have been confirmed more recently by other authors of the same Romanian school (Nicolesco & Lelutin, 1967) by employing both the antigens Patoc 1 and Sao Paulo in the complement-fixation test (Sao Paulo was less sensitive than Patoc 1). In dogs the results were different in different years and it has been suggested by the above authors that good agreement between the serodiagnosis obtained using pathogenic leptospirae and that obtained using saprophytic strains indicated that the infections were probably recent and that the lack of good agreement in other years indicated that the infections were probably older.

Agreement of 98% between agglutination tests using the pathogenic strains and tests using the saprophytic strain Patoc 1 has also been found by Mailloux (1967) in human, murine and dog sera. This author also observed that immune-rabbit sera prepared with pathogenic leptospirae can agglutinate the saprophytic strains Patoc 1, Sao Paulo, and Saada, while the sera anti-Patoc 1 and anti-Sao Paulo agglutinated 12 different serotypes of pathogenic leptospirae which he examined.

Recently Torten et al. (1966), using the indirect immunofluorescence test, were able to obtain positive results regularly with strain Patoc 1 in 30 human sera positive for pathogenic leptospirae (mainly of the serotype grippotyphosa). On the contrary, of 77 negative sera, only 4 were weakly positive (1 : 50) for the same saprophytic strain.

**MATERIALS**

**Sera**

Our studies were carried out on human and animal sera sent to us for diagnosis from various regions of Italy. Some of them were not accompanied by a clinical history and some reached us in poor condition; these points are important in the evaluation of the results. Some of the sera that we considered negative, however, came from zones where leptospirosis was endemic. In addition to these negative sera, we also examined other human sera that were obtained specially for our tests from places in Italy where leptospirosis does not exist or is very rare, and these sera are of particular value in the evaluation of the results of our investigations.

**Antigen for complement-fixation tests**

The antigen for complement-fixation tests was prepared with the strain Patoc 1, which had been repeatedly observed to be the most suitable for its sensitivity and specificity. Cultures in Korthof medium, 10–12 days old, were centrifuged for 1 hour at 12,000 rev/min. The sediment was resuspended in salt solution containing 1 : 10,000 thiomersal sodium at a concentration equal to one-twentieth of the original culture, and stored at 4°C. The antigen was titrated for its specific power of fixation and its non-specific activity against positive and negative human sera. The specific titre was 1 : 256 and it remained stable for 2 years.

**Antigen for agglutination test**

The agglutination test was carried out with live strains of leptospirae incubated for 10 days in Korthof medium. The following strains were employed: *icterohaemorrhagiae* Bianchi 1; *copenhageni* Wijnberg, canicola Alarik, pomona Mezzano I, *bataviae* Pavia 1, *grippotyphosa* Moskva V, *australis* Ballico, *zanoni* Zanoni, *saxkoebing* Mus 24,
*pathogenic leptospirae* carried at a certain stage of our studies we also used the strain Sao Paulo, in order to see whether this strain was more reliable than Patoc 1.

**METHODS AND EVALUATION OF THE RESULTS**

The complement-fixation tests were carried out using the technique usually employed in our laboratory. To the serial dilutions of the serum in Wassermann test-tubes, we added 4 units of antigen and 2 units of complement.

In the evaluation of the results we usually considered as positive the reactions in which there was no trace of haemolysis in the first test-tube (dilution of the serum 1:4).

In the agglutination tests the serum was diluted 1:10 with salt solution; then 0.05-ml aliquots of the diluted serum were put into test-tubes and 0.45 ml of the live *Leptospira* culture was added to each tube. The mixture was then incubated for 2 hours at 37°C. For the reactions which appeared positive after the first screening under dark-field illumination, we established the final titre the next day.

The evaluation of the significance of an agglutinating titre is not easy and is partly a matter of experience. In our preliminary studies we observed that the titre for the strain Patoc 1 was usually lower than that for pathogenic leptospirae. For these reasons, and also because the sera from subjects known to be negative do not agglutinate the strain Patoc 1 even at a low titre, we normally consider as positive those reactions for water leptospirae with a titre of at least 1:100. With the pathogenic leptospirae we prefer to consider a titre of 1:500 or more as positive because titres of 1:100 are at times found in subjects with no past history of leptospirosis infection. These low titres (1:100) we regard as doubtful cases.

