GUIDELINES FOR THE CONTROL OF

LEPTOSPIROSIS

Edited by

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WORLD HEALTH ORGANIZATION
GENEVA
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FOREWORD

These guidelines have been prepared because of concern for the human patient with leptospirosis. Often it is the illness in such a patient that first draws the attention of his medical attendants to this disease, and then eventually to the animal sources of that particular case and the need for diagnosis, management and control of the infection in both man and animals. Thus, these guidelines begin with a consideration of the clinical problem, and they conclude with the requirements of control programmes, such as local prophylactic measures at the primary health level, essential secondary services for laboratory diagnosis at various levels, epidemiological analysis, local and national plans for prevention, and international cooperation.

It is not intended that these guidelines should be a textbook about leptospirosis, but rather that they should concentrate on advice on the practical aspects of recognition, diagnosis, management and control of this disease in man and animals. Since leptospirosis is a worldwide disease, its occurrence presents problems in various cultural, social and geographical environments, for which different solutions are needed, appropriate to these various circumstances. Considering this need, the guidelines have been prepared so as to be useful to clinicians, veterinarians, laboratory microbiologists and epidemiologists. The book should permit people of many different educational levels in each of these groups to find appropriate recommendations to help them with their task in the teamwork of disease control.

None of the groups of users can operate independently to control leptospirosis. The guidelines need to be at once self-sufficient for each group including users in remote rural areas, as well as comprehensive enough for the coordination of control activities. Hence there are deliberate repetitions so that sections are relatively complete in themselves; however, extensive cross-references indicate the sections where further information is given for those who need it. In the main, technical details have been placed in a separate section.

Similarly, there is extensive detail on matters that are applicable universally, as in the clinical and laboratory sections, but a more general approach has been adopted to emphasize the principles in the areas of epidemiology and administration, where the details of any programme must be adapted to meet local conditions.

The references were selected - from among those available to the contributors - for their general content, or suitability for further reading in greater depth, or for specific details of information and techniques to which reference is not easily available in standard texts.

The World Health Organization is greatly indebted to all who contributed to these guidelines, and particularly to Professor S. Faine who not only undertook to assemble and edit the contributions but also created a new orientation so that these guidelines could provide help and advice to all who are confronted with leptospirosis and are concerned in its treatment, control and prevention.
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Several colleagues, notably K. Bögel and L.H. Turner, made valuable critical comments on the draft.
PREFACE

These guidelines are designed for use by those who are not experts in leptospirosis, to help them with the following:

1. Diagnosis at the bedside or in the field (by local practitioners), even if full medical services are not available.
2. Clinical action, including treatment.
3. Laboratory and administrative support for accurate diagnosis and the compilation of surveillance data to be used as a rationale for control measures.
4. Selection and implementation of preventive measures according to local needs.

They provide current information about clinical, laboratory and epidemiological aspects that are not generally available in one source. Local adaptations may be required, because social, economic and environmental differences in various parts of the world make it impossible to give ideal advice applicable to every situation.
HOW TO USE THESE GUIDELINES

The contents of these guidelines are arranged in 4 main sections, A-D.

Section A contains a concise summary of leptospirosis. It covers the main features of leptospirosis in man and animals, the causal agent, the means of transmission and epidemiology. It does NOT deal with details of how to recognize leptospirosis clinically, make a diagnosis, investigate an outbreak, collect epidemiological data or set up control programmes. This information will be found in Sections B, C and D.

Section B contains the main body of information in detail about clinical presentation, clinical and laboratory diagnosis, epidemiological investigation and action for control of leptospirosis in man and animals. It does NOT contain detailed methods and instructions for carrying out clinical, field or laboratory tests, which are to be found in Section C.

Section C is solely technical, and presents in detail the clinical, field, laboratory and statistical methods and techniques that are referred to in Section B. These technical methods have been segregated so as not to encumber reading of the text of Section B.

Section D is concerned with the aims, organization and administration of control programmes, using the information set out in Sections B and C.
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<th>A</th>
<th>ALT</th>
<th>alanine aminotransferase (SGPT)</th>
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<tbody>
<tr>
<td>APD</td>
<td>average pore diameter</td>
<td></td>
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<tr>
<td>approx</td>
<td>approximately</td>
<td></td>
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<tr>
<td>AST</td>
<td>aspartate aminotransferase (SGOT)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>BP</td>
<td>British Pharmacopoeia</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
<td></td>
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<tr>
<td>C</td>
<td>°C</td>
<td>degree Celsius (centigrade)</td>
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<tr>
<td>CF</td>
<td>complement fixation</td>
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<td>CFT</td>
<td>complement fixation test</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
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<tr>
<td>CSE</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>cu mm</td>
<td>cubic millimetre</td>
<td></td>
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<tr>
<td>D</td>
<td>DF</td>
<td>dark-field (microscopy)</td>
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<td>E</td>
<td>e.g.</td>
<td>for example</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EM3H</td>
<td>Ellinghausen-McCullough-Johnson-Harris media (TA medium)</td>
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<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
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<tr>
<td>ESS</td>
<td>erythrocyte sensitizing substance</td>
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<td>et al</td>
<td>and co-authors</td>
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<tr>
<td>F</td>
<td>°F</td>
<td>degree Fahrenheit</td>
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<tr>
<td>FA</td>
<td>fluorescent antibody</td>
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<td>G</td>
<td>g</td>
<td>gram</td>
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<tr>
<td>g</td>
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<td>gel filtration</td>
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<td>gamma glutamyl transpeptidase</td>
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<td>glutamyl pyruvate transaminase (d-glutamyl transferase)</td>
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<td>H</td>
<td>HA</td>
<td>haemagglutination</td>
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<tr>
<td>HE</td>
<td>haematoxylin and eosin</td>
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<tr>
<td>HL</td>
<td>haemolytic test</td>
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<td>Hz</td>
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<td>ic</td>
<td>intracerebrally</td>
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<td>i.e.,</td>
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<td>IE</td>
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<td>IF</td>
<td>immunofluorescence, immunofluorescent</td>
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<tr>
<td>IFT</td>
<td>immunofluorescence test, technique</td>
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<td>lg</td>
<td>immunoglobulin</td>
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<td>IHA</td>
<td>immune haemagglutination</td>
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<td>im</td>
<td>intramuscular(ly)</td>
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<td>in</td>
<td>inch</td>
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<tr>
<td>ip</td>
<td>intraperitoneally</td>
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<tr>
<td>lu</td>
<td>international unit(s)</td>
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<tr>
<td>IUMS</td>
<td>International Union of Microbiological Societies</td>
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<tr>
<td>iv</td>
<td>intravenous(ly)</td>
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<tr>
<td>K</td>
<td>kg</td>
<td>kilogram</td>
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<td>L</td>
<td>l</td>
<td>litre</td>
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<tr>
<td>lb</td>
<td>pound (weight, imperial measure)</td>
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<tr>
<td>M</td>
<td>MAT</td>
<td>microscopic agglutination test</td>
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<td>mg</td>
<td>milligram</td>
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<td>min</td>
<td>minute(s)</td>
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<td>mol</td>
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<td>μg</td>
<td>microgram</td>
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<td>μl</td>
<td>microlitre</td>
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<tr>
<td>μm</td>
<td>micrometre</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>normal (concentration)</td>
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<tr>
<td>na</td>
<td>numerical aperture</td>
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<tr>
<td>nm</td>
<td>nanometre</td>
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<tr>
<td>O</td>
<td>OD</td>
<td>optical density</td>
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<td>OE</td>
<td>outer envelope</td>
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<td>oz</td>
<td>ounce (weight or volume, imperial measure)</td>
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<tr>
<td>P</td>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
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<td>PC</td>
<td>protoplasmic cylinder</td>
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<tr>
<td>R</td>
<td>RBC</td>
<td>red blood cells (erythrocytes)</td>
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<td>rpm</td>
<td>revolutions per minute</td>
<td></td>
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<tr>
<td>S</td>
<td>sc</td>
<td>subcutaneous(ly)</td>
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<tr>
<td></td>
<td>sec</td>
<td>second</td>
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<tr>
<td></td>
<td>SGOT</td>
<td>serum glutamate oxaloacetate transaminase</td>
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<tr>
<td></td>
<td>SGPT</td>
<td>serum glutamate pyruvate transaminase</td>
</tr>
<tr>
<td></td>
<td>sp, spp</td>
<td>species (singular and plural)</td>
</tr>
<tr>
<td>T</td>
<td>TA</td>
<td>tween-albumin</td>
</tr>
<tr>
<td></td>
<td>TSA</td>
<td>thermostable antigen(s)</td>
</tr>
<tr>
<td></td>
<td>TSCL</td>
<td>Taxonomic Subcommittee on <em>Leptospira</em></td>
</tr>
<tr>
<td></td>
<td>TLA</td>
<td>thermolabile antigen(s)</td>
</tr>
<tr>
<td>V</td>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
</tr>
<tr>
<td></td>
<td>vol</td>
<td>volume(s)</td>
</tr>
<tr>
<td>W</td>
<td>WBC</td>
<td>white blood cells (leucocyte count)</td>
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<td></td>
<td>wt</td>
<td>weight</td>
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SECTION A: AN OVERVIEW

1. THE INFECTION

1.1 HOW TO RECOGNIZE LEPTOSPIROSIS CLINICALLY IN MAN

"Leptospirosis" is an etiological description of a disease with a number of possible clinical presentations and courses (1).

The common characteristic features of this infection are:

- febrile illness of sudden onset;
- headache, continued fever, and prostration;
- severe myalgia and conjunctival suffusion.

The classical signs of haemorrhage and jaundice may appear in very severe cases, which are rare and depend on the type of leptospire, as well as other circumstances.

Clinical recognition is based on the clinician's knowledge of the varied presentations of the disease and on:

- a high "index of suspicion", and
- knowledge of local epidemiological probabilities.

In addition to the above clinical features, which are nearly always present, there may be:

- a rash (eruption) on the palate, and a fine macular exanthem;
- haemorrhages;
- meningism;
- jaundice;
- renal failure;
- mental depression.

If the illness is not treated within the first 2-3 days, it may progress in severity. Meningitis, anuria, iritis, liver failure and toxic delirium may develop, depending on the infecting serovar and other factors (see section B 1.1, p. 43). The severe form of illness may be fatal, death being due to renal failure. Usually the patient recovers after a prolonged convalescence.

Clinical laboratory or bedside tests to aid the diagnosis are those for albuminuria, which is usually positive, and bilirubin. Liver function and renal function tests (urea, creatinine) show raised levels (see section B 1.6.2.2, p. 49). There is a relative lymphopenia, reduced platelet count and, in severe cases, anaemia and jaundiced serum.

1.2 HOW TO RECOGNIZE LEPTOSPIROSIS IN ANIMALS

In all animals, the initial symptoms of acute leptospirosis are essentially similar and not characteristic (2, 3). They are the symptoms of an acute febrile illness - such as malaise, depression, anorexia, and conjunctivitis, accompanied by fever. The main clinical features are shown in Table 1. If the disease progresses, symptoms and signs appear which are more characteristic of leptospirosis, such as bleeding and its consequences; jaundice; central nervous system (CNS) involvement, and liver and renal failure (Table 1). Abortion, stillbirth and mastitis in lactating animals occur late in the acute stage.

Chronic infection is usually localized in the kidneys and may occur in animals which have passed through the acute stage, with or without detectable clinical evidence of illness. Leptospires are excreted in the urine in the carrier state which may be of short or long duration, and either intermittent or continuous, depending on the animal and the infecting serovar. Chronic infection may be detected only by laboratory tests if there are no clinical signs. The symptoms, when present, are those of nephritis, with usually the passing of large amounts of urine of low specific gravity.
Table 1. MAIN CLINICAL FEATURES OF LEPTOSPIROSIS IN ANIMALS

<table>
<thead>
<tr>
<th></th>
<th>Cattle (Bovines)</th>
<th>Horses (Equines)</th>
<th>Sheep and Goats (Ovines and Caprines)</th>
<th>Pigs (Swine)</th>
<th>Dogs (Canines)</th>
<th>Rodents</th>
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</thead>
<tbody>
<tr>
<td>Acute infection (initial):</td>
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<tr>
<td>fever (temperature up)</td>
<td>1-2.5°C 2-5°F</td>
<td>+</td>
<td>0.5-2°C 1-4°F</td>
<td>+</td>
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<tr>
<td>malaise</td>
<td></td>
<td>+</td>
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<tr>
<td>weakness</td>
<td></td>
<td>+</td>
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<tr>
<td>depression</td>
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<td>+</td>
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<tr>
<td>listlessness</td>
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<td>+</td>
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<tr>
<td>anorexia</td>
<td>+</td>
<td>+</td>
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<tr>
<td>vomiting</td>
<td></td>
<td>+</td>
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<tr>
<td>diarrhoea</td>
<td>+</td>
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<tr>
<td>convulsions</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>conjunctivitis</td>
<td>+</td>
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</tr>
<tr>
<td>haemorrhages</td>
<td>+</td>
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<td></td>
<td></td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>anaemia</td>
<td>+</td>
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<td></td>
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<tr>
<td>jaundice</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>anuria</td>
<td></td>
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<td></td>
<td>±</td>
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<tr>
<td>haemoglobinuria</td>
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<td></td>
<td>±</td>
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<tr>
<td>mastitis, agalactia</td>
<td></td>
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<tr>
<td>Acute infection (late):</td>
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<tr>
<td>pneumonia abortion/stillbirth</td>
<td>+ + 1-3*</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Chronic infection:</td>
<td></td>
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<tr>
<td>nephritis</td>
<td></td>
<td>+</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>periodic ophthalmia</td>
<td></td>
<td>+</td>
<td></td>
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<tr>
<td>encephalitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>grey-white spots on kidneys post-mortem</td>
<td>+</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* weeks after onset of initial infection
+ regularly present
± occasionally present
2. THE ETIOLOGICAL AGENT

2.1 MORPHOLOGY

The etiological agent is *Leptospira interrogans* (2, 4). The genus *Leptospira* comprises thin (0.1 μm x 6-20 μm), tightly coiled spirochaetes which are characterized by very active motility, both rotating ("spinning") and bending. Usually one or both ends of the cell are bent or hooked, but straight forms also occur which rotate and travel more slowly than the hooked forms. Because of their narrow diameter, the leptospires are best visualized by dark-field illumination or phase-contrast microscopy and they do not stain readily with aniline dyes.

The genus *Leptospira* shares the following basic morphological features with other spirochaetes. Surrounding the leptosporial cell is a 3-5 layered membrane, which is referred to as the outer membrane or outer envelope (OE). The term protoplasmic cylinder (PC) describes the cellular components enclosed by the OE. The PC consists of a peptidoglycan layer and a cytoplasmic membrane which enclose the cytoplasmic contents of the cell. Two flagella are located between the OE and PC, one at each end of the cell. They are attached to the PC in a subterminal position and the free ends extend toward the middle of the cell but do not overlap. The flagellar basal bodies resemble those of Gram-negative bacteria, with the exception of *L. illini* (an unusual leptospire of uncertain taxonomic status) whose basal bodies are similar to those of Gram-positive bacteria. The cytoplasm contains nuclear material, ribosomes, mesosomes and inclusion bodies. *Leptospira* are not known to contain endotoxin. The free-living (*L. biflexa*) and parasitic leptospires (*L. interrogans*) are morphologically indistinguishable.

The unique motility of *Leptospira*, combined with their narrow diameter, flexibility and shape, allows them to pass through 0.1-0.45 μm pore-size membrane filters and to migrate within media solidified with up to 1% agar. Few other nonspirochaetal bacteria have these capabilities.

2.2 CULTURAL CHARACTERISTICS

Leptospires are obligate aerobes. When cultivated in a suitable aerated medium at 30°C their generation time varies between 7-12 hours and yields of 6-8 x 10^9 cells per ml may be obtained. The nutritional requirements of *Leptospira* are unique although simple. Vitamins B_1_ and B_1_2__ and long-chain fatty acids are the only organic compounds that are known to be required. Fatty acids are their major source of energy and carbon and are also required as a source of cellular lipids since *Leptospira* cannot synthesize fatty acids de novo. Owing to the inherent toxicity of free fatty acids, these must be supplied to the leptospires either bound to albumin or in a nontoxic esterified form. Carbohydrates are not a suitable source of energy or carbon. Even though amino acids are utilized to a limited extent, they cannot satisfy the nitrogen requirements of these organisms. Ammonium salts are an effective source of cellular nitrogen. The nonessential nutrient, pyruvate, enhances the initiation of growth of the parasitic leptospires, particularly serovars such as hardjo and ballum. Leptospires incorporate purine bases but not pyrimidine bases into their nucleic acids. Because of this, they are resistant to the antibacterial activity of the pyrimidine analogue, 5-fluorouracil. This compound is used in selective media for the isolation of leptospires from contaminated sources.