**RESULTS**

**Negative human sera from zones of Italy free or almost free of leptospirosis**

We examined 988 human sera negative for pathogenic leptospirae coming from mountain zones or from other zones where leptospirosis is practically unknown. None of these sera would agglutinate the strain Patoc 1. Among a further 712 sera negative for pathogenic leptospirae, coming from other regions of Italy where leptospirosis is rare, in only 3 cases have we had a low agglutination titre (1:100) for the strain Patoc 1.

**Negative human sera from zones of Italy where leptospirosis is endemic**

Altogether, we examined 6857 negative human sera coming from zones of Italy where leptospirosis is endemic. Included in this total are the sera which agglutinated pathogenic leptospirae at a titre of 1:100. A total of 377 sera included in this group agglutinated the strain Patoc 1 at a titre which varied between 1:100 and 1:500 (5.5%). This percentage positiveness for the non-pathogenic water strain, however, is very different if it is divided into two separate groups (see Table 1): the sera which did not agglutinate pathogenic leptospirae at all and those which did agglutinate them at a low titre (1:100).

Table 1 thus shows that it is rare for sera completely negative for pathogenic leptospirae to agglutinate the strain Patoc 1. The few sera which do so, or at least some of them, may come from very recent cases of infection. In fact we were able to show that in the initial stages of leptospirosis, the agglutination of the strain Patoc 1 became positive before the appearance of agglutinins for the pathogenic strains responsible for the infection.

The sera whose agglutination with pathogenic strains we have considered doubtful, cover a rather

<table>
<thead>
<tr>
<th>Titre for pathogenic leptospirae</th>
<th>Total no. tested</th>
<th>Positive agglutination tests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 50 000</td>
<td>141</td>
<td>89.3</td>
</tr>
<tr>
<td>1: 10 000</td>
<td>374</td>
<td>86.1</td>
</tr>
<tr>
<td>1: 5 000</td>
<td>539</td>
<td>81.5</td>
</tr>
<tr>
<td>1: 1 000</td>
<td>612</td>
<td>74.0</td>
</tr>
<tr>
<td>1: 500</td>
<td>471</td>
<td>53.2</td>
</tr>
<tr>
<td>1: 100</td>
<td>782</td>
<td>26.4</td>
</tr>
<tr>
<td>&lt;1: 100</td>
<td>7775</td>
<td>2.8</td>
</tr>
</tbody>
</table>

TABLE 1

**RELATION BETWEEN THE RESULTS OF AGGLUTINATION TESTS WITH THE STRAIN PATOC 1 AND THE TITRE FOR PATHOGENIC LEPTOSPIRAE**
high percentage of samples which contained agglutinins for the water strain. Therefore, we think that these titres of 1:100 for pathogenic leptospirae represent a certain category of case, i.e., those who were infected a long time ago. We also consider that the sera which are slightly positive for pathogenic leptospirae and negative for the strain Patoc 1 might perhaps be from subjects who had overcome a leptospirosis infection a long time ago. It is understandable that in these cases the titre for the saprophytic strain, which is usually lower than that for parasitic strains, should be the first to become negative. We carried out the complement-fixation test on 267 sera belonging to this group and obtained a positive result in only 2 cases (0.7%).

**Human sera positive for pathogenic leptospirae**

We examined 2137 human sera positive for pathogenic leptospirae; the serotypes which are most frequently agglutinated in Italy being *icterohaemorrhagiae, copenhageni, pomona, bataviae, canicola*. The complement-fixation test was carried out on 205 sera belonging to this group and positive results were obtained in 166 cases (81.0%).

We show in Table 1 the ratios between the titres of the sera for pathogenic leptospirae and the percentages of positivity for strain Patoc 1. As mentioned above the specificity of this agglutination test was excellent (97.2%) when the titre for pathogenic leptospirae was less than 1:100 (sera considered negative). On the other hand, Table 1 shows that the sensitivity of the test was not so high with sera positive for pathogenic leptospirae. In fact the sensitivity varied from 53.2% to 89.3% (with an average of 74.4%) and was directly related to the titres for pathogenic leptospirae.

This variation is due to the fact that the time for a positive reaction to become manifest with the saprophytic strains does not correspond with, and is usually shorter than, that with the pathogenic leptospirae. In consequence, low titres for pathogenic leptospirae are often due either to a very recent infection or to an infection contracted a long time ago. In these circumstances the positivity for the strain Patoc 1 might not yet have appeared or, alternatively, might have dropped to an undetectable level.

**Human sera from persons vaccinated against leptospirosis**

We examined the sera of 47 rice-field workers immunized by a vaccine prepared from *icterohaemorrhagiae* and *bataviae* serotypes. These sera had titres between 1:500 and 1:5000 for these 2 serotypes but only 8 of them agglutinated the strain Patoc 1 at a low titre (1:100) and were positive in complement-fixation test: the other sera were completely negative for the saprophytic strain.

**Non-human sera**

Sera from immunized rabbits, and from cattle, horses, swine, sheep, dogs and guinea-pigs were also examined by the agglutination test, with the non-pathogenic water strain Patoc 1, and the results are shown in Table 2.

As the results of these tests showed that the animal sera did react only very slightly with the antigen Patoc 1, we decided to study other saprophytic strains, belonging to the same group as Patoc 1. In some preliminary studies we tried the agglutination test with horse, cattle and swine sera using the strains Sao Paulo, B Sh, Madida, Veldrat Semarang 173 and Lublin. Poor results were obtained with all these strains except Sao Paulo, and further studies were limited to this strain.

Of the 32 horse sera tested, which were positive with pathogenic strains, 22 agglutinated Sao Paulo (68.8%), and of the 264 positive swine sera tested, 68 gave a positive reaction with this strain (25.8%). The strain Sao Paulo was, therefore, shown to be more sensitive than the strain Patoc 1 in revealing animal leptospirosis, but we were still very far from being able to utilize it for serodiagnosis of animal leptospirosis.

We next wanted to see whether Sao Paulo was also more sensitive than Patoc 1 to positive human sera. We therefore carried out comparative studies between the 2 strains—including the Sao Paulo strain with the pathogenic strains and the strain Patoc 1 in our serodiagnosis tests. The sera that we examined with the strain Sao Paulo were some of the group of sera mentioned above, which were originally tested with Patoc 1. The results showed that among the 1187 human sera positive for pathogenic leptospirae which were tested, the strain Patoc 1 was agglutinated in 838 cases (70.6%), and the strain Sao Paulo in 676 cases (56.9%). There was agreement between the results with the 2 strains in 875 cases: both strains gave positive results with 601 sera and both gave negative results with a further 274 sera. However, in the case of 237 of the sera tested only the strain Patoc 1 gave a positive result and in the case of a further 75 sera only the strain Sao Paulo gave a positive result.


| Species        | Agglutination test with pathogenic leptospi 
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agglutination test</td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune-rabbit</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Immunized against different serotypes of pathogenic leptospires.
b Of this total 4 were positive at the titre of 1:100 and 1 at the titre of 1:1000.
c Included in this total were several sera with titres of 1:100 for pathogenic leptospires.

In addition we found that in tests with 4499 human sera, which were negative for pathogenic leptospirae, the strain Patoc 1 was agglutinated in 243 cases and the strain Sao Paulo in 132 cases. These results show that the strain Sao Paulo was less reactive with human sera positive for pathogenic leptospirae than the Patoc 1 strain.

**DISCUSSION**

Our results showed that with human sera positive for pathogenic leptospirae, 74.4%-81.0% of the agglutination and complement-fixation tests carried out with the strain Patoc 1 were positive, irrespective of the serotype responsible for the infection: in our case these were mainly *icterohaemorrhagiae, copenhageni, pomona, bataviae* and *canicola*. Unfortunately we could not study sera from patients infected by other serotypes, such as *grippotyphosa*, which is responsible for many cases of disease in other countries, but is very rare in Italy. Perhaps this is the reason why our results were not in complete agreement with those obtained in some other countries.