The types of media used for the isolation and cultivation of leptospires (see section C 3.2.4, p. 123) are media enriched with rabbit serum or bovine serum albumin (BSA), and protein-free media. Liquid media are converted to the semisolid form by the incorporation of 0.2% agar and to the solid form by the addition of 1% agar. Liquid media are necessary for growing the cultures (for serological diagnosis of the infection) and for typing the isolates. Semisolid media are generally used for isolating strains and for the maintenance of stock cultures. Growth is readily initiated in these media and usually is easily visualized as one or more rings of dense growth, several mm to cm below the surface of the medium. The lack of rings of growth does not necessarily mean the absence of leptospires. Solid media are useful for cloning the strains and for isolating leptospires from contaminated sources (see section B 3.2.3.1, p. 70). The colonies in 1% agar are subsurface and become visible within 7-14 days in most serovars. The shape of the colony of motile strains changes with time and, for a given strain, the colony size is directly related to the agar concentration. Subsurface colonial morphology has not proved to be a useful characteristic.
for differentiating the strains of *Leptospira* in routine laboratories. Media containing pyruvate are required to obtain colonial growth of some of the more fastidious serovars. Surface colonies have been described recently (2) on 2% agar but the significance of their occurrence and morphology is not yet clear.

The BSA and the rabbit serum component of the leptospire medium are the most expensive and variable ingredients. A protein-free chemically-defined medium, composed of detoxified Tween 80 (polyborates), vitamins B₁ and B₁₂, inorganic salts and an organic buffer, has been described recently (3) (see section C 3.2.1.3, p. 126). In experimental infections, growth may be detoxified by charcoal treatment. High yields of leptospires can be obtained with suitable batches.

### 2.3 Pathogenesis of Infection

#### 2.3.1 Source and Entry of Leptospires

The source of infection is water or soil contaminated with infected urine, the infected urine itself or tissues from infected animals. The leptospires enter through cuts or abrasions in the skin or mucosal surfaces, through the conjunctiva, or by inhalation (into the lungs) of droplets or aerosols of fluids containing leptospires. In naturally acquired infections, the leptospires do not cause a local inflammatory reaction at the site of infection.

#### 2.3.2 Spread and Growth

Leptospires spread immediately via the bloodstream. Avirulent organisms fail to multiply in the body and are removed from the bloodstream within a day or two of infection. Virulent organisms multiply until they are opsonized and phagocytosed at a stage when agglutinating antibody is just detectable by microscopic agglutination tests; they are then cleared rapidly by the reticuloendothelial system. The growth rate in vivo is equivalent to a generation time of about 6–8 h in experimental infections. In fully susceptible experimental animals, growth continues until high concentrations of leptospires may be found in all tissues, especially blood, liver, kidney, lung, brain and adrenals. At this time the clinical illness, nitrogen retention, and pathological changes in many tissues are evident. If an animal is pregnant, the fetus or fetuses may be infected during this leptospiraemic phase. The pathogenesis of infection and the pathological changes in the fetuses resemble essentially those in adult animals, but may be relatively more severe owing to lack of immunity in the fetus.

#### 2.3.3 Lesions

Lesions occur when there is a threshold concentration of leptospires in the body. The primary lesions are damage to the endothelium of small blood vessels, leading to extravasation of blood, emigration of leptospires into the tissues, and local relative anoxia, these then leading to secondary ischaemic damage to organs such as kidneys, liver and adrenals. Renal tubular necrosis follows and then uraemia. In severe cases, haemoglobinuria and haemoglobin casts may be found. Liver cell necrosis leads to disordered liver function and jaundice in severe cases. Haemorrhages may occur in almost any organ or tissue, but especially in the muscle and subcutaneous tissues where there is movement. Anaemia may occur for reasons which are not clear. A haemolysin is produced by some serovars, notably, *pomona*, but not by other serovars which are capable of producing severe illness. In very severe or terminal haemorrhagic illness the pathological changes resemble those of disseminated intravascular coagulation.

Although several authors have shown indirect evidence of the action of a postulated toxin, a toxin capable of accounting for the lesions of leptospirosis has not been characterized.

Animals which survive may become renal carriers, but prolonged renal excretion after convalescence has very rarely been reported in man.
The pathogenesis of the carrier state is obscure. Systemic infection is a prerequisite for the carrier condition, which develops some 7–28 days after the initial infection, at about 2-3 weeks after the onset of recovery from the clinical illness. Leptospires survive in the proximal renal tubules, adherent to and closely associated with the luminal border of the cells. Antibody to the homologous serovar of leptospires is detectable in the urine and kidney, but there is sometimes no evidence of inflammation in the renal parenchyma. The pathological changes in the kidneys of carriers differ according to the animal species. For example, the kidneys of dogs show much more scarring and infiltration with inflammatory and plasma cells than those of many other species.

Leptosporal antigen, with or without the presence of recognizable leptospires, may be detectable in the urine of carrier animals of all species.

2.3.4 IMMUNITY AND RECOVERY FROM INFECTION

Recovery from infection may follow the appearance of lytic and opsonic antibodies and phagocytic clearance of leptospires from the blood and tissues. In carriers, the leptospires may continue to survive in the kidney as well as in the anterior chamber of the eye and in the brain. Recovery depends on the relative size of the dose of infecting leptospires, their rate of multiplication, the number of leptospires in the tissues and the extent of damage to vital organs, especially the kidneys and liver; and on the immunological competence of the host animal, especially the rate at which an IgM, B-cell-mediated antibody response occurs. Immunologically immature fetuses and young animals are much more susceptible than adults. Immunologically suppressed adults, without B-cell function, are also susceptible while comparable immunologically competent animals are susceptible.

2.4 SEROLOGY AND IDENTIFICATION

The morphological and cultural properties of different leptospires are relatively uniform so that they have been used much less for purposes of identification and classification than have the serological characteristics of leptospires. The system of agglutination by antiserum (from laboratory rabbits) has revealed so far about 180 major serological varieties, each one described, named, and designated as a serovar, which is currently the basic taxon. The serovars are distinguished on the basis of agglutinogen characteristics as disclosed in cross-agglutination and agglutinin absorption reactions (see sections B 3.3, p. 70 and B 3.4, p. 76), the end-points of which are read microscopically (see section B 3.4.1.1, p. 76). For practical purposes, related serovars are collected into convenient serogroups.

Microscopic agglutination techniques are also used for typing of cultures. Serological typing entails an initial screening of the isolate with a group of 12 or more selected antisera (group sera), encompassing most of the known cross reactions, and usually serves to determine serogroup affinities (see sections B 3.3.2.1, p. 71; B 3.4, p. 76; and C 3.1.4.1, p. 115). The isolate is then tested with antisera to different serovars which make up one or more serogroups in order to narrow the number of possibly related serovars. Finally, on the basis of observed cross reactions, representative strains of serovars are chosen for reciprocal agglutinin-absorption tests for definitive identification. According to current criteria, two strains are considered to belong to different serovars if, after cross-absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titre regularly remains in at least one of the two antisera in repeated tests. Definitive typing is usually carried out in reference laboratories. Preliminary culture typing, with use of approximately 12 screening antisera, can be carried out in diagnostic laboratories and will usually provide a presumptive serovar identification of the isolate, especially if information is available on the source of infection and on locally prevailing serovars.

In addition to a taxonomic role (see section A 2.5, p. 20), the agglutinogen characteristics are currently the most widely used basis for serological diagnostic procedures, identification of isolates, epidemiological studies, and for vaccine prophylaxis. The approximately 180 serovars have been arbitrarily assembled into 18 serogroups on the basis of shared major agglutinogen components. The serogroup is not a recognized taxon but serves as a useful guide for the selection of antigens and antisera, for the examination of sera, and for preliminary serological characterization of isolates. Various other antigens, active in complement fixation or other serological reactions, have been identified and may be used for the diagnosis of infection (see section B 3.4, p. 76).
2.5 CLASSIFICATION

The genus leptospira comprises 2 species: the pathogenic leptospiris (L. interrosana) and the so-called 'saprophytic' leptospiris (L. biflexa) which are found predominantly in fresh surface water and occasionally in sea water. Unlike L. interrosana, the strains of L. biflexa are rarely associated with infection in man or other mammals and are avirulent for laboratory animals. L. interrosana can be differentiated further from L. biflexa by its DNA composition, more fastidious growth requirements, reluctance to grow at 13°C, greater susceptibility to the growth-inhibitory action of divalent cations and of 8-azaguanine, and by its serological characteristics (see sections C 3.2.3.2, p. 127 to 3.2.3.4). L. illini, a possible third species, is phenotypically similar to L. biflexa, but is remarkably different from all other leptospiris in its DNA base composition, serological characteristics, and some morphological characteristics. Accordingly, pending additional studies, the Taxonomic Subcommittee on Leptospira (TSCL) proposed that this third species should be classified provisionally as Leptospira illini, species incertae sedis (species of uncertain position).

Included within each of the species L. interrosana and L. biflexa are a large number of serological types designated as serovars. The serovar is the basic taxon.

3 EPIZOOTIOLOGY AND EPIDEMIOLOGY

3.1 CYCLES OF INFECTION IN ANIMALS

Leptospiriosis is characterized by the spread of infection within species or groups of animals in a cyclical fashion. Usually a carrier animal, the survivor of an acute infection, infects its young. Alternatively, the urine from a carrier contaminates moist soil and the nesting or foraging areas in the perimeter of the animal's habitat. Young animals of the same species, within the same area, become infected because they frequent the same habitat. The pollution of surface waters leads to the risk of infection of other animals, whether rodent or domesticated.

Certain serovars are often found in association with particular hosts. The classical examples are the relationships between rats and interrohaemorrhagiae, and between field mice and grippotyphosa. Researchers have sought specific biological relationships between leptospiris and hosts, or leptospiris and host immune mechanisms, in order to explain the apparent "host of predilection" phenomenon, but no acceptable hypothesis has emerged. The phenomenon might be, in fact, an ecological result of relationships between leptospiris and the various animal species which share the environment. Furthermore, changes with time have been observed in the main serovar, found associated with particular host species. A list of animal species and serovars of leptospiris isolated from them has been published (3). In general, there is a wider range of rodents and other small mammal species in tropical areas, and they provide reservoirs for a greater variety of serovars than are found in temperate climates. Hence infection in man in tropical areas may be caused by many more serovars than are experienced generally in temperate areas.

3.1.1 LARGE ANIMALS

3.1.1.1 Infections between Farm Animals.

Here the examples are infection cycles between cattle and cattle, or within a sheep population, or amongst pigs. There are two means of transmission. The first is by a congenital or neonatal infection, followed by recovery and a continuing carrier state. The second, which is more important, is spread from the urine of carriers onto farmyard floors, muddy ground, or sources of drinking water. Human infection arises from contact with the above animals, and reflects the prevalent serovar.
3.1.1.2 Infections between Farm Animals and Rodents.

Rodents, especially rats, may infect both farm animals and their own species. This is a common cycle for infection of cattle and pigs, particularly if they are housed indoors. Man may be infected from either animal source.

3.1.1.3 Infections between Farm Animals, Water and Rodents.

An extension of the above epidemiological pattern is seen where the rodent carriers contaminate water or soil, which then becomes the source of infection for pigs, cattle or sheep, which in turn become carriers and excretors, thus infecting other rodents or more of their own species. The contaminated water is an extra problem to be controlled because it remains a potential source of infection for man. This is the common epizootiological-epidemiological pattern in the rice-growing parts of the world.

3.1.1.4 Interaction with Feral Rodents.

The infection cycles confined to feral rodents are self-maintaining, and are related to the territorial limits of individuals, families and species of animals in their natural habitats. The intrusion of man into these habitats involves him in the risk of leptospirosis, as a result of contact with the animals themselves or with the surface water contaminated with infected urine. Domestic animals intruding into the uncultivated habitats (such as the fringes of the forest) run similar risks and, conversely, the incursions of foraging feral animals into cleared, populated areas pose risks for both people and farm animals. Sometimes the infecting serovars in feral populations and in the geographically associated farm animals are quite distinct. An example is the presence of hardjo infection in cattle in areas of New Zealand, while possums (found in the neighbouring bushland, and foraging onto farms) are infected with balcanica, a serovar which is serologically similar to hardjo. Only by careful surveillance, based on isolation from both the domestic and feral groups, is it possible to elucidate such relationships.

3.1.2 SMALL ANIMALS

Similar principles apply to the transmission of infection among and between species of small animals. Dogs have been studied most, the main source of infection being urine from other dogs and rat urine that has polluted wet areas frequented by dogs.

3.2 EPIDEMIOLOGY OF HUMAN LEPTOSPIROSIS

Human leptospiral infections primarily result from direct or indirect exposure to the urine of infected animals. Other modes of infection, such as handling infected animal tissues, animal bites, and ingestion of contaminated food and water, are far less important. Human leptospirosis patients present little risk to other persons. Person-to-person transmission of leptospirosis is extremely rare.

Leptospira can enter the body through breaks in the skin, including even small scratches, and through the lining of the mouth, nose, and eyes. Leptospiral organisms can also penetrate skin which has been immersed in water for a long time. Thus, persons who work with their feet in contaminated water have a higher risk of contracting the infection.

3.2.1 PATTERNS OF OCCURRENCE

Persons of all ages and sexes are susceptible to infection. Adult men, however, are more frequently infected because they tend to work in higher-risk jobs.

Leptospirosis infections can occur in any month of the year, but the frequency of cases usually fluctuates with season. In temperate climates, infections are more common in the warm months. In subtropical and tropical climates, seasonal fluctuation of cases may also occur in association with factors such as periods of heavy rainfall and crop-raising cycles.
The number of cases in a region often fluctuates from year to year. This fluctuation may be due to variations in rainfall, flooding, density of rodent populations, and incidence of leptospiral infections in animals. But, more often than not, the basis for fluctuations in the incidence cannot be determined.

Leptospirosis infections tend to occur as individual or small clusters of cases. Although the patients may have had a similar type of exposure (such as working in rice fields), their infections often appear to be isolated events unrelated to a specific exposure site. Infections may also be isolated in time, in the sense that the patients were infected by a single source but on widely separated dates. These patterns of occurrence are typical of endemic leptospirosis.

Leptospirosis, less frequently, occurs as large outbreaks or epidemics over a limited period of time. Large outbreaks typically involve a group of people who have been immersed in flood waters, or who have gathered together to engage in a common activity such as harvesting crops or swimming.

3.2.2 ANIMAL SOURCES OF HUMAN INFECTION IN FARM AND HOME ENVIRONMENTS

A wide variety of animals may serve as sources of human infection. The relative importance of a given species varies from area to area depending on the population density of the species, the type and character of human housing, and the occupational and leisure activities of the local residents. In some instances, a wild animal species presents little direct risk to human health but indirectly causes human illness by transmitting leptospirosis to an intermediary domestic animal species.

Mammals bearing hair or wool are the most important sources of human infection. Most domestic mammals including cattle, sheep, goats, water buffalo, pigs, horses, dogs and cats may be infected. Wild mammals are also commonly infected. Rodents (such as rats, mice, voles, gerbils, and coypu) are the most important wild mammal source of human leptospirosis. Carnivores (jackals, mongoose, civets, skunks), marsupials (bandicoots, possums), insectivores (hedgehogs, shrews), rabbits, and deer are also carriers of leptospirosis.

Reptiles and amphibians (turtles, tortoises, snakes, frogs, and toads) may be infected, but they (with birds and insects) are not generally recognized to be of direct importance in the epidemiology of human leptospirosis, although toads are believed to be important in the Caribbean (see section A 3.3.3, p. 25).

Infected animals shed large numbers of leptospires in their urine. Domestic animals often shed leptospires for weeks to months following infection, and wild rodents may be carriers for more than a year. Because leptospires can survive for weeks in soil and water, environmental contamination may reach high levels in areas where carrier animals frequently urinate. More human cases result from this mechanism of indirect transmission than from direct contact with the urine of infected animals.

Human sources of infection. Only one outbreak of leptospirosis in man from contact with the urine of infected humans has been recorded (8). Transmission from human to humans is considered to occur rarely and exceptionally, and not to be of general practical importance.

3.2.3 WATER-BORNE INFECTION: GEOGRAPHICAL AND SOCIAL FACTORS

3.2.3.1 Occupational Hazards. In most areas of the world, leptospirosis is primarily an occupational disease. Agricultural workers have the highest risk of infection, but persons who work in sewers, mines and other wet, rodent-infested environments are also at risk.

Agricultural manual workers account for more leptospirosis cases than any other occupational group. The raising of wet land crops such as rice and taro is particularly hazardous. Rice and taro field-workers often work with their bare feet and hands immersed in water for prolonged periods of time. The skin changes, resulting from prolonged immersion in water, and the small cuts
and abrasions (commonly present on the skin of these workers) provide portals of invasion for leptospires. The relative risk of infection for rice and taro field-workers varies from area to area depending on factors such as water pH, soil type, and rodent density in the fields.

Persons involved in the raising of dryland crops such as sugar cane, vegetables, and various grains may also be infected. With these crops, the risk of infection is greatest during harvesting when the workers have considerable hand and bare-foot contact with moist soil. The relative risk of infection is highest in fields with slightly acid to alkaline soil (pH 6.2 to 8.0) and abundant rodent populations. Rainfall during times of field work also increases the risk.

Persons who raise (or take care of) livestock may be infected from exposure to their animals' urine. The exposure may be direct (e.g., dairy farmers who are splattered with urine while milking) or indirect (e.g., from walking barefoot in wet or muddy animal pens). Infection may also result from helping an infected animal to give birth (while not wearing protective rubber gloves), or from contact with discharges from the reproductive tract of infected female animals which have given birth to dead or weak offspring, or while cutting up infected dead animals.

The raising of fish and prawns in fresh-water ponds has been associated with human leptospirosis. Infections are typically acquired while wading during maintenance of the ponds or while harvesting their contents.

Leptospirosis is also an occupational disease among workers in poultry and fish processing plants and slaughter-houses. Poultry and fish are not inherently infectious. Rat infestation of the processing plants, however, could lead to contamination of the working-areas during non-working hours. The wet environment of these processing plants allows the leptospires to survive and to infect the workers. In the case of slaughter-house workers, leptospirosis may result from direct contact with the tissues of animals which may not appear to be infected. Rat infestations of slaughter-houses will further increase the risk of infection.

Mine- and sewer-workers are at risk of infection because they also work in wet environments that are often infested with rats. Veterinarians, laboratory workers, construction workers, military personnel, animal trappers and fresh-water fishermen are further examples of occupations at risk of leptospirosis.

3.2.3.2 Home and Leisure Activity Hazards.

Infections acquired in or near homes generally are the result of environmental contamination or exposure to infected pet animals. Rodent infestations of the home environment are of particular importance. The contamination by infected rodent urine, of food preparation surfaces, well water, soil, and stagnant pools of water near homes commonly leads to infection. Infection may also result from rodent bites.

Pet animals, particularly dogs, are another common source of infection. Dogs are frequently infected without obvious signs of disease. Vaccination of dogs will not prevent shedding of leptospires in their urine.

Leisure activities may also present risks of infection. Numerous isolated cases and epidemics have been associated with swimming in fresh-water streams and ponds. Swimming in slow-moving, polluted water is particularly hazardous. Swimming in the sea presents little risk because L. interrogans can survive only a very short time in salt water. Fishing in fresh water, canoeing, hunting, and hiking in wet areas are further examples of leisure activities that have resulted in leptospiral infections.

3.3 MODEL ENVIRONMENTS

3.3.1 "DRY" FARMING

"Dry" farming refers to methods of farming in temperate or cold climates where the animals are stabled and husbanded indoors, and where they are fed and watered by hand. The fodder may be
freshly cut in the growing season, or dry in the form of hay or silage in the winter. Both the animals - mainly cattle and pigs - and their attendants are at risk.

The animals may become infected when new stock is introduced. Leptospirosis may not be apparent in chronic carriers. The first manifestation of the disease may be the occurrence of abortions or stillbirths, or the passing of "redwater" by calves (see section B 2.1.2, p.55). Rodents, especially rats and mice are another source of animal infection.

 Handlers of the animals (stable-hands, farmers, milkers, butchers) may become infected from the urine, abortion products, or the carcasses of slaughtered animals. They may also become infected from the urine or field-mice which may contaminate the wet, green feed that is cut for hand-feeding in the summer. In this last case, the infecting serovars will be the same as those found in the feral rodent population.

3.3.2 "WET" FARMING

Wet farming provides the most important factors necessary for the survival of leptospires, namely animals and water. The introduction of farming, whether of livestock or crops, to an area can drastically alter the balance between the environment and leptospirosis as a disease entity, in the first case because the livestock themselves perpetuate the organism, and in the second case because new food sources encourage the expansion of rodent populations and their predators. Further, some ancillary farming practices (such as irrigation and ditching) favour the survival of leptospires, so that most categories of farmers are at risk. As an example, one survey in the Caribbean region found that 45% of sugar-cane farmers, 33% of rice workers, 36% of vegetable and fruit farmers, and 20% of animal farm-hands had been exposed to the disease.

Because high concentrations of animal carriers produce high concentrations of leptospires in the environment, the stables, pens, kennels, paddocks and grazing grounds are the likely areas of infection. Not only farmers but also smallholders who usually keep domestic pets and cows, sheep and goats tethered near their homes are likely to find their open water, soil, grass, animal bedding and feedstuffs infected. Among farm animals, pigs and cattle are particularly associated with leptospirosis, though all types are susceptible, and abortion is particularly likely in pregnant animals. The commonest portals of entry for pathogenic leptospires include the mouth, nose or eye; urine or cut skin that has been softened by prolonged contact with water; the mucus membranes of the nose, muzzle or mouth; the conjunctivae; and the moist snout of dogs and grazing animals. Standing in (or drinking) contaminated water is probably the commonest mode of infection and is particularly hazardous to thorn feeders; in the case of pigs, inhaling splashed urine or getting it in the eyes is an especial danger. Infection can be transmitted transplacentally, venereally, or through milk. Farmers should be aware of these transmission routes and take steps to circumvent infection, wear protective clothing, take care when handling carcasses (especially bladder and kidney tissues), and maintain a high standard of hygiene.

The list of crops, whether tropical tree crops, cereals, legumes, root crops, vegetables or fruit, which can be severely damaged by vertebrate pests, particularly rodents, is extensive, as is the list of pests themselves. Besides the ubiquitous *Rattus norvegicus*, *R. rattus*, *R. r. frugivorus*, *R. r. alexandrinus*, and numerous subspecies throughout the world, well-known rodents which are known to carry leptospires include *Akodon* (South American field mouse), *Nectomys squamipes* (water rat), *Oryzomys* (rice rat), *Proechimys semispinosus* and *P. guayannensis* (spiny rats), and *Zygodontomys* (cane rat), all from South and Central America. Notable from Africa are *Arvicachis niloticus* (Nile rat), *Cricetomys gambianus* (African pouched rat) and *Hastomys natalensis* (multimammate rat); from the Far East, *Bandicota* species (bandicoot) and *Rattus rajah* (spiny-backed rat); and from South-East Asia, *Suncus murinus* (house-shrew). *Rattus exulans* (Polynesian rat) and *R. conicus* (Australian canefield rat) are well-known carriers in the Pacific area and Australia, respectively. It has been estimated that two shudder rats of a good carrier species per hectare can maintain leptospirosis within that population. In Europe, the harvest mouse (*Micromys minutus*), the field vole (*Microtus arvalis*) and the house mouse (*Mus musculus*) have been responsible for epidemics of leptospirosis in farm workers. Other rodents, including squirrels and porcupines, are also known to be disseminators of leptospirosis, while jackals, foxes, mongooses and other small carnivores are noteworthy because the presence of rodent populations encourages their presence in farming areas. Buffaloes, donkeys and horses, which are often used by farmers for carrying produce and occasionally for threshing and other purposes, may add to the contamination of cultivated areas.
Agricultural workers, particularly those in some single crop cultivations (e.g., rice, sugarcane, pineapple and legumes), have been frequently and regularly infected with *Leptospira*, giving rise to reports of "cane-cutters' disease", "ricefield workers' disease", "harvest fever" (Danube Basin), "autumn fever", "swamp fever" (Germany), "water fever" and "mud fever" (Germany). Rice farmers in both tropical and temperate regions are particularly vulnerable because flooded fields provide the ideal environment for transmission. Major epidemics can occur when seedlings are transplanted into flooded fields by farmers who work for long periods bare-footed and bare-handed, and when crops that are particularly vulnerable to attack by rodents are harvested. Wet soil and heavy early morning dew, mixed with urine voided at night by nocturnal rodents or infected livestock in pastures, present a serious hazard to early morning field workers, particularly in the tropics. The heavy field work is done in the heat of the day. The cutting and handling of crops like sugar-cane and pineapples frequently cause skin abrasions which may increase the possibility of leptospiral infection, since strong protective clothing is not always available to workers in the low-income groups (see section A 5.1.6, p. 32). Cane cutters are often bare-footed or poorly shod, and scratches on their feet and legs are particularly likely to come into contact with animal urine. However, boots into which water has seeped (as when working in deep rice fields) may become hazardous than protective, and gloves that are wet by rain or dew permit continued contact of leptospires with moist and softened skin. Further, the wearing of gloves is impracticable for the harvesting of some legumes and fruits. Although the burning of ripe cane prior to harvesting has many disadvantages, and mechanical harvesting may be undesirable in labour-intensive areas, burning not only facilitates the cutting but also drives out rodents, snakes and amphibia, kills leptospires on the soil, in surface waters and on the damp vegetation, and helps to dry the ground surface. Both burning and mechanical harvesting of cane should be seriously considered in areas of high endemicity where rodent damage is excessive and surface moisture is abundant.

3.3.3 WET TROPICAL REGIONS

The optimum temperature range for the survival of leptospires in the environment is 28°C to 32°C, though the organisms can live at mammalian body temperatures, which may be higher, and survive subzero temperatures as well. Tropical, unpolluted, nonsaline waters with a slightly alkaline pH (7.2-8.0) provide an ideal year-round environment. Where limestone exists in the soil (e.g., in the coral islands of the Pacific, in Barbados, or in the alkaline natural waters of the tropical rainforests), survival may be prolonged. For example, the waters of Java are far more acid and leptospires are comparatively few or absent. Survival is less likely in tropical areas where rainfall is low to moderate and seasonal as well; but in high temperature zones where the annual rainfall is over 200 cm and the vegetation is luxuriant, leptospires thrive in fresh-water ditches, drains, ponds, damp soil, mud, and in association with damp vegetation. However, urine, decomposing vegetable matter, leaf mold, and swamp mudds tend to be acid. While pathogenic leptospires can survive for a few hours in acid, concentrated urine, they survive for very much longer when the urine is diluted and less acid. Survival times for leptospires in the environment have been found to vary from a few hours in dry soil to six months under flooded conditions.

Flooding after heavy tropical rains is particularly favourable to leptospires. It elevates the water table, allowing saturation of the environment by subsurface leptospires; it prevents animal urine from evaporating or penetrating the soil so that the leptospires may pass directly into the surface waters; and it "tops up" swampy zones, causing invasion by aquatic rodent or carnivore populations from neighbouring cultivated fields. When pastures remain flooded for several days or even weeks, animals, particularly cattle and horses which often lie down or roll about, are especially vulnerable to infection. Analysis of the incidence of cases shows annual peaks associated with tropical seasonal rainfall, and higher than average rates during periods of flooding or exceptionally heavy rains.

The presence of suitable animal hosts is necessary for the long-term survival of leptospiral organisms. Besides domestic animals and livestock, a wide range of species (whether in scrub, forest, cultivated areas, towns or villages) can act as carriers. The majority are rodents but numerous other species, particularly ground-dwelling types, can be significant disseminators, and loci of leptospirosis can be found where wildlife is common, particularly in bush or forest with still water, large pools and slow-flowing streams. Well-known carnivore and marsupial carrières which may extend into the tropical belt include the jackal, opossum species, the striped skunk, palm civet, raccoon, and several species of bats. Species like the common toad (*Bufo marinus*) and the small Indian mongoose (*Herpestes auropunctatus*), which is now found on all the major Caribbean islands, on parts of the South American mainland, Hawaii and Fiji, as well as in its indigenous habitat, are of
especial significance because of their close association with man. Reptiles and wading birds can be disseminators of leptospires, and it has been suggested that migrating birds, particularly waders and those that feed on small rodents, could become long-range distributors.

Socioeconomic and ethnic factors are also important in the spread of leptospirosis and largely account for the high prevalence of the disease in the tropics. Most tropical and subtropical countries are underdeveloped or developing in comparison with those in temperate zones, and the standard of living and habits of the people in these communities may have changed little. Limited sanitation facilities for both man and animals, lack of pipe-borne water and good drainage, poor abattoir and animal-handling techniques, inadequate public health supervision, the use of animals (particularly oxen or water buffalo) as beasts of burden, and the ever-present stray dog and peri-domestic rodent problems in both rural and urban societies increase the likelihood of exposure to leptospires. Children in such communities are frequently bare-legged and bare-footed, and may be exposed to Leptospira from a very early age. A survey in the Caribbean found 8.0% of 5-6-year-olds, 9.9% of 7-9-year-olds, 12.2% of 10-12-year-olds to be seropositive. The highest figures in this series was 36.6% of seropositive children among 13-14-year-olds in Barbados. Year-round outdoor activities, including swimming and playing in river beds or ditches, present more opportunities for exposure in the tropics than in temperate lands. In temperate zones, the incidence of leptospirosis tends to be seasonal, the higher incidence in the summer and autumn being attributable both to the longer survival and greater numbers of organisms in the warmer weather, and to an increase in outdoor activities by people wearing lighter, less-protective clothing. These activities include swimming, hunting and fishing (among recreational pursuits), as well as hedging, ditching, irrigating, planting and harvesting. In the tropics, these activities tend to be more uniform throughout the year, resulting in a less obvious seasonal pattern except where periods of heavy rainfall may produce a flush of leptospires into the environment.

Surveys in the Caribbean and tropical Americas have shown a striking difference in exposure rates between rural and urban dwellers. In one such survey, 11% of an urban control group were seropositive, compared with 28% of rural family groups. Particularly at risk are those in agricultural occupations, vertebrate-pest control workers, zookeepers, veterinarians, meat processors, soldiers with training or fighting in subtropical areas, fishermen, those who live or work on inland waterways or whose activities bring them into contact with fresh water, particularly where there are concentrations of animal sheds, and others such as foresters and charcoal burners working in damp vegetation. The immersion of the body in lakes and rivers as practiced by Hindus, some Baptists and other sects for religious purposes can greatly increase the risk of exposure to leptospires, while individuals engaged in ditching, clearing drains, and constructing and maintaining soakaway pits and pit lavatories in the tropics are also at high risk.

3.3.4 MINING AREAS

It is well known that mines and underground tunnels are frequently wet from underground water seepage and cooling-water leakages, and are also often infested with rats. Thus, leptospirosis is notoriously endemic among miners in many areas. The main source of infection is rat urine contaminating the ground water in which miners may be obliged to walk, crawl or lie. The leptospires enter through the inevitable cuts and abrasions in the skin.

3.3.5 FOOD INDUSTRY WORKERS, ANIMAL HANDLERS AND PROCESSORS

Persons handling animals destined for the meat industry, prior to slaughter, may become infected from contact with urine or contaminated ground water and mud in marshalling yards, stables and saleyards. The risks run by milkers and dairy farm workers are mentioned elsewhere (sections A 3.2.3.1 p. 22 and B 4.2.4.2 p. 94). In the abattoirs those at risk are slaughtermen, dressers of carcasses and handlers of offal, meat inspectors, and veterinarians. The main sources of infection are the urine and kidneys of apparently uninfected animals (cattle, pigs, sheep and goats). Sick animals slaughtered in knackeries and their carcasses are not known to be important sources of infection. The serovars responsible for human infection reflect those that are prevalent in the animals handled. Another source of infection is the rat population which is almost invariably found in places associated with the food industry (see section A 5.1.1 p. 30). Infections from rat urine reflect the serovars infecting these animals.
4. DIAGNOSTIC METHODS AVAILABLE

The diagnosis of leptospirosis, based only on clinical signs, is difficult because these signs are not pathognomonic in man and they vary extensively in all domestic animals. Therefore, a diagnosis is dependent upon an evaluation of individual and herd histories, clinical signs, lesions, and laboratory findings. An understanding of the pathogenesis of the disease is important in the selection of appropriate samples for each stage of the disease. In the acute disease, during the febrile response, leptospires may be isolated from the blood by cultivation in appropriate liquid or semisolid media (see sections B 3.2.1, p. 68 and C 3.2.2, p. 129) or by inoculation of certain laboratory animals (see section B 3.2.2, p. 69).

Although leptothes have occasionally been isolated from the milk and semen of animals during the initial stages of the disease, most attempts have been unsuccessful. Urine samples are the most appropriate source from which to isolate leptothes either by culturing or by animal inoculations.

4.1 CULTURE OF LEPTOPIRES

In general, it takes several days to several weeks to culture leptospires from clinical specimens, so that only a retrospective diagnosis can be made. However leptothes may sometimes be visualized microscopically.

Leptospires are present in the blood during the first few days of illness and may be cultured during this time (section C 3.2.2, p. 129). They can occasionally be observed directly by dark-field microscopy but the numbers present are too low to allow observation in most cases. Care and great-precision are necessary to avoid mistaking proteinaceous strands (pseudo-spirochaetes) in blood for leptothes. Leptospires may be concentrated by centrifugation but the percentage of positive observations remains low. Direct microscopy of blood is not recommended as a routine procedure.