There were a few cases in which a serum, positive for pathogenic leptospirae, gave a negative result with water leptospirae. However, because of this result we should not conclude that the saprophytic strains cannot be used for serodiagnosis of leptospirosis as many of our tests were performed with sera from patients whose history was not always well known. It is probable that antibodies for the water strain may not take the same time to become manifest as the pathogenic strains and this, we think, may explain, in many cases, the lack of agreement between the two reactions. This has already been noted by Kmetty (1957-58) and also by Combiescu et al. (1960), but we were not able to study the time
of appearance and disappearance of the antibodies for the two types of leptospirae, as we seldom had at our disposal 2-3 specimens of serum drawn from the same patient at different stages of the disease. On the few examples we did have we were able to show that the agglutination of the strain Patoc 1 usually became positive before the agglutination of the pathogenic strain.

We also found that a certain number of human sera which were negative for pathogenic leptospirae did, however, agglutinate the water strain. In this connexion it should be remembered that most of our sera came from rice-fields, where leptospiroses were very widespread. Consequently this negativeness, especially when referred to sera with an agglutinin titre of 1:100, cannot exclude the possibility of an earlier leptospirosis infection in some of the subjects. In fact, although the titre for pathogenic leptospirae is usually higher than the titre for the strain Patoc 1 immediately after the infection, sometimes we find the opposite. Thus it is possible that, later on, the level of the former may become too small to be measured, while the latter may still be at a significant level. This hypothesis is supported by the fact that with negative human sera we found 5.5% of the agglutination tests with the strain Patoc 1 positive, whereas complement-fixation tests with the same strain gave positive results in only 0.7% of cases.

It is well known that the agglutination test can detect antibodies over a longer period than the complement-fixation test and that the sera of subjects who have recovered from an earlier leptospirosis infection are positive in the agglutination test more frequently than in the complement-fixation test. It is also important that none of the 988 negative human sera that came from mountainous zones, where leptospirosis is practically unknown, agglutinated the strain Patoc 1. Of the 712 negative sera that came from other regions where leptospirosis is rare, only 3 cases gave a slight agglutination with the strain Patoc 1.

These results indicate that the few cases in which human negative sera agglutinate the strain Patoc 1 are related either to a previous leptospirosis infection or to a very recent infection in which the positivity for Patoc 1 preceded that for the pathogenic strains.1

The fact that sera containing antibodies for pathogenic leptospirae, after vaccination, seldom agglutinated Patoc 1 is very interesting, and should be investigated further using a larger number of sera.

On the basis of our results we believe that the degree of agreement between the tests performed with the strain Patoc 1 and the agglutination tests with pathogenic leptospirae (92.7% of the 9912 sera tested) is high enough to regard this new test as reliable (sera positive for pathogenic leptospirae at 1:100 and sera from vaccinated people have been excluded in calculating this figure): this percentage should, perhaps, be even higher, if earlier qualifying remarks are taken into consideration. The simplicity of the technique, which uses only a single, non-pathogenic and easily cultured strain, makes it particularly useful when serodiagnosis must be carried out in small laboratories with little equipment.

Using this test it is not possible to identify the serotype responsible for the infection, but to the practising physician that is only of relatively minor interest; he wants to know rapidly whether or not the patient is affected by leptospirosis. In any case the agglutination of the strain Patoc 1 can be considered as a first screening, and in positive cases, it can be followed by an agglutination test with pathogenic strains. This reaction can also be particularly useful for serodiagnosis in countries where the serotypes of leptospira are not known. In this connexion we tested 180 human sera sent from Laos where no research studies on leptospirosis have ever been performed. Of these sera, 32 agglutinated the strain Patoc 1 and 52 agglutinated the strain Sao Paulo. Of those that were positive for the water strains, 29 sera agglutinated some pathogenic strains at a titre of 1:500 or more and another 9 sera gave a slightly positive reaction at 1:100.

We cannot give a definite judgment about the absolute validity of the new test, however, as our studies have almost always been made with sera positive for the serotypes icterohaemorrhagiae, copenhageni, bataviae, pomona or canicola: our results should be confirmed on an adequate number of sera containing antibodies for other serological types. It is also necessary to investigate more thoroughly the time of appearance of antibodies for Patoc 1 in comparison with those for the responsible pathogenic

1 This is clearly shown in the case of a patient affected by a severe Leptospira infection. The first serological test was performed on this patient 5 days after the onset of the illness and at this time the agglutination test was negative for all the pathogenic leptospirae tested, but was positive, at 1:100, for Patoc 1. When the tests were repeated 5 days later, the serotypes icterohaemorrhagiae, copenhageni, canicola, pomona and poi (paradoxical reaction) were all agglutinated, but the titre was only 1:100 (not significant according to our rules): Patoc 1 was agglutinated, however, at 1:1000 and Sao Paulo at 1:500. The patient died the next day with a picture typical of Weill's disease.
serotype. According to Kmety (1957-58) and to our own observations, the antibodies for Patoc 1 should be present before those for the pathogenic serotypes, but this requires confirmation in larger numbers of patients. We cannot say definitely at this moment whether it is preferable to use the strain Patoc 1 or Sao Paulo. The former seems to be preferable for human sera and the latter for animal sera.

As to the possibility of using the agglutination reaction instead of the complement-fixation test, our results indicate that the two tests give somewhat different indications because the persistence of the two types of antibodies in the sera of convalescent patients is different. A positive agglutination indicates a past infection, perhaps a long time ago; the complement-fixation, however, is positive only if the infection is relatively recent.

Our results indicate that the antigens Patoc 1 and Sao Paulo are not useful for serodiagnosis in animals as a high percentage of animal sera gave negative results with the water strains, both in agglutination and in complement-fixation tests. This different behaviour of human and animal sera is also known in other pathological conditions. In serodiagnosis of leptospirosis it has already been noted by Cox, Stover & Treik (1958); their antigen, extracted with alcohol from the water strain CDC, reacted well with human sera, but gave very little reaction with cattle and horse sera. We have also noted with animal sera that some of those considered negative for pathogenic leptospirae show a positive reaction with the strain Patoc 1, but here again it must be remembered that the agglutination of pathogenic leptospirae was often at a very low titre (1:100) and therefore must be considered as relating to an old Leptospira infection.

Our results with immune-rabbit sera do not agree with those of Combiescu et al. and with those of Mailloux (1967). In our research studies such sera were almost always negative and, in our opinion, the results obtained by Combiescu et al. and by Mailloux with immune-rabbit sera should be accepted with some reserve. In studies of the systematics of the leptospirae some of the saprophytic strains employed by these authors have been studied by other workers by the cross-agglutination test, and in some cases also by the complement-fixation test, and have seldom given positive reactions. However, Kmety found that 6 immune-rabbit sera agglutinated the strain Patoc 1, in one case at the titre of 1:6400.

We think that these differences in the results with immune-rabbit sera should not be attributed to differences in the preparation of the sera, as suggested by the Romanian authors (the use of killed leptospirae in Romania, and live leptospirae in our studies), but rather to differences between the animals. In considering whether an immune-rabbit serum is positive for a water strain of Leptospira, we must bear in mind its titre: this soon becomes extremely small in relation to its titre for the homologous pathogenic strain.

With animal sera, the strain Sao Paulo gave us better results than the strain Patoc 1 but it was still not good enough to be of practical use in diagnosis. In the future, however, another water strain may be isolated which gives even better results.

How are we to interpret the reactions of non-pathogenic strains with human sera containing antibodies for pathogenic leptospirae? The Romanian authors consider this positivity for saprophytic strains as a "paradoxical reaction", that is a paraspécific reaction, which concerns serotypes not involved in the infection. This type of reaction regresses and disappears when specific antibodies appear in the serum. We think that it must be considered similar to a paradoxical agglutination but not identical. In fact the water strains can be agglutinated also by sera of patients who were infected a long time ago or by sera from convalescents. We did not observe the swift reduction in titre which is characteristic of the so-called paradoxical reaction, and which gradually occurs during the course of the disease.