Leptospires in urine may be visualized and/or cultured after the second week of illness in acute cases and over prolonged periods (up to a year or more) in animals, especially dogs and pigs which are renal carriers. Leptospires are killed or lysed by acid conditions, and may be inactivated in as short a time as about 3 h in undiluted urine. The urine should be alkalized and examined within 1 h or as soon as practicable after voiding, using direct microscopy, culture or animal inoculation. Media containing antibiotics (see section C 3.2.1.4) are essential to reduce contamination of urine cultures.

Leptospires may be observed and isolated from cerebrospinal fluid during the end of the first week of illness. However, this procedure is not usually performed.

In fatal cases of human and animal leptospirosis, the organisms may be seen and cultured from ground post-mortem specimens of tissues (sections C 1.1.6, p. 103 and C 1.2.6, p. 104); liver, kidneys and brain are the tissues most suitable. Leptospires may also be successfully isolated from aborted animal fetuses (see section C 1.2.5, p. 104) in this manner; the tissues should not be frozen.

In addition to observation of human and animal tissues by dark-field microscopy, they may be stained by a silver staining technique (section C 3.2.5.1, p. 128). However, recognition of leptothes in such preparations is more difficult than the observation of motile leptothes by dark-field. Immunofluorescence (section C 3.1.4.2, p. 118) is also helpful in detecting leptothes in urine or tissues.
4.2 SEROLOGY OF INFECTION IN ANIMALS AND PATIENTS

4.2.1 TESTS AVAILABLE

The basic technique is agglutination of live or formalized leptospires by titrated amounts of serum from animals or patients. Tests are usually "microscopic", i.e., the end-point is read microscopically under dark-field illumination. Various macroscopic tests performed in tubes or on slides have also been described.

The specificity of the tests depends on the animal species whose serum is being tested. The reference standard for specificity is hyperimmune rabbit serum, prepared according to an empirical immunization schedule recommended by the Taxonomic Subcommittee on Leptospira (TSCL) of the International Committee for Systematic Bacteriology (ICSB) of the International Union of Microbiological Societies (IUMS) and revised from time to time. This method is used to identify the isolates of leptospires and classify them according to their serovars (sections B 3.3.2, p. 71 and B 3.4, p. 76). Serovars thus identified may be used in agglutination tests. Authentic strains may be obtained from reference laboratories where stock culture collections are maintained (sections C 3.2.4, p. 127 and Annex 1, p. 134).

Human immune sera cross-react between serovars to a much greater extent than sera from domestic or laboratory animals, and they show an almost universal cross-reaction with *L. biflexa* serovars patoc and andamana. This property of human immune sera can be used for screening the sera from convalescents and in surveys of people for evidence of leptospirosis. A proportion of "false" positive and negative reactions, compared with specific agglutination tests, reduces the usefulness of human immune sera in the diagnosis of illness in individual patients.

The reaction of human sera with individual serovars of *L. interrogans* must be tested whenever an attempt is to be made to identify the homologous infecting serovar. Animal sera, which are much less cross-reactive, must be tested initially with the homologous serovar in the absence of suitable screening tests. When the homologous serovar is not known, a battery of several locally prevalent serovars is required (see sections B 3.3, p. 70 and B 3.4, p. 76).

Various techniques have been developed to simplify and reduce the time, skill, and expense required for serological testing. They have not been accepted universally, but are nevertheless useful, both for human and for animal infections. They include incubation of dilutions of sera with red blood cells of various species, sensitized by absorption of essentially polysaccharide antigens, sometimes after pretreatment with formaldehyde, glutaraldehyde or pyruvaldehyde. The end-point is haemagglutination (section B 3.4, p. 81). Various antigenic preparations for use in complement-fixation tests have been described (see sections B 3.4.3, p. 80 and C 3.1.3.2, p. 110).

ELISA procedures can be used to identify either IgM, IgG or all antibodies reacting with the antigen (see section C 3.1.4.3, p. 120). Counter-current immuno-electrophoresis and immunofluorescence have also been applied as useful rapid diagnostic tests. Agglutination has also been used to detect urinary antibody in animals which are carriers or have residual renal damage.

The main methods available and in general use (Table 2) are as follows (the techniques are described where indicated):

\*(i) Screening tests\*

1. Agglutination of *L. biflexa* Patoc I or Andaman by human sera, using a macroscopic or a microscopic method (section B 3.4, p. 76).
2. Macroscopic or microscopic agglutination using a panel ("battery") of locally prevalent serovars (section B 3.4.1, p. 76).
3. Indirect haemagglutinating screening test (HA), which is a genus-specific test and a haemolytic variant HL (section B 3.4.4, p. 81).
4. Complement fixation tests (section B 3.4.3, p. 80).
5. Latex particle agglutination, which is a genus-specific test (section C 3.1.3.3, p.114).
6. Immunofluorescence (section B 3.4.2, p. 79).
7. Immunoelectrophoresis (section B 3.4.3, p. 81).
8. ELISA (section C 3.1.4.3, p. 120).

(2) Specific tests

Specific diagnostic serological tests can be used with confidence for the presumptive identification of the infecting serovar only in animal (not in human) infections.

1. Microscopic agglutination test (MAT). This test is the current reference standard test for serology (section B 3.4.1.1).
2. Immunofluorescence (section B 3.4.2).
3. ELISA (section C 3.1.4.3, p. 120).

4.2.2 INTERPRETATION OF SEROLOGICAL TESTS

4.2.2.1 Response to Acute Infection. IgM antibodies are produced relatively early in infection and are detectable by microscopic agglutination test (MAT) and the immune haemagglutination (HA), complement fixation test (CF) or enzyme-linked immunosorbent assay (ELISA) test for IgM. Later, IgG antibodies may appear, and may be detected by MAT or ELISA for IgG (section C 3.1.4.3, p. 120). Thus in the period of the acute infection and subsequent convalescence, the transient IgM response will be detected by HA and CF, or by ELISA (IgM). These tests will be found to be negative later in the illness, or after recovery. They will not detect residual antibodies from previous infections or immunizations.

4.2.2.2 Index of Previous Infection. MAT, or ELISA (IgG) will also detect IgG antibodies (if they appear, later on in the infection) and IgG residual antibody in patients or animals who have recovered or who have been immunized. In general, at least in man and rabbits, the IgG antibodies tend to be more serospecific for the infecting serovar.

Table 2. USE OF VARIOUS DIAGNOSTIC SEROLOGICAL TESTS FOR LEPTOSPIROSIS

<table>
<thead>
<tr>
<th>Sera of</th>
<th>Agglutination</th>
<th>CF</th>
<th>HA, HL</th>
<th>IF</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microscopic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nonspecific (Patoc or similar)</td>
<td>Specific</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Dog</td>
<td>?</td>
<td>++</td>
<td>+</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Cattle</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Swine</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Horses</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Sheep</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>?</td>
<td>+</td>
</tr>
</tbody>
</table>

++ = widely used  + = useful  - = not useful  ? = little or no information
CF = Complement fixation  HA = Immune (indirect) Haemagglutination
HL = Immune haemolysis  IF = Immunofluorescence
5. MEASURES FOR CONTROL

5.1 CONTROL OF INFECTION IN MAN

5.1.1 RODENT CONTROL IN AREAS OF FOOD PRODUCTION

Few rodent species have adapted well to a commensal existence and whilst some may be of importance regionally as commensals (e.g., *Hastomys natalensis* in Africa or *Bandicota bengalensis* in India), only three species have achieved worldwide association with man. These are *Rattus norvegicus* (the brown, Norway or common rat), *Rattus rattus* (the black, ship or roof rat) and *Mus musculus* (the house mouse). All three species can occupy a wide range of habitats and are omnivorous in their feeding. As a result they are pests at all stages of food production, causing damage to growing crops as well as to stored products; in both spheres they are important from both the economic and the public health aspects. Field control is described in section B 4.2.2, p. 91. The largest rodent populations are concentrated in areas of stored food, so that this is where rodent control is usually most needed.

Food stores tend to increase in size as economic forces compel the handling of larger quantities and hence the scope for rodents within these bulk storage areas increases. Rodents eat relatively little but they spoil large quantities, and the house mouse is a particularly wasteful feeder because it discards large amounts of partly eaten food. Storage bags are damaged by rodents that use them for nesting material and the costs of re-bagging are high.

The most effective method of controlling commensal rodents is to deny them access to food and living sites. Furthermore, it avoids the use of poisons, which are undesirable in food production areas. The design and maintenance of premises are vital for excluding rodents; the basic principles in this approach have been described (9, 10). New buildings for food processing and storage should be made rodent-proof and, where possible, be situated where the chance of infestation is low.

Maintaining a high level of hygiene has repeatedly been shown to be the best form of rodent control, both inside and outside food premises. Waste food left outside will attract commensal rodents and this should be avoided. Similarly, dense vegetation should be cleared from areas around the storage building so as to leave a space which rodents would be reluctant to cross. Within the stores, scrupulous cleanliness and attention to packaging are essential. Rodent stacks should not be more than 10m wide and should be kept well away from the walls. The provision of clearways round all stacks will enable inspection to be carried out easily and facilitate control measures. Early detection of infestations will prevent the breeding and dispersal of rodents. The presence of rodents may be detected by leaving dust patches to reveal their footprints.

Bags and cardboard boxes are vulnerable to rodents and loose cereals should be stored in bulk containers.

If these hygienic measures fail, rodent infestations in food stores will have to be controlled in other ways. The most common method is to administer poison in some suitable food or in water. The acute and chronic poisons used to control commensal rodents in various parts of the world have been described (11, 12; also see section B 4.2.2.2, p. 92).

Since some rodenticides present problems when used in food storage or processing areas, anticoagulants are preferred in these situations. Poison dusts, which are ingested by the rodents during grooming, are not generally recommended for use in food stores or processing plants because they could be blown about and contaminate the food; but they could be used in conjunction with other control techniques when they should be placed in wall cavities, beneath floors and in pipe ducts.

In warm, dry environments where there is no water, commensal rodents may be controlled with poisoned water. Solutions of very acute poisons, such as sodium fluoroacetate, are too dangerous to use in open areas and so water-soluble anticoagulants, especially the sodium salts of warfarin and pival, are often used (see sections A 3.2.1, p. 33 and B 4.2.2.2, p. 92). To control mice in food stores, anticoagulant water baits should be surrounded by areas with anticoagulant dust.
In cases where rodent infestations in food stores have reached a high level, fumigation may be used. This method, which should only be carried out by professional operators, consists in covering the foodstuffs with a gas-proof sheet and passing the fumigant under the sheet. Methyl bromide is a common fumigant and carbon dioxide has also been used.

Finally, where only small numbers of rodents are involved, traps may be used (see section C 2.1.1, p. 104). These can be of the live-catch variety or snap traps which kill outright (13, 14).

5.1.2 RODENT CONTROL IN THE DOMESTIC ENVIRONMENT

The transmission of leptospirosis to man is likely in rodent-infested domestic premises where infective urine may contaminate food and drinking water and where human contact with infected rodents is more likely because of their close proximity.

The house mouse is a cosmopolitan pest which is found in cities and small settlements in all climates, as well as living outdoors in many parts of the world. The brown rat is the common rat of temperate climates where it lives in towns and countryside and commonly infests sewer systems, coal mines and other man-made environments. In the tropics, it is the chief rat of towns and ports but may spread inland where local species of competitors are absent. The black rat inhabits human dwellings throughout the tropics and, being a better climber than the brown rat, is found at all levels in high buildings. In some areas this rat seems unable to compete with field species of rodents and is not found far from dwellings, although on islands where there are no competitors it is common throughout forests and fields. In temperate latitudes the black rat is confined to warm buildings because it does not tolerate cold conditions.

The most effective control is achieved by a combination of sound, rodent-proof constructions and good hygiene. The principles of construction design are referred to in section B 4.2.2, p. 91. On the other hand, rodent infestation of domestic premises is greatly facilitated by any of the following circumstances:

(1) When the accommodation is part of a large complex (e.g., a residential block of flats or apartments), which is in poor repair and has become progressively rodent infested and in whose fabric rodents live;

(2) When the premises are adjacent to infested food stores or farms;

(3) When the dwelling house is close to an outdoor source of food for rodents, such as occurs in the keeping of poultry;

(4) When structural damage occurs to sewers, enabling rats to escape into buildings.

When infestations occur in dwellings, many of the techniques outlined above (see section A 5.1.1) may be used. All food should be kept in rodent-proof containers, and potential nesting material removed. The chronic (anticoagulant) poisons are more suitable for use in the domestic environment, because there is too great a risk with the acute poisons in case children and pets may contact them or the food may become contaminated. If the infestations are light, then the poison - always a hazard in houses - may be replaced by traps. Snap-killing traps and live traps are very effective when set close to the room edges where rodents run (see sections A 5.1.1, p. 31; A 5.2.1, p. 33; and C 2.1.1, p. 104). Rodenticidal dusts may be of value in houses as a supplement to other control methods which are laid in the same places, as described above. Where free water is scarce, as in food stores, poisoned water sources may be especially useful.

In the open, single-storeyed village houses of tropical countries, the normal construction methods (e.g., roofs made of leaves and thatch) provide accommodation for the black rat. In these cases, poisons can be safely laid at roof level and the occupants encouraged to keep the food for themselves and their animals in rodent-proof containers. Where the rodent species are found in scrubland or forests adjacent to villages, an area cleared of vegetation for some distance from the houses, together with semi-permanent poison bait stations around the perimeter, can be an effective barrier.
5.1.3 CONTROL OF EXPOSURE IN AN INFECTED ENVIRONMENT

5.1.3.1 Urine. Contamination of the environment is mainly due to infected urine. In the handling of domestic animals, direct contact with the urine must be avoided, whenever possible. In animal houses, such as milking sheds, sale yards and piggeries, there should be provision for rapid drainage of urine with minimal contact with humans. Ideally, the floors should be made of an impervious material, and the drains should be situated some distance away from areas where people work and should be kept covered. When these places are cleaned, care should be taken to protect workers from water and urine splashes during the cleaning procedures.

Effluents from animal houses should be discharged in areas which are not frequented by domestic livestock or people. Pasture areas and holding pens, which are subject to high stocking density and frequent human contact, must be well drained to prevent accumulation of surface waters contaminated with urine.

5.1.3.2 Animal Tissues. The only time when the tissues of an infected animal, apart from kidneys and urine, are likely to contain leptospires is during the acute bacteraemic stage of the disease. Any animal which is suspected to have died from the disease and all aborted fetuses and membranes must be rapidly and hygienically disposed of, by cremation or burial. Care must be taken to bury such animals in an area from where drainage cannot contaminate the places that are frequented by animals and people. Similarly, a site to carry out an autopsy must be chosen with care and suitable protective clothing must be worn.

Milk is not regarded as a vehicle for the transmission of leptospirosis; it has been shown to be leptospirocidal, even when diluted.

5.1.3.3 Water and Mud. Leptospires are capable of surviving for several months in a wet environment, providing that temperatures are not too low. In areas where leptospirosis is at a high endemic level in domestic stock or wild animals, good drainage of pastures and other agricultural land should be undertaken whenever possible. Efforts should be made to keep wild rodent populations and dogs under control. In agricultural areas which require constant irrigation or a very wet environment, efforts should be made to provide dry footpaths for persons not actually working in the wet area. It must be remembered that the probability of infection depends on the degree of exposure to infection. Although complete environmental control will usually be impossible, a reduction of contamination is likely to have a very significant effect on the reduction of infection contracted by this means.

5.1.4 OCCUPATIONAL HYGIENE

Leptospirosis is usually contracted by penetration of the organisms through skin wounds and abrasions, and through intact mucous membranes (especially the conjunctiva). Occupational hygiene should therefore be based on preventing infection by these routes and reducing the number of leptospires in the environment.

One of the most important measures must be to keep any laceration well covered with a waterproof dressing. Any wound sustained while working should be immediately treated and covered with a suitable dressing. Protective clothing should be worn whenever possible; such clothing must include waterproof footwear which should be possible in almost all circumstances (see section A 3.3.2, p. 28 and B 4.2.4, p. 94). In the abattoir it should be possible to wear impervious aprons and other protective clothing; this is often a legislative requirement. For those at special risk, such as meat workers who handle kidneys and urinary bladders, disposable plastic gloves could give added protection. When potentially infected premises are being cleaned, care must be taken (when using water hoses) not to splash the eyes with urine-contaminated water. People working directly with live animals should avoid urine splashes and attempt to wash away such contamination when it occurs.

Veterinarians involved with clinical cases of leptospirosis or the examination of animals post-mortem must wear protective clothing and adopt normal professional standards of hygiene. Drainage and sewage workers should wear impervious boots and gloves (see section B 4.2.4.2, p. 94).
5.1.5 IMMUNIZATION

An effective non-toxic vaccine, suitable for human use, has been sought since leptospires were first isolated. Early vaccines consisted of killed suspensions of leptospires grown in media containing animal serum. They were injected either subcutaneously or intravenously and both local and severe systemic clinical reactions were common, although in general the vaccines appeared to be both immunogenic and protective.

In an attempt to eliminate these allergic reactions, Shenberg & Torten (15) prepared a whole-cell vaccine of serovars grippotyphosa and schwajizak, grown in chemically-defined protein-free medium. This vaccine produced only mild localized reactions in volunteers, in whom a 57% seroconversion rate was observed. The vaccine is being tested in field trials but the results have not yet been reported. Recent large-scale immunization in man includes the wide use in China of a vaccine made from a suspension containing one or more serovars of leptospires, grown in a derivative of Shenberg's protein-free medium (15); the immunization is repeated annually. A washed suspension of leptospires, grown in 5% rabbit-serum medium and given intradermally, was used in Vietnam. Very little has been published about the efficacy or toxicity of either of these vaccines. An "outer envelope" vaccine (16) has been shown to be highly immunogenic and protective in several animal species but has not been tested in humans.

Thus, at present there is no human vaccine available which is nontoxic and of proven efficacy in a field situation.

5.2 CONTROL OF INFECTION IN ANIMALS

5.2.1 RODENT CONTROL

Housed livestock may have contact with house rats and mice. Destruction of the latter in animal houses is essentially similar to their destruction in private houses. Grazing animals may have contact, either directly or indirectly, with a variety of wildlife including rodents. The elimination of leptospiral carriers in the wildlife populations from pasture lands is impossible. However, limited success may be achieved by the following methods for reducing the number of field mice and voles.

5.2.1.1 Poisoning (see section B 4.2.2.2, p. 92). Zinc phosphide is an effective and safe poison for foxes, the natural enemy of rodents. The minimum lethal dose is small, and the poison can be distributed in pellets in the shape of feed.

Sodium monofluoracetate and thallium sulfate have also been used successfully. They are very toxic to non-target animals, and foxes may be poisoned by eating mice and voles which have died from sodium monofluoracetate. They should be used only by specially trained staff. Thallium sulfate penetrates unbroken skin, and its use is discouraged by a WHO Expert Committee on the Safe Use of Pesticides (17).

5.2.1.2 Use of Predators (see section B 4.2.2.3, p. 93). Foxes, weasels, martens, hawks, owls, kites and snakes, which are known to be natural enemies of rodents, must be protected and preserved. Leaving weasels at large was found to be effective in reducing the number of voles in a relatively small island. However, the transmission of leptospirosis through the predator chain from rodents to foxes, skunks and opossums has been shown. Such transmission of leptospirosis, induced by the predator chain, only causes other problems.

5.2.1.3 Other Methods. Trapping (see section A 5.1.1, p. 30 and C 2.1.4, p. 104) is rather ineffective. However, the trimming of grass, followed by burning, greatly reduces the population of rodents temporarily.

If the elimination of carriers in the wildlife population is impossible, the separation of grazing animals from wildlife must be considered as the next best policy. Suitable but expensive measures are fencing of the grazing animals with double palisades, and immunization.
5.2.2 CHEMOTHERAPY FOR DOMESTIC ANIMALS

The disease in infected animals can be diminished or eliminated by the administration of dihydrostreptomycin, tetracyclines and penicillin (see section B 4.2.5.1, p. 94). Of the chemotherapeutic agents used against leptospirosis, only dihydrostreptomycin is believed to be completely effective in freeing an animal from infection. It must be given by injection, usually over a period of days. Although tetracyclines may provide a good response in acute infections and reduce renal shedding to a nondetectable level, they are considered inadequate for eliminating the infection. Penicillin is ineffective for renal leptospirosis but often brings about a rapid improvement in acute leptospirosis, particularly when administered in the early stage. Oxytetracycline mixed with feed has been used chemoprophylactically in some countries in swine for the purpose of preventing abortion due to *pomona* infection. Drug-resistant leptospires appear very rarely.

5.2.3 ISOLATION OF DOMESTIC ANIMALS

Domestic animals (either diseased or recently converted to serologically positive) may be separated from uninfected animals and kept in isolation. The isolated animals are treated with chemotherapeutic drugs in order to eradicate leptospires from their kidneys. Floors, soil, tools and anything else that has been contaminated with the urine of the animals must be washed with disinfectants. Pigs must be isolated from cattle, sheep or goats because infected pigs apparently shed leptospires in their urine in greater numbers and for longer periods of time than do cattle and other domestic animals. Dogs on farms should be isolated from other domestic animals for the same reason; if they cannot be isolated from livestock, they should be immunized with appropriate vaccines.

5.2.4 SLAUGHTER OF DOMESTIC ANIMALS

Slaughter of infected domestic animals may be undertaken to make the herd free from leptospirosis. Animals that have died of leptospirosis or that have been slaughtered during the systemic infection should be buried or burnt. In the abattoir, the slaughter of animals showing symptoms and clinical signs of leptospirosis (see sections B 2.1.2, p. 55 and B 2.9.1, p. 63) should not be permitted. In apparently uninfected animals, the gross lesions of renal leptospirosis, such as focal necrosis and haemorrhages in the cortex, may nevertheless sometimes be seen. Such kidneys may contain viable leptospires and should not be used for food (section B 4.2.1.(3), p. 91).

5.2.5 IMMUNIZATION OF DOMESTIC ANIMALS

Since many of the epizootiological factors that maintain leptospirosis infections in domestic animals are not amenable to control (e.g., wildlife carriers), it is extremely important that immunological control should be as complete as possible. Immunological studies have demonstrated that leptospiral bacterins induce reasonable production of antibodies for a period of at least 6 months following vaccination. Studies in cattle indicate that *pomona* bacterin induces primarily an IgG response which persists for as long as 12 months. Revaccination or exposure to a live culture results in an increase in IgG antibodies but only causes a primary IgM response. As this protection is serovar-specific, the bacterins should contain the antigens of all the serovars prevalent in a particular geographical area in order to achieve the broadest protection. This in turn requires a good diagnostic and surveillance service (section B 4.1.2, p. 86).

Bacterins prepared with leptospires killed by various means (such as formalin, phenol, freezing and thawing) have been used with varying degree of success in different species of animals. Ideally a bacterin should afford protection against not only the acute disease but also infection, subsequent leptospirosis, and complications such as abortion. Bacterin prophylaxis, however, may not prevent infection and leptospirosis in all treated animals, although the spread in herds can be markedly reduced. A "component" vaccine prepared from the OE of leptospiral cells has proved to be safe and protected animals against renal shedding. A vaccine prepared from living avirulent leptospires may also protect from both the clinical and the renal forms of leptospirosis. How to prolong the duration of the immunity induced by vaccination needs further investigation. Female animals should be vaccinated before the breeding period in order to provide the greatest protection during pregnancy. It seems essential to immunize the whole herd annually.
5.2.6 ECOLOGICAL CONTROL OF FERAL POPULATION

Depopulation of one animal species often results in the increase of other species. For example, fox depopulation may cause an increase in rodents which are prominent leptospiral reservoirs. Recently there has been a trend to destroy the natural enemies of rodents. A method of ecological control of a feral population, which will also control the leptospiral carriers, awaits future investigation. A device and method for oral vaccination of wildlife may be necessary. See also section B 4.2.3, p. 93.

5.3 GENERAL CONTROL MEASURES

5.3.1. CHEMICAL TREATMENT OF WATER, SOIL, AND RICEFIELDS

The chemical treatment of water and soil appears to be useless in open pastures and crop-farming areas. Attempts to disinfect soil polluted by leptospires in open and unbounded areas, such as ricefields, using calcium cyanamide, hypochlorite or copper sulfate, have also been unsuccessful. To be effective, copper sulfate, which kills leptospires at a concentration of 1,400 g/ha, has to be sprayed daily on ricefields at a calculated dosage of 1,900 g/ha/ha (see section B 4.2.3.2, p. 93).

Furthermore, leptospirosis in rural populations engaged in pasture farming and agriculture is often characterized by a benign, frequently asymptomatic, course. In these environments, the severe form is seen in general in visitors to the area, or is related to the occasional or massive inhalation of polluted waters. Thus attempts to kill leptospires in soil are not feasible and are likely to benefit only a small number of people.

Chemical treatment of the environment may be considered for the control of leptospires from animal excreta in farms and from sewage waters in built-up areas.

In conventional European animal husbandry ("dry" farming - see section A 3.3.1, p. 23), the animals are stabled on straw litter. The dung heaps collected from this litter develop temperatures so high that all vegetative pathogenic microorganisms likely to be present in the excreta are killed. After three weeks of conservation, these dung heaps are considered to be disinfected and may be used for agricultural purposes, i.e., as fertilizer. This "dump packing" procedure is regarded as the optimum means for disinfecting the animal excreta so as to control the spread of infectious diseases among the animals to be reared.

In the raising of industrial livestock, the animal excreta are collected as "liquid manure" (a mixture of urine, dung, forage remains, and splashed water, which is also called "slurry"). This liquid manure is stored in underground or surface containers but fails to generate sufficient heat to reach a temperature that will rapidly inactivate the pathogenic microorganisms (mean European winter and summer temperatures of 8°C and 17°C respectively) during the time in which the slurry is stored in the tanks.

In industrial pig-husbandry, the regular persistence of pathogenic microorganisms, including leptospires, has been demonstrated in the "sludge" (i.e., the solid residue that is left after liquid manure treatment). Experimental addition of leptospires to the oxidation trenches for liquid manure showed that the leptospires survived for at least 136 days.

Chemical treatment before disposal into the water-soil biotope may be useful to eliminate pathogenic microorganisms, including leptospires, from urban sewage waters and slurry from the excreta of industrial livestock. No specific recommendation can be made at present. Further research in these two sectors is necessary, and should be included in programmes for the control of communicable diseases.
5.3.2 OCCUPATIONAL HYGIENE

The main measures for the protection of farm-workers and meat-workers involves reduction of disease in the animals they handle. Specific occupational hygienic measures have already been described (see section A 5.1.4, p. 32).

5.4 RELATIVE EFFICIENCY AND COST-BENEFIT

Little has been published about the relative efficiency of control measures, or about their cost benefit. The methods by which such an analysis might be made are described in section B 4.3, p. 96.
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SECTION B: LEPTOSPIROSIS

1. THE ACUTE INFECTION IN MAN

1.1 GENERAL DESCRIPTION

Leptospirosis, a zoonosis, is an acute febrile illness caused by microorganisms of the genus *Leptospira*. Leptospires are bacteria infecting a large variety of domestic and wild animals, which excrete them in their urine (see section A 1.2). Rodents (especially rats), pigs, cattle and dogs are the animals most often involved in human disease. Man becomes infected when he comes into contact with an infected animal, either directly or more frequently indirectly through environmental contamination with infected urine. The entry of the organism is through damaged skin and through the mucous membrane of the oropharynx, the conjunctiva, or the genital tract.

There are a large number of serological varieties (serovars) of pathogenic leptospires (see section A 2.5, p. 20 and Table 5, p. 72). All serovars that are pathogenic for animals can cause human disease, the severity and the clinical manifestations of which depend on the dose and the virulence of the infecting serovar, the host susceptibility, and the organ systems predominantly involved. The human infection varies greatly in severity; in the mildest form, the infection may be subclinical or a benign self-limiting nonspecific febrile illness, in the most severe form, it is a fulminating and fatal illness with hepatorenal failure (Weil's disease or syndrome). Historically, several clinical syndromes have been described and often linked to certain serovars, but it is clear now that there are no serovar-specific syndromes and that the pathogenesis of all cases of leptospirosis is similar. Any serovar can cause a mild or a severe and fatal illness, but infections with *pomona*, *hardjo* or *grippotyphosa*, which are common causes of leptospirosis in temperate climates, are seldom severe or fatal, and almost never cause the severe hepatorenal type.

1.2 MAIN SYMPTOMS AND SIGNS

The clinical manifestations vary greatly. The disease may present as a febrile, meningeal or severe icteric illness. It is not possible to give an accurate figure for the incidence of clinical features, because experience varies. The incidence of any symptom mostly depends on the frequency with which mild infections are diagnosed and included in the statistics. If the mild infections are not diagnosed, the relative incidence of severe symptoms may appear to be much higher than it really is.

1.2.1 FEBRILE ILLNESS OR ANICTERIC LEPTOSPIROSIS

The illness may be mild with fever, headache and body pains. The onset may be sudden, with low-grade fever, and the headache is seldom severe. Anorexia and nausea may occur. The illness may last from one day to several days. Often medical advice is not sought and, even when sought, the features are so nonspecific that the diagnosis is not suspected and the condition is labelled as "Influenza" or "viral illness".

The more severe infection is a biphasic febrile illness ("saddle" type). The first (septicaemic or leptospiraemic) phase is characterized by acute systemic infection and by the presence of leptospires in the blood and cerebrospinal fluid. This phase lasts for 4-7 days, followed by a 1-3 day afebrile and asymptomatic period and then the second or immune phase with recurrence of fever, meningitis and leptospiuria.

The incubation period is 7-14 days but ranges from 2 to 21 days. The onset is abrupt with chills, rigors, rapidly rising fever, and increasing prostration, severe headache and body pains. Fever is always present and high, rising to a maximum temperature of 38-40°C (100-105°F). The headache is severe and persistent, and resistant to the common analgesics. Prostration may be so marked that the patient has to stop work and frequently takes to bed. Body pains are severe and most marked in the lower limbs, especially in the calves and thighs. The pain and weakness make walking difficult; occasionally the gait may be shuffling. Severe pains in the back, neck, abdomen and
upper limbs are frequent. Anorexia, nausea and vomiting are frequent and there is either constipation or diarrhoea. When the abdominal symptoms are severe, the clinical picture may mimic gastro-enteritis, enteric fever, or acute abdominal emergencies such as cholecystitis, appendicitis and pancreatitis.

Epistaxis may occur during the early stage. Chest pain, dry cough or slightly blood-stained sputum may occur. Frank haemoptysis is rare but may be massive. Sore throat is frequent during the septicemic stage. Mental symptoms of restlessness, confusion, delirium, and hallucinations and occasionally psychotic behaviour may be prominent features in some patients. Signs of meningeal irritation and photophobia may be present.

The most characteristic findings on examination are conjunctival suffusion and severe myalgia. A transient macular, maculopapular, erythematous, purpuric or urticarial rash may occur, usually on the trunk but it may be localized on the upper limbs or on the shins. A transient palatal enanthem may be observed. The pharynx may be injected but without exudates and without cervical lymphadenopathy. Generalized lymphadenopathy, hepatomegaly and splenomegaly are not common features and may be present in up to 10% of cases. Tachycardia is the usual finding but relative bradycardia and arrhythmias may be present in some patients. Rarely parotitis or arthritis may be present, and in occasional cases the sclera may show slight icterus.

The septicemic phase subsides after 4-7 days. The temperature then becomes normal and the patient feels well. The second or immune phase is short, and at its peak the fever goes down and is of short duration. Severe headache may suggest meningeal involvement. This diphasic course may not be seen in all patients.

1.2.2 SEVERE ILLNESS WITH HEPATORENAL FAILURE

In some patients the septicemic illness, instead of subsiding, progresses to a severe icteric illness with renal failure. The fever may fall slightly but the diphasic course is rarely seen. Jaundice appears between the fourth and sixth days and deepens. Oliguria occurs in the second week but may occur as early as the fourth day of the illness. The longer the anuric period and deeper the jaundice, the worse the outcome. Anorexia and vomiting worsen, and hiccup may occur. Meningeal symptoms are frequent but overshadowed by hepatic and renal features. Confusion, restlessness, hallucinations, delusions and convulsions may occur. Severe bleeding and cardiac and pulmonary complications are frequent. Moderate anaemia is invariably. Towards the end of the second week the patient is deeply jaundiced, uraeic, haemorrhagic, and becomes comatose. Death occurs at this time or early in the third week from renal failure. Occasionally sudden death may occur from arrhythmias, cardiac failure or adrenal haemorrhage. Massive bleeding from the alimentary or respiratory tract may also end fatally. Death is rarely due to hepatic failure, but is virtually limited to icteric cases and in untreated severe cases the mortality rate may be as high as 15-60%.

In those who are not severely ill, recovery takes place in the second or third weeks. Diuresis occurs and the blood urea level falls gradually. The fever subsides, the general condition improves, and appetite returns. The jaundice takes several weeks to clear and convalescence is prolonged. The long-term outlook is good and there are no late complications other than uveitis.

1.2.3 MENINGITIS

In some cases - this happens relatively frequently where other mild presentations are not diagnosed - the leptospiral infection may present as meningitis with headache, fever, photophobia and vomiting with signs of meningeal irritation (i.e., neck stiffness, and Kernig’s and Brudzinski’s signs). The cerebrospinal fluid shows lymphocytic pleocytosis, raised protein (1.0-2.0 g/l), and normal sugar; the lymphocytic cells are in the range 0.01-1.0 x 10⁹/l (10-1000/ mm³).

Convulsions, focal neurological signs, myelitis, polynuropathy and encephalitis are rare. Prognosis in the meningitic illness is excellent. Very rarely death may occur from encephalitis.

In leptospiral meningitis, some distinctive features (such as myalgia, conjunctival suffusion and evidence of bleeding) are frequently present, if sought carefully.
1.3 SPECIAL CLINICAL CRITERIA AND GUIDES

The possibility of leptospirosis should be considered in any febrile illness of abrupt onset and in the presence of any or all of the following - marked prostration, myalgia, conjunctival suffusion and a bleeding diathesis.

The distinctive features of leptospirosis are the abrupt onset, severity of the symptoms, and the characteristic findings. The headache is severe, constant and generalized; it may be localized to the frontal, temporal or occipital regions and may be throbbing. The fever is high, spiking or persistent throughout the septicemic phase, with maximum temperatures reaching 41°C (106°F). Prostration is so marked that walking is difficult, and body pains are also severe. Blood pressure is usually normal unless there is dehydration, when it may fall slightly. Other frequent symptoms are photophobia, sore throat, abdominal symptoms, palatal enanthem, and exanthem.

The most characteristic clinical findings are conjunctival suffusion and myalgia.

Conjunctival suffusion occurs in the first 3 days and lasts from one day to more than a week. It is bilateral, most marked on the palpebral portion, and usually associated with unilateral or bilateral conjunctival haemorrhage. There is no inflammatory exudate and true conjunctivitis does not occur.

Myalgia is severe. Examining or even touching the muscle causes intense pain. It is most commonly observed in the lower limbs, especially the calves.

Haemorrhages occur, especially in severe and icteric infections, where there is also evidence of bleeding tendencies such as petechiae, purpuric spots, conjunctival haemorrhages and bloodstained sputum. Sometimes no more than a few purpuric spots are seen on the thorax, abdomen and upper limbs. There is increased capillary fragility measured by the tourniquet (Hess) test (see section C 1.1.2, p. 102). The bleeding, clotting and prothrombin times are usually normal.

A variety of skin rashes may occur, including erythematous, macular, maculopapular and urticarial. The skin lesions may be generalized or localized to the trunk, the shins or other areas. The skin rashes are transient but occasionally may persist for a week or longer.

Jaundice rarely causes any problem in clinical recognition. When slight, it is best seen in the sclera, which should be examined carefully and in the daylight. Jaundice occurs between the fourth and sixth days of the illness, but may occur as early as the second day.

1.3.1 AUTOPSY APPEARANCES

Patients seldom die unless they have had a severe type of infection, usually then dying from renal failure. Autopsy should be carried out soon after death because the leptospires are rapidly lysed with post-mortem contamination of the tissues. The most characteristic findings are the deep bile-staining of the tissues and widespread haemorrhages in the skin, mucous membranes and internal organs and tissues. The kidneys are slightly enlarged, bile-stained, with haemorrhages in the capsule, and the renal pelvis may be filled with blood. Bleeding in the lungs, alimentary tract, bronchial tree, pleura, pericardium and peritoneum is a constant feature. There may be unilateral or bilateral adrenal haemorrhage. The liver may be normal or enlarged, and frequently there are subcapsular haemorrhages.

1.4 SPECIAL OCCUPATIONAL AND GEOGRAPHICAL CRITERIA AND GUIDES

People at high risk of leptospirosis are mainly those whose occupations or way of life bring them close to animals. Thus those most exposed to risk are agricultural and livestock farmers; workers in rice fields, sugarcane fields, sewers and mines; canal workers; those involved in ditching, and road building and forestry; and abattoir workers, meat and animal handlers, veterinarians, and meat and livestock inspectors.
These occupationally exposed and predominantly rural or abattoir workers are usually young adult males. Most acquire the infection during or shortly after the warm rainy season. The risk for urban and city dwellers is increased when they enter the rural areas and the habitats of reservoir hosts during recreational activities, such as swimming, canoeing or fishing in fresh water streams, rivers, ponds and lakes, as well as during picnics, camping, hiking and hunting. Recreational exposures are seasonal and may be a common source of outbreaks of leptospirosis.

Infection may be acquired in the home from family pets, especially dogs. The exposure is constant and the family members most commonly affected are those with most contact with the pets, i.e., children and housewives. It should be realized that immunization of dogs prevents clinical disease but gives no protection against the renal carrier state.

Others at risk are those involved in diagnostic work such as veterinarians, soil scientists, and laboratory workers. Rarely exposure may occur by accident, e.g., by falling into sewers, rivers, ponds, or canals.

The epidemiological history should include the details of the occupation, recreational activities in the previous three weeks, pet animals at home, and any accidental exposures from mishaps.

Leptospirosis requires a wet environment for transmission. Conditions are ideal in the warm wet climates of some tropical regions where there is much vegetation, rainfall and wildlife, and where rice cultivation and other kinds of farming favour contact with water which may be contaminated with infected urine. A variety of carrier animals and of serovars are found to infect man in these conditions, the result often being severe disease (see section A 3.3.3, p. 25). In temperate zones there is a strong seasonal incidence related to rainfall, microclimate, nesting season for rodents and reproductive season for farm animals, and seasonal rural activities such as calving, milking and cropping.

1.5 COMPLICATIONS

1.5.1 KIDNEYS

Renal involvement is the most serious complication and is the commonest cause of death. The kidney manifestations range from abnormal urinalyses to fatal renal failure. Proteinuria is the most frequent abnormality noted in nearly all patients at some stage of the illness. Pyuria, haematuria, and hyaline and granular casts are often present. Red cell casts do not occur.

Oliguria or anuria may occur, usually in the second week but sometimes as early as the fourth day of the illness. It may be shortlived (lasting a day) or prolonged for up to 2 weeks. Most patients with renal failure also have significant hepatic involvement. With prolonged oliguria, the renal excretory functions are impaired, metabolic wastes accumulate, uraemic symptoms occur and worsen in already severely ill patients. The blood urea may exceed 70 mmol/l (400 mg/dl), and serum creatinine 1.32 mmol/l (15mg/dl). Death occurs towards the end of the second week or early in the third week. Sometimes renal failure may occur when there is a normal urine volume (non-oliguric renal failure).

The onset of diuresis is a good sign indicating the beginning of the recovery of the renal damage. Urinary abnormalities do not necessarily indicate the severity of the renal damage; severe and fatal renal failure may occur with minimal urinary abnormality.

Renal failure follows from acute tubular necrosis as a result of renal ischaemia, possibly because of a direct toxic effect of the leptospires. Renal failure in leptospirosis is reversible; it does not lead to chronic renal failure, and in survivors the long term prognosis is excellent.
1.5.2 EYES

 Conjunctival suffusion is a constant feature of the septicaemic phase and is usually associated with photophobia and conjunctival haemorrhage. It subsides within a week without any complications and no specific treatment is required.

 More important, but less common, is the late complication of anterior uveal tract inflammation which presents clinically as iritis, iridocyclitis and rarely as chorioretinitis. The main presenting complaints are ocular symptoms developing as early as the second week. The symptoms may be delayed for up to a year but are most frequent in the first 6 months. Uveitis may be unilateral or bilateral and the course is variable (i.e., an acute benign episode, recurrent episodes, or a chronic process). The ultimate prognosis is good but chronic uveitis may cause blindness from cataract formation and hypopyon in the anterior chamber. Uveitis may occur even after mild leptospiral infection.

1.5.3 LIVER

 Jaundice is the most important clinical indication of the severity of the illness. Jaundice occurs between the fourth and sixth days but may occur as early as the second day or as late as the ninth day, and deepens rapidly, reaching a peak within a week. The liver is often enlarged and is tender.

 Jaundice is mainly due to hepatocellular damage. However, hepatocellular necrosis is usually mild and additional factors in the pathogenesis of jaundice include an increased bilirubin load from absorption of blood from tissue haemorrhage and possibly intravascular haemolysis, intrahepatic cholestasis and decreased bilirubin excretion. Even in cases where treatment is begun in the initial leptospiraemia phase, it is not unusual to find elevated serum AST, GGT and ALT levels.

 Death is rarely due to hepatic failure and there is complete recovery of the liver damage Most of the severe renal, cardiac, pulmonary and bleeding complications occur in icteric patients.

1.5.4 OTHER COMPLICATIONS

 Cardiac complications are frequent in severe leptospirosis. They are usually mild and are observed as electrocardiographic abnormalities ranging from low voltage tracing, non-specific ST and T wave changes, conduction defects, and arrhythmias. Atrial fibrillation is the most common of the arrhythmias. Rare but more severe manifestations are cardiac dilatation, cardiac failure, and severe arrhythmias from haemorrhagic myocarditis. Sudden death may occur from cardiac failure or arrhythmias. All cardiac abnormalities revert to normal within 2-3 weeks.

 Pulmonary. Severe haemorrhagic pneumonitis may occur, usually in the second week but occasionally as early as 24-48 h after the onset, and often suddenly with haemoptysis, chest pain, respiratory distress and cyanosis. Massive haemoptysis may cause asphyxiation. Radiological abnormalities occur more frequently in those with haemoptysis and range from a single ill-defined opacity, through multiple areas of infiltration, to a large area of consolidation. These clear up within two weeks without any residual damage.

 Bleeding. Bleeding is a constant feature of leptospirosis and is believed to be mainly due to vascular damage. It is usually mild in anicteric cases but more common and severe in icteric cases. Bleeding may occur from the respiratory, alimentary, renal and genital tracts, and occasionally into the subarachnoid space and adrenal glands. Death may occur from massive bleeding, usually gastrointestinal or into internal organs.

 Leptospirosis during pregnancy. The hazards of leptospirosis during pregnancy include intra-uterine infection with fetal death and abortion, stillbirths, premature labour, and signs of congenital leptospirosis within a week or two of the delivery. Leptospiras may be secreted in the milk of lactating mothers who, during the septicaemic phase, should be regarded as potentially infective for breast-fed infants.
Psychiatric changes. Psychotic changes, irritability and behavioural disturbances, and depressive illness have been reported to follow mild infections in up to 15% of convalescents.

1.6 DIFFERENTIAL DIAGNOSIS

1.6.1 CLINICAL FACTORS

Leptospirosis, with its varied manifestations, may mimic a large number of disease processes. Most frequently it is misdiagnosed as influenza, viral illness, pyrexia of unknown origin, aseptic meningitis, or viral hepatitis.

In any febrile illness, leptospirosis should be suspected when the initial clinical diagnosis is influenza, viral illness, rickettsioses (Q fever, typhus), enteric fever, brucellosis, relapsing fever, toxoplasmosis, malaria, pyelonephritis and any other locally prevalent febrile illness. With a positive epidemiological history and distinctive signs and symptoms, most of the above conditions can be differentiated clinically, especially once the clinician has seen a number of laboratory confirmed cases of leptospirosis.

In those presenting with meningitic illness, leptospirosis has to be differentiated from bacterial and viral meningitides, encephalitis and non-paralytic poliomyelitis. In bacterial meningitis, the onset may be gradual, the patients are more toxic, and the diagnosis is readily confirmed by spinal fluid examination. Leptospiral meningitis is clinically indistinguishable from viral meningitis. However, the possibility should be considered in cases resembling viral meningitis when there is marked prostration, myalgia, conjunctival suffusion, evidence of bleeding, or a history of contact with domestic or wild animals. The diagnosis is confirmed by serological tests.

Severe icteric leptospirosis may be confused with other febrile icteric illnesses such as viral hepatitis, yellow fever, septicaemia with jaundice, and malaria. Viral hepatitis is the most likely condition to cause difficulty. In leptospirosis the onset is abrupt, severe headache, myalgia and conjunctival suffusion are constant features, and proteinuria is common, whereas in viral hepatitis the onset is gradual, headache and myalgia are mild, and proteinuria and conjunctival suffusion are absent. Also haemorrhages and chest symptoms occur more frequently in the former. Fever, a universal feature of leptospirosis, is rarely present in viral hepatitis after a day or two from the onset of jaundice.

Certain organ systems are commonly involved in leptospirosis. When one of these is predominant, the illness may present as pneumonia, gastroenteritis, acute abdominal emergency, acute nephritis, or a bleeding disorder. Once the possibility of leptospirosis is considered, then the correct diagnosis will rarely pose any difficulty because some distinctive features will usually be present in the history and physical findings.

1.6.2 LABORATORY TESTS

Laboratory tests are rarely helpful in early diagnosis, which is clinical, and based on the epidemiological history, characteristic signs and symptoms, and is supported by the presence of leucocytosis, a raised ESR, and proteinuria.

In all suspected cases, the diagnosis should be confirmed by laboratory tests, by sending appropriate specimens (see below) at the right time and under proper conditions. Leptospires may be present in the blood and the CSF in the first 7-10 days of the illness, and in the urine from the tenth to fourteenth day for a period of up to a month or longer. Agglutinating antibodies appear in the blood towards the end of the first week, reach a peak in the third and fourth weeks, and then gradually decline with low levels persisting for an indefinite period. Occasionally antibody response is delayed for some weeks.

A screening test (see sections A 4.2.1, p. 28; A 4.2.2, p. 29; B 3.4.1.2, p. 79; and C 3.1.3, p. 107) is useful in the first instance, since the important diagnostic question for the individual patient is whether or not he has leptospirosis. A serovar nonspecific test helps to establish this quickly. A
positive result can be confirmed later by specific tests to ascertain the likely infecting serovar, but see section B 3.4.1.1 (3) (b), p. 78. This latter information is necessary for prognostic and epidemiological reasons.

1.6.2.1 Diagnostic tests. (i) Blood cultures (sections B 3.2.1, p. 68 and C 3.2.2, p. 125). Blood cultures should be done in the first 10 days of the illness and before antibiotics are given. Venous blood is collected by aseptic technique and inoculated at the bedside. Small inocula of 1, 2, 5 and 10 drops of blood are inoculated into 4 tubes, each containing 3–10 ml of suitable semisolid medium or TA medium. Large inocula will inhibit the growth of leptospires. If bedside inoculation is not possible, the blood is mixed with anticoagulant and sent to the laboratory immediately (see section C 3.2.2.1, p. 126).

(ii) Cerebrospinal fluid (section B 3.2.1, p. 68). Cerebrospinal fluid, if collected in the first 10 days of the illness, should be cultured; 0.5 ml of CSF is inoculated in 5 ml of semisolid medium.

(iii) Urine. Urine should be sent for culture and animal inoculation after 10–14 days of the illness. Fresh midstream urine is collected and inoculated immediately. Ideally, the urine should have been made alkaline by medication, but if this was not done, it may be collected in sterile buffered saline. One drop of undiluted urine is inoculated in the first tube containing 5 ml of semisolid medium. Five more tubes are similarly inoculated with urine in increasing 10-fold dilutions. If possible, the procedure should be carried out at the bedside, or the patient should be sent to the laboratory. As leptospires are shed intermittently in the urine, the urine culture may have to be repeated.

(iv) Blood, CSF and urine for dark-field microscopy should be sent to the laboratory immediately, at the same time as the above specimens are collected for culture. The value of this examination is questionable but see section B 3.1, p. 67.

(v) Blood for serology. It is always necessary to send paired sera. The first specimen is sent when the patient is first seen. It is essential to record on the specimen the day of the illness on which it was collected. The second specimen should be collected 14–21 days after the first. If the second specimen is negative or not diagnostic, then a third specimen is collected about 2–3 weeks after the second specimen (section B 3.4.1.1 (3) (b), p. 78).

1.6.2.2 Other tests. (1) Liver function tests are usually normal in anicteric illness. In icteric cases the serum bilirubin is raised, often markedly, the greater fraction of which is direct-reacting. Other tests show mild and inconsistent abnormalities. Serum aminotransferases (transaminases) (AST, ALT) are either normal or raised 2–3 times normal. A raised serum bilirubin with virtually normal transaminases is highly suggestive and should immediately indicate the possibility of leptospirosis, which is further strengthened by finding a raised blood urea level. In viral hepatitis the serum aminotransferases (transaminases) are invariably raised.

(2) Full blood count and erythrocyte sedimentation rate. The level of haemoglobin is normal in anicteric cases unless there is bleeding. A moderate degree of anaemia is a constant feature of icteric cases and is aggravated by the bleeding diathesis. Slight leucocytosis (WBC 11–20 × 10^9/l [11 000–20 000/mm^3]) is the usual finding and may be marked (WBC up to 40 × 10^9/l [40 000/mm^3]) in severe icteric cases. Occasionally in icteric cases the total WBC may be normal, but even then neutrophilia (neutrophils, over 75%) is almost invariably. Leucocytosis is a very useful clue in differentiating leptospirosis from a viral illness or viral hepatitis, in which leucopenia is a common finding.

Platelets may be normal but in mild (as well as in severe) icteric cases, slight thrombocytopenia (80–150 × 10^9/l [80 000–150 000/mm^3]) is not infrequent and is occasionally severe with platelet counts dropping to 5 × 10^9/l [5000/mm^3]. The ESR is raised, often markedly.

(3) Blood urea and renal function tests. Renal failure should be assessed in all patients. Blood urea may be slightly raised, even in the absence of other signs of renal involvement. Markedly raised levels indicate renal failure. Values may rise to above 70 mmol/l (400 mg/dl). Blood urea may however be raised owing to dehydration. The serum creatinine gives a more reliable indication of renal function. For clinical purposes, serial estimations of blood urea,
Table 3. LIKELIHOOD OF THE DIAGNOSIS OF LEPTOSPIROSIS IN MAN

This check-list is designed for those who deal directly with the patient. To use the list, note the main clinical features listed, mark the box "Yes" or "No", and write the appropriate score in the right-hand column.

A presumptive diagnosis of leptospirosis may be made if:

<table>
<thead>
<tr>
<th>Part A, or Parts A and B score</th>
<th>26 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A, B and C total</td>
<td>25 or more</td>
</tr>
</tbody>
</table>

A score between 20 and 25 suggests leptospirosis as a possible but unconfirmed diagnosis.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Has the patient:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache of sudden onset?</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Fever?</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>If &quot;Yes&quot;, is the temperature 39°C or more?</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Conjunctival suffusion (bilateral)?*</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Meningism?*</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Muscle pains (especially calf muscles)?*</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>*Are all 3 features (conjunctival suffusion, muscle pains and meningism) present together?</td>
<td>Yes</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Jaundice?</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Albuminuria or nitrogen retention?</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
</tbody>
</table>

Total score for Part A
Score from Part A ..........................................................

B. Epidemiological factors:

Has there been contact with animals at home, work, leisure, or in travel, or contact with known (or possibly) contaminated water?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

C. Bacteriological laboratory findings

Isolation of leptospires in culture - diagnosis certain

Positive serology - leptospirosis endemic:

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>single positive, low titre</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>single positive, high titre</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>paired sera, rising titre</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Positive serology - leptospirosis not endemic:

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>single positive, low titre</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>single positive, high titre</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>paired sera, rising titre</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Total:
serum creatinine, and electrolytes are necessary for reviewing the progress and the manage-
ment of renal failure. Usually creatinine clearance and other renal function tests are not
necessary.

(4) Urinalysis. (i) Albumin is present in the urine of most patients, both anicteric and
icteric, at some stage of the illness. It is slight (less than 1 g/day), may be transient, and
is frequently associated with pyuria and microscopic haematuria.

(ii) Bile pigments, both bilirubin and urobilin, are always present in the urine of icteric
cases and occasionally in the absence of any obvious clinical jaundice.

(iii) Casts (mainly hyaline, granular or cellular) are frequently present in the urine; red cell
casts are not a feature of leptospirosis.

(iv) Urine volume should be watched carefully. Oliguria (urine volume less than 500 ml/day)
may be due to dehydration, hypotension or acute tubular necrosis. Persistent oliguria indica-
tes kidney damage. The onset of diuresis is a good prognostic sign. Occasionally severe
renal failure may occur in the presence of a normal urine volume.

1.6.3 LIKELIHOOD OF DIAGNOSIS

The probability of making a diagnosis of leptospirosis depends on the clinical features, the labora-
tory findings, and the epidemiological situation. These factors have been scored (Table 3 p. 90)
so that they may be used to help assess the likelihood that the diagnosis is 'leptospirosis' in an
acutely ill patient. Table 3 may also be used as a checklist for clinical signs and investigations.

1.7 TREATMENT

The treatment is the same for infections with all serovars of leptospires and includes chemother-
apy, as well as symptomatic and supportive therapy.

1.7.1 CHEMOTHERAPY

The main aim of the chemotherapy is to prevent complications and this is possible with early
treatment. Leptospires are sensitive to most antibiotics. Penicillin is the most effective when
given early, in the first 4-5 days of the illness, or before jaundice occurs. Large doses, 6-8 mega-
units of benzyl (crystalline) penicillin may be given in divided doses, preferably iv, for 5-7 days.
Another successful regimen recommended is 4-5 megaunits daily of equal parts each of crystalline
and procaine penicillins im, dropping to half the dose after the fever subsides, usually for a total
of 5-6 days. Procaine penicillin, 1.3 megaunits im should be continued for 2 days after the
albuminuria ceases. Penicillin will cut short the course of the illness and prevent complications.
The fever subsides within 24-36 h and the biphasic course is rarely seen. There may be a slight
temporary exacerbation of symptoms with penicillin (Hersheimer type reaction) but it is not frequent.
Persons allergic to penicillin may be treated with tetracyclines or erythromycin, but both are less
effective than penicillin. Tetracyclines are contra-indicated if there is evidence of renal failure.

The doses of tetracycline recommended are 500 mg immediately, followed by 250 mg 8-hourly im
or iv for 24 h, and then 250-500 mg 6-hourly orally for 6 days. Erythromycin is given in doses of
250 mg 6-hourly for 5 days.

1.7.2 OTHER TREATMENT

1.7.2.1 Immunotherapy. This treatment is not recommended and is not generally available. It is
not a substitute for chemotherapy. (Specific antileptospiral gamma-globulin, given in addition to
chemotherapy, appears to be available only in the USSR where some authors advocate its use in a
total dose of 10 ml given im once a day, over 3 days, after preliminary desensitization. This
treatment should be given as soon as possible. It is not contra-indicated by hepatitis or renal insuf-
iciency, but hypersensitivity to the serum limits its usefulness. Furthermore it is necessary to
know exactly the infecting serovar at the time of the acute illness and contemplated administration of immunotherapy. This information can only be surmised on epidemiological grounds. Administration of antileptospiral specific antiserum suppresses the formation of agglutinins, both group-specific and cross-reactive).

1.7.2.2 Symptomatic and supportive. Pain is treated with analgesics, but in many patients more potent analgesics (pethidine or morphine) may have to be used. Headache is usually relieved with potent analgesics but if still troublesome, lumbar puncture will give prompt relief. Restlessness and anxiety are controlled with sedatives, fever with antipyretics, and anaemia with blood transfusion. Intravenous diazepam is very effective in controlling convulsions. Fluid and electrolyte disturbances from vomiting and diarrhoea are frequent and should be treated vigorously. Patients with hepatic and renal failure need intensive supportive therapy.

1.8 MANAGEMENT

1.8.1 MILD ILLNESS

The management of the acutely ill patient is like that of any acute fever. The patient should be put to bed and will generally benefit if the room is darkened. Steps are then taken to supply adequate hydration, care being taken, if there is oliguria, not to overload the circulation. A routine administration of analgesic, anti-emetics and sedatives will probably be required. The fever, particularly after the loading dose of penicillin, may require tepid sponging; at this stage, delirium is not uncommon and the relatives should be warned of this possibility. The patient must have absolute rest from work for at least one and preferably two weeks. Early resumption of work appears to lead to prolonged aches, headaches, general debility, and occasional relapse. It has also been noted that sometimes the psychiatric sequelae are more common in those who return to work too soon. When the patient resumes work, he is warned to stop work at the first sign of tiredness or headache and to start again in a graduated manner. Providing he has had adequate therapy and followed these instructions, recovery is generally complete, although in some patients it may be wiser to administer a tricyclic compound such as amitryptiline (25 mg to 50 mg) at bedtime. Frank psychosis has occurred and been reported to be of a schizoid nature but this is uncommon.

1.8.2 SEVERE ILLNESS

The management of severe icteric leptospirosis is an exercise in intensive care. The baseline investigations should include blood count, serum electrolytes, liver function tests, serum transaminases, urinalysis, and bleeding, clotting and prothrombin times; also chest x-ray and electrocardiogram, and the patient's blood should be grouped. The blood pressure, temperature, pulse and respiratory rates are recorded at regular intervals (hourly, then 4-hourly) and the urine volume should be carefully measured.

Any fluid deficit and hypotension are treated promptly with isotonic saline, plasma or blood. Oliguria may be due to dehydration, hypotension, or acute tubular necrosis. In the former two, diuresis invariably occurs after correction of the dehydration and hypotension alone or when combined with the potent diuretic, furosemide, in doses up to 500 mg. Persistent oliguria is due to acute tubular necrosis. In some cases, renal failure may occur without oliguria. The progress of the liver and the kidney damage is monitored by clinical signs and by daily serum bilirubin, blood urea, and serum electrolyte estimations.

Hepatic failure is treated along the standard lines, i.e., elimination of nitrogen-containing materials from the gut; protein-free diet; Neomycin 1 g 4-hourly; correction and maintenance of fluid and electrolyte balance and avoiding hepatosuppressive drugs. Mild renal failure is treated conservatively with fluid restriction, administering enough only to replace the losses, and with a high carbohydrate and protein-free diet.
Severe and worsening renal failure is treated with dialysis. It is advisable to institute dialysis early, as soon as the blood urea reaches 35 mmol/l (200 mg/dl). Peritoneal dialysis is preferred to haemodialysis, as it is simpler, quicker to institute, does not require complicated equipment and highly trained personnel, and avoids the dangers of bleeding from the use of heparin in an already haemorrhagic patient. Renal function has to be supported with repeated dialysis until recovery takes place.

Bleeding is treated with blood transfusion. Occasionally thrombocytopenia is severe and symptomatic and is treated with a transfusion of fresh blood or preferably platelets. A short course of steroids is very effective.

In these severely ill patients, the cardiac and pulmonary status should be carefully watched. Mild arrhythmias are usually temporary and subside without treatment. In general, cardiotonic drugs are avoided as they may precipitate fatal arrhythmias. Respiratory complications are treated symptomatically.

Besides the above measures, the successful outcome depends on the standard of nursing care. With adequate support of renal function, death from renal failure is preventable and the overall mortality of 15-40% formerly observed in these severely ill patients is reduced to about 5%. All centres dealing with severe leptospirosis should have peritoneal dialysis facilities.

It is important - for epidemiological, preventive, surveillance and public health reasons, and in some countries for reasons connected with workers' compensation - to ensure that the patient has follow-up serology carried out at the times indicated above (section B 1.6.2.1. (ii), p. 49) in order to ensure that his legal rights are protected, because the compensation authorities are reluctant to accept a clinical diagnosis, even when this is made by experienced practitioners. As outlined above, serology may be negative by reason of good treatment and it is therefore vital to have blood cultures available as well.

2. LEPTOSPIROSIS IN FARM AND DOMESTIC ANIMALS

Although a wide variety of leptospiral serovars (serotypes) have been isolated from domestic animals, relatively few are repeatedly associated with each host species. The serovars most frequently associated with cattle are pomona and hardjo, (grippotyphosa in Eastern Europe); with swine, pomona and tarassovi; with sheep and goats, pomona and grippotyphosa; with horses, pomona, icterohaemorrhagiae and autumnalis; and with the dog, canicola and icterohaemorrhagiae. However, the leptospires endemic in regional wildlife may also influence the serovars that are present in domestic animals of that geographical region. In some countries, vaccination has apparently reduced the incidence of the homologous serovars, thereby allowing other regional serovars to increase in incidence.

2.1 CATTLE

2.1.1 GENERAL DESCRIPTION

The first isolations of leptospires from cattle were made in 1946 in the USSR and in Israel. The following serovars have been isolated from cattle, at first in the countries cited in parenthesis and then in other countries: grippotyphosa (USSR, Israel, 1946), pomona (USA, 1948), icterohaemorrhagiae (Argentina, 1947), hebdomadis, kremastos, autumnalis, australis (Japan, 1953, 1955, 1960), canicola (Israel, 1955), hardjo (USA, 1960), sejroe (Belgium, 1952), tarassovi (USSR, 1958) and bataviae (China, 1960). The prevalence of bovine leptospirosis is now well known to be worldwide.

Although pomona and hardjo are most frequently reported in cattle, grippotyphosa and other serovars, especially from the hebdomadis serogroup have been detected with increasing frequency in recent years. The incidence of bovine leptospirosis has been most often recognized in large herds in the warmer and wet climatic regions where leptospirosis have an increased opportunity of survival.
2.1.2 RECOGNITION OF INFECTION

The disease may either run a rapid course, or develop gradually, or remain clinically inapparent. In acute and subacute cases there is usually fever (1-2.5°C above normal), lasting up to 4-5 days, with malaise, depression, loss of appetite, general weakness, conjunctivitis, anaemia and diarrhoea at the onset. In more severe cases, haemoglobinuria, which is due to haemolytic anaemia, is often the first noticeable clinical sign. The urine becomes dark red or almost black. Jaundice and encephalitis may be observed. The mortality rate is variable, according to the age of the beast and the serovars involved. Death occurs after several days, as a result of severe renal degeneration with accompanying hepatic necrosis.

During acute infections in lactating cattle, the milk produced is at first yellow and clotted; this is followed by agalactia which generally persists from a few days to 2 weeks. Lactation in most cows returns to a normal level in 2-3 weeks, but some fail to attain full production during the affected lactation period.

Encephalitis probably occurs in many infections because leptospires can constantly be isolated from brain tissue; but clinical signs of encephalitis are observed infrequently.

Clinical evidence of nephritis may be evident in both the acute and chronic forms of the disease. In acute leptospirosis it is recognized by haemoglobinuria or haematuria. Decreased specific gravity of the urine is the primary evidence of nephritis in chronic leptospirosis.

2.1.3 COMPLICATIONS

2.1.3.1 Abortion. The most common signs of bovine leptospirosis are abortions and stillbirths, which occur 1-3 weeks after the onset of clinical signs. Interruption of gestation is more frequent in infections originating during the last third of the gestation period, at a time when the fetal attachments are less secure. Infected fetuses can produce an antibody response detectable by the microscopic agglutination test.

2.1.3.2 Congenital Infection. Infected newborn calves are often weak and succumb to either liver and kidney degenerations or to secondary infections. As the disease progresses, the infection often becomes localized in the kidney, resulting in subacute and chronic lesions which are evident grossly at post-mortem as white foci on the surface.

2.1.3.3 Infertility. Infertility has frequently been associated with the less severe infections caused by members of the hebdomadis serogroup. Although uterine lesions are not extensive, annual vaccination of the entire herd with a homologous serovar vaccine (bacterin) reduces the infertility problem.

2.1.4 DIAGNOSIS

2.1.4.1 Clinical diagnosis. The main symptoms and signs, described above (see section B 2.1.2), are useful for the clinical diagnosis of bovine leptospirosis. The disease, however, cannot be diagnosed solely by clinical observation.

Post-mortem findings. The initial lesion occurs in the liver, usually in the form of scattered necrosis of hepatic cells, bile retention, and infiltration with lymphocytes. Generally, the liver lesions are not extensive, but in fatal infections they may be diffuse with extensive necrosis together with considerable bile retention caused by occlusion of the bile ducts with cellular debris.

Generally the most extensive changes in acute leptospirosis are the kidney lesions, with major changes occurring in the glomeruli and proximal convoluted tubules. The lesions consist of cell necrosis, and petechial and ecchymotic haemorrhages in the cortical tissues.
Microscopic lesions in the kidneys in chronic cases include atrophy of glomerular tufts, interstitial infiltration of lymphocytes, plasma cells and fibroblasts, casts of cellular debris, and dilatation and atrophy of some tubules.

Leptospires actively invade the uterus and fetus during the acute infection. The lesions associated with abortions and stillbirths consist predominantly of oedema of the placental tissue with some necrosis of the cotyledon tissues. Retained placentas occur in cattle infected late in the gestation period. Infertility has been associated with chronic leptospiral infections. Uncomplicated infections do not produce inflammatory reactions in the mammary gland, although agalactia and abnormal milk are common clinical findings. Owing to lack of appropriate pathological studies, adequate information is not currently available concerning the histological changes.

Signs of acute pneumonic changes occur in experimental infections but are usually not detected in natural infections. The lesions consist of haemorrhages and some inflammatory cell infiltrations. Although lung lesions are usually not extensive, the activation of latent bacterial infections or secondary infection can initiate more severe lesions.

2.1.4.2 Laboratory Diagnosis. Leptospires are present in the blood and other tissues (e.g., liver, spleen, kidney), and can be isolated by culture or animal inoculation procedures (see sections B 3.2, p. 68 and C 1.2.6, p. 104). One or two drops of blood, obtained at the febrile stage, are inoculated into 5-6 ml of liquid or semisolid medium in a screw-capped tube. It is advisable to take specimens more than once during the early febrile stage. The inoculated tubes are sent to the diagnostic laboratory. One or 2 ml of blood from the animal may also be inoculated intraperitoneally into guinea pigs or hamsters (see section B 3.2.2, p. 69); if the sample is infected, these animals will have fever for several days after the inoculation. The blood of the guinea pigs or hamsters is then cultured similarly. Kidney (cortex) and liver tissue may also be cultured (see section B 3.2.2, p. 69). The tissues must not be frozen (below 0°C) before examination although they may be refrigerated (1-4°C) for a few hours. Each of the small pieces (1-2 mm²) of the tissues is cultured into 5-6 ml liquid or semisolid medium and the cultures are sent to the laboratory for examination. Leptospires are seldom isolated from either aborted or stillborn calves because of autolytic changes. However, the serum from aborted fetuses or stillborn calves may contain agglutinins which are diagnostically significant, if present.

Serological testing (section B 3.4, p. 76) is the laboratory procedure most frequently used to confirm the clinical diagnosis. Leptospiral antibodies appear in the serum within a few days of the onset of illness and persist for weeks or months and, in some cases, years. Demonstration of an agglutination response in serum dilutions of 1:100 or greater is indicative of exposure to leptospiral antigens.

In the absence of vaccination, MAT titres provide relatively reliable evidence of a current or previous leptospiral infection. A rising titre in paired serum samples indicates a current illness when acute and convalescent samples are tested. Generally, acute serum samples are not obtained because the illness is often not recognized prior to an abortion in cattle and swine. Titres in excess of 1:1000 in a number of animals in a herd usually are indicative of a current or previous infection. Titres of 1:100 in unvaccinated animals are also diagnostic. However, as individual animals vary in their antibody response, the presence of a high titre is not indicative of the severity of the disease. It is also known that some animals fail to respond with a titre of 1:100, even though leptospires can be isolated from their urine. Therefore, evaluation of a herd test provides a more reliable diagnostic procedure than individual testing.

The widespread use of current leptospiral bacterins, which can stimulate relatively high titres (1:100 to 1:1000) in some animals for periods of 1-2 months following administration, has increased the problems of serological diagnosis. It is important to consider the vaccination history in the interpretation of the serological results.

2.1.5 TREATMENT

Treatment with dihydrostreptomycin in a dose of 11 mg/kg (5 mg/lb) 12-hourly for 3 days, or 5 g twice daily for three successive days, may be effective in curing the acute disease and eliminating the carrier state.
2.1.6 MANAGEMENT

Proper herd management, including an immunization programme, is necessary for the control of leptospirosis in cattle. All cattle should be vaccinated during the last two-thirds of gestation. Cattle assembling for feed lots should be immunized with vaccines and held in isolation for 2 weeks before they are mixed with other cattle. When the disease is diagnosed during the early phase of an enzootic, prompt vaccination and/or treatment with two separate injections of 25 mg dihydrostreptomycin/kg body weight may be carried out on the entire herd.

2.1.7 PREVENTION OF SPREAD OF INFECTION

Sound animal husbandry practices may greatly help to prevent or minimize exposure (see section B 4.2.3, p. 93). The following routines are advised for prevention of the spread of infection: (1) Record daily the temperatures of all animals after the onset of outbreaks; segregate those animals with a temperature above 39.5°C and treat them with dihydrostreptomycin. (2) Decontaminate or remove all excreta, aborted fetuses and fetal membranes. (3) Provide a safe source of drinking water; the water supplies of clean herds must not be contaminated by the urine of infected animals. (4) Eliminate swampy areas where practicable. (5) Eliminate excessive crowding in the feeding, housing, and watering of animals. (6) Examine the serum of all animals at the onset of the outbreak and again one month later; isolate and quarantine the animals that have serologically converted during this period. (7) Make no additions to the herd for at least 6 months, and keep unaffected stock isolated from the infected herd for 6-9 months after the last case occurred. (8) Minimize or eliminate contact with other domestic animals, rodents and other wildlife. (9) In artificial insemination programmes, only semen derived from bulls proved to be free of infection should be used. (10) Early isolation of pregnant animals from the rest of the herd is recommended.

2.2 PIGS

2.2.1 GENERAL DESCRIPTION

Leptospirosis affects swine of all ages but is most frequently recognized through its interruption of pregnancy in the sow. From an epidemiological point of view, leptospirosis in pigs probably constitutes a greater and more important public health hazard than the disease in cattle. In many parts of the world, pigs are recognized as one of the most important mammals associated with leptospirosis in man and domestic animals. Pigs frequently are not obviously infected but shed leptospires in the urine in large quantities, for a relatively long period of up to a year following infection. The following serovars have been isolated from pigs, at first in the country cited in parenthesis and then in other countries: icterohaemorrhagiae (Netherlands, 1987), pomona (Australia, 1939), tarassovi (originally designated hyos; Argentina, Australia, 1945), canicola (Czechoslovakia, USA, Chile, Israel, 1956), sejroe (Czechoslovakia, 1956). Of these, pomona seems to be the most prevalent in pigs, but infection with tarassovi, grippotyphosa, canicola and icterohaemorrhagiae are common. Infection hazards among pigs are especially enhanced because of their habits of wallowing in swampy soil and mud holes, rooting, sniffing excreta and organic matter, and splashing urine in the presence of other animals.

2.2.2 RECOGNITION

Most serovars produce a similar disease pattern. Infected young pigs may show clinical symptoms and signs, which are fever (0.5-1.5°C above normal), anorexia, weakness, conjunctivitis, jaundice, haemoglobinuria, and central nervous disturbances (convulsions). Infection in non-pregnant pigs is usually benign or inapparent. Gross manifestations are jaundice in various organs, petechial haemorrhages (particularly in the cortex of the kidneys and lung) and focal necrosis of the liver. The lesions in the kidney increase in severity with the chronicity of the disease. Fetal membranes from aborted sows show only minor changes consisting of small haemorrhages and oedema. Initial awareness of leptospirosis in swine usually occurs as a result of abortion, or the birth of weak or dead pigs. The survivors may be sickly, fail to gain weight as expected, and continue to excrete leptospires, with or without other symptoms.
2.2.3 COMPLICATIONS

2.2.3.1 Abortion. In pregnant sows, infection may result in abortions, stillbirth and neonatal diseases after 14-30 days from the onset of the acute infection. During leptospirosis in the dam, the leptospirae migrate from the maternal blood, through the placental tissue to the fetus. The leptospirae can produce a systemic infection in the fetus resulting in the death of the fetus, or in a neonatal infection that persists after birth. The course of the disease is dependent on the stage of gestation at the onset of the infection and on the serovars involved. Infections occurring during the first third of gestation seldom interrupt fetal development. The most critical time is during the last third of the gestation period which is the period when abortion or stillbirth may occur. Leptospirae are present in many tissues and body fluids of the aborted fetus. Serovars tarassovi and canicola are known abortifacient agents in swine leptospirosis. Other serovars may also produce abortions.

2.2.4 DIAGNOSIS

2.2.4.1 Clinical Diagnosis. The symptomatology of porcine leptospirosis is not characteristic for this disease. Diagnosis is, therefore, contingent on the demonstration of the organisms or significant antibody levels in infected animals. At the time of abortion, leptospirae may be found in the fluids and tissues of the fetus.

Post-mortem findings. Stillborn piglets may show evidence of maceration if the deaths occur a week or more prior to birth, or they may appear to be normal if death is just before birth. The body fluids are often blood-stained and the tissues oedematous and jaundiced. Small necrotic areas may be present in the liver and the kidneys, or the tissues may appear grossly normal. Histological changes consist of focal necrosis in the liver and in the tubular epithelium of the kidneys.

In adult swine, white foci in the kidneys and oedema of the renal lymph nodes occur in leptospiral infections. Haemorrhages may be present in severe acute infections. Histological changes consist of glomerular degeneration, degeneration of tubular epithelium, and interstitial infiltrations with lymphocytes, plasma cells and macrophages. Proliferation of connective tissue and fibrosis occur in sub-acute and chronic infections. Hepatic lesions present in some of the pigs consist of focal degenerative changes. Meningoencephalitis has been observed in experimentally infected young pigs. The most extensive lesions have been observed in tissues of swine with chronic infections persisting for 4 weeks or more.

2.2.4.2 Laboratory Diagnosis. Leptospirae present in the blood and other tissues should be detected as described in section B 3, p. 67 and C 3.2.2, p. 125. In addition, leptospirae can be detected from aborted fetuses and fetal membranes (see section C 1.2.5, p. 104).

2.2.5 TREATMENT

A significant decrease in the number of abortions and neonatal deaths may be obtained by administering to a group of gestating sows and gilts a ration containing either 500 g oxytetracycline per tonne of feed for 14 days, commencing 1 month before farrowing; or 400 g chlortetraacycline per tonne of feed, for 10 days, commencing 2 months before farrowing.

The administration of dihydrostreptomycin at a dosage of 25 mg/kg (12 mg/lb) of body weight, daily for 3 days, early in an outbreak has effectively reduced the signs and prevented abortion losses. Administration of a combination of antibiotic therapy and vaccination with a bacterin of the appropriate leptospiral serovar(s) can be an effective procedure if a herd is treated early in an outbreak.
2.2.6 MANAGEMENT

Three-month old piglets and pregnant sows and gilts may be protected by the administration of vaccines. Breeding swine should be immunized with vaccines at 6-monthly intervals (see section B 4.2.5.2, p. 95).

2.2.7 PREVENTION OF SPREAD OF INFECTION

For general sanitary measures, see section B 4.2.3, p. 93.

2.3 DOGS

2.3.1 GENERAL DESCRIPTION

Canine leptospirosis has been reported in almost every country where it has been sought. It is more common in males than females owing to their behaviour. Infected dogs may continue to excrete leptospires in their urine for months or years after recovery from the acute infection. The most common serovar is canicola; but severe, even fatal illness, may result from icterohaemorrhagiae infections. Other serovars have also been isolated from dogs.

2.3.2 RECOGNITION

Two symptomatically different clinical manifestations of the disease have been described. In both, the first signs are usually fever, conjunctivitis, vomiting and slight weakness. They then develop as either the acute and subacute types due to canicola; or the peracute, icteric and subacute types due to icterohaemorrhagiae.

2.3.2.1 Infection with canicola. The acute form is the syndrome known as Stuttgart disease, characterized by acute vomiting, rapid dehydration and collapse, occasionally the passage of blood-stained faeces, and if the dog survives long enough, rapid necrosis and sloughing of the buccal mucosa and tongue. The mortality rate is high, death occurring in 36 hours to 4 days. The subacute form, which is commoner in canicola infection, is characterized by symptoms of nephritis; a rise in temperature, followed by depression, vomiting, blood-stained faeces, albuminuria, palpable swelling of kidneys, collapse and death. The mortality rate varies.

2.3.2.2 Infection with icterohaemorrhagiae. The peracute form is characterized by a sudden onset with a rise in temperature, severe depression, shivering and sometimes vomiting. The temperature then begins to fall and haemorrhages develop in and around the mouth, lips and conjunctiva. Collapse and death may occur within a few hours or days after the onset of symptoms. The icteric form is characterized by similar initial symptoms, followed by a severe jaundice. Severe cases terminate in death after a few days. Less severe cases may not die until 7-10 days after the onset of symptoms, and a few may survive. The subacute form is similar to those of subacute infection by canicola.

2.3.3 COMPLICATIONS

Chronic nephritis may result from an initial renal infection in leptospirosis.

2.3.4 DIAGNOSIS

2.3.4.1 Clinical Diagnosis. Peracute and icteric forms of icterohaemorrhagiae infection, and the acute form of canicola infection may be diagnosed from the symptoms, in view of the characteristic severity and features. The subacute disease cannot be diagnosed as leptospirosis on clinical
grounds alone. Laboratory diagnosis is recommended (see section B 3.1, p. 67 and B 3.2, p. 68), including culture (section B 3.2.1, p. 68), immunofluorescence (section B 3.4.2, p. 79) and serology (section B 3.4.1, p. 76).

Post-mortem findings. The acute stage of the disease is dominated by severe dehydration and icterus, with many petechiae on the pleura, peritoneum, nasal and oral mucosa, and kidneys. Lymphnodes and spleen are usually grossly enlarged and may contain areas of oedema and haemorrhage; haemorrhages and areas of oedema may be seen in other organs including the lung. Dogs that survive or escape the acute form of the disease may show dehydration and emaciation, and give a strong uraemic odour. The renal lesions are most significant; the kidneys are grossly enlarged, the capsule is tense and white or greyish, sometimes with haemorrhages. In chronic leptospirosis, the kidneys show the most important lesions. Both glomerular and interstitial nephritis are often present, with loss of glomeruli and infiltrations by mononuclear cells, and fibrosis. Some mononuclear cell infiltrations may be present in areas of the brain in some dogs.

2.3.4.2 Laboratory Diagnosis. See sections B 2.3.4.1, B 3, C 2 and C 3.

2.3.5 TREATMENT

2.3.5.1 Chemotherapy. A combination of penicillin and dihydrostreptomycin (given parenterally) has been generally accepted as the best treatment. Most veterinarians administer dihydrostreptomycin (11 mg/kg, or 5 mg/lb) every 8 h and a single dose of penicillin 100 000 units/kg (50 000 units/lb) body weight for 3-4 successive days.

2.3.5.2 Other Treatment. Immune serum against icterohaemorrhagiae (or/and canicola) may be used, particularly at the late stage of the disease when antibiotics have no effect.

2.3.6 MANAGEMENT

The vaccine used in dogs is usually given to puppies when they receive their initial vaccination for distemper and canine viral hepatitis. Annual booster doses to maintain an adequate level of immunity are necessary. Besides specific chemotherapy, good supportive care is essential. This includes transfusion of blood if the haematocrit is low, intravenous therapy with fluids and electrolytes during the oliguric phase to avoid excessive dehydration, peritoneal dialysis if the animal becomes oliguric and anuric, and giving B-complex vitamins.

2.4 HORSES

2.4.1 GENERAL DESCRIPTION

Despite the fact that serological evidence of subclinical infection in horses by some serovars has been found in Europe, USA and other parts of the world, few acute infections or outbreaks have been reported. The serovars isolated from horses are pomona (Yugoslavia, Hungary, Romania, USSR, USA, 1953-1963), icterohaemorrhagiae (France, 1955), sejroe (Hungary, Yugoslavia, 1958), and canicola (USSR, 1961).

2.4.2 RECOGNITION

Acute leptospirosis usually produces a mild disease in horses which is marked by pyrexia and associated anorexia and, therefore, usually not recognized. However, in more severe infections, the pyrexia may be accompanied by conjunctivitis, petechiae on the mucosae, haemoglobinuria, icterus, depression, and muscular weakness. The illness may last 3-18 days. Acute leptospirosis may cause deaths in neonatal foals infected in utero and occasionally in adult horses. The infected neonatal foal often shows lesions in the liver, kidneys and lungs.
Leptospiral infections in pregnant mares have often been associated with abortions. Leptospires have been isolated from the urine of aborting mares and from fetal tissues of stillborn and aborted foals. Not enough is known of the significance of leptospirosis on the equine reproductive system to establish its economic impact on horse breeding.

2.4.3 COMPLICATIONS

2.4.3.1 Periodic ophthalmia. Horses infected with leptospirosis may show periodic ophthalmia (recurrent iridocyclitis, uveitis, "moonblindness"). The incidence of the syndrome is not uniform, but has been as high as 45% in some enzootics. The syndrome usually appears after a considerable latent period, 2-8 months after the acute illness, as a non-purulent panophthalmitis with a preponderance of uveitis and a tendency to relapse. Sometimes total blindness results. Although leptospirosis is not the sole cause of periodic ophthalmia, the role of leptospiral infection has been strengthened by the detection of leptospires in the eye lesions for extended periods, demonstration of high concentrations of agglutinin in both the blood and in the aqueous humour of the eye, and experimental induction in dogs of chronic hypersensitivity ocular reactions by injecting inactive soluble leptospiral antigens into the eye. Periodic ophthalmia is commonly found in Europe, the USA and Asia.

2.4.3.2 Abortion. See section B 2.4.2 above.

2.4.4 For DIAGNOSIS, TREATMENT, MANAGEMENT and PREVENTION OF SPREAD OF INFECTION, please see the relevant sections under Cattle (Section B 2.1).

2.5 SHEEP AND GOATS

2.5.1 GENERAL DESCRIPTION

Leptospirosis in sheep has been reported from Africa, Australia, Brazil, Bulgaria, Hungary, India, Iran, Italy, New Zealand, USSR, USA and Yugoslavia, and in goats in some of these and other countries where both sheep and goats are raised. Infections with different leptospiral serovars have been reported from different countries, e.g., serovar grippotyphosa in sheep and goats in Israel; pneumonia in sheep in New Zealand and USA; hardjo in Australia and New Zealand, although recent evidence suggests that infections with other serovars also exist. Awareness of other serovars, especially of the hebdomadis group (e.g., hardjo), is becoming increasingly apparent, perhaps because of the recognition of this serogroup as a major cause of infertility problems in cattle. The prevalence of a particular serovar in sheep and goats may depend on the presence of the same serovar in the surrounding animal population.

2.5.2 RECOGNITION

Leptospirosis in sheep and goats is characterized by increase in temperature, icterus, haemoglobinuria, anaemia, infertility, abortion, stillbirth and perinatal deaths. The clinical picture of the disease, however, is influenced by the virulence of the infecting serovar, age of the animal, susceptibility and physical condition of the animal, and the amount of initial inoculum.

In the acute phase of leptospirosis in sheep and goats, there is anorexia, depression, elevation of temperature by 0.5-2°C, and polyptena. The febrile response, appearing 4-6 days after exposure, lasts for 4-5 days. Leptospiraemia is detectable during 3-8 days after exposure, when the organisms may be found in various tissues. Haemoglobinemia and haemoglobinuria may be observed in some animals. Marked reduction in haemoglobin