The first hypothesis that occurred to us was that all pathogenic leptospirae, and at least some of the water strains, have a common antigen. This could be located deeply in pathogenic leptospirae and sometimes superficially in water strains. If this were so, then since the agglutination test and the complement-fixation test are both surface reactions, which use whole spirochaetes as antigens, the hypothetical common antigen could not react with pathogenic leptospirae, but could do so with the water strains. To test the feasibility of this hypothesis we performed adsorption tests using human positive sera with Patoc 1 and with the pathogenic strain responsible for the infection and obtained the following results. When adsorbed with the pathogenic strain, the serum completely lost the antibodies for the pathogenic as well as for the water strain; when adsorbed with the latter, the antibodies for the pathogenic strain decreased, sometimes very markedly, without, however, disappearing entirely. These results do not support the hypothesis suggested
above which consequently must be incorrect. Evidently the reaction is much more complex and there are many strong links between the specific antigens and those of the Semaranga group. It is possible that the antigen responsible for the reaction may be the same as the one that is active in the Chang & McComb haemagglutination test (1954) and in Cox, Stover & Treik haemolysis tests (1958). In these 2 tests the antigen also reacts with human sera and not, or only slightly, with animal sera as we have reported above for the water strains. To understand this problem it will be necessary to study in more detail not only these paradoxical phenomena, but also the paraspecific easy agglutinability which at times is present in some pathogenic serotypes, such as *poi* and *bratislava*.

**RéSUMÉ**

Dans l'espoir de définir une épreuve simple pour le diagnostic sérologique de la leptospirose, on a procédé à une étude comparative de tests d'agglutination utilisant soit des souches de leptospires pathogènes soit des souches de leptospires aquicoles non pathogènes (souches Patoc 1 et Sao Paulo).

Sur 1700 sérum humains négatifs pour les souches pathogènes, en provenance de régions d'Italie où la leptospirose est quasiment inexistante, 3 seulement ont présenté une réaction positive à faible titre avec la souche Patoc 1. Sur 6857 sérum humains négatifs pour les souches pathogènes, expédiés de régions d'Italie où la leptospirose est endémique, 377 (5,5%) ont fourni une réaction positive avec la souche Patoc 1, les titres variant de 1: 100 à 1: 500. Les tests d'agglutination effectués avec la souche Patoc 1 sur 2137 sérum humains positifs à des titres plus ou moins élevés pour les leptospires pathogènes ont été positifs dans une proportion variable: dans 89,3% des cas, si les titres de la première épreuve étaient élevés (1: 5000); dans 53,2% des cas s'ils étaient faibles (1: 500). En moyenne, pour les titres s'éteignant de 1: 5000 à 1: 500, la sensibilité du test d'agglutination avec l'antigène Patoc 1 a atteint 74,4%.

On a également procédé à des tests d'agglutination au moyen de la souche Patoc 1 sur des sérum d'animaux. Ici, l'essai comparatif a été très peu concluant, un grand nombre de sérum positifs pour les souches pathogènes ne réagissant pas avec la souche Patoc 1. Des résultats plus favorables, mais encore très insuffisants, ont été obtenus en employant comme antigène une autre souche de leptospires saprophytes, la souche Sao Paulo.

Pour les auteurs, la concordance (92,7%) entre les résultats de l'épreuve d'agglutination avec la souche Patoc 1 et l'épreuve utilisant des souches pathogènes est suffisamment grande pour que le premier de ces tests puisse être considéré comme une méthode sûre de diagnostic de la leptospirose humaine. Il offre surtout l'avantage d'être simple; étant donné qu'il n'utilise qu'une seule souche, non pathogène et aisément cultivable, on peut l'effectuer dans les laboratoires qui ne disposent que d'un équipement réduit. Il ne permet évidemment pas d'identifier le sérotype en cause, mais peut rendre de grands services pour le dépistage. Par contre le test d'hémagglutination pratiqué avec les antigènes Patoc 1 et Sao Paulo ne semble d'aucune utilité pour le diagnostic sérologique de la leptospirose chez l'animal.

**REFERENCES**

Acanfora, G. (1939) *Ann. Igiene*, 39, 1
Combiescu, D., Sturdza, N., Sefer, M. & Radu, I. (1958a) *Arch. roum. Path. exp.*, 17, 245
Coppola, A. M. & De Lorenzo, F. (1940) *Rif. med.*, 56, 72
Erber, B. (1932) C. R. Soc. Biol. (Paris), 109, 165
Gardner, G. (1928) Bull. Acad. Méd., 100, 961 (cited by Combiescu et al., 1958a)
Kujumgiev, I. (1957) Vet. ital., 8, 490
Pettit, A. (1928) Presse méd., No. 2, p. 1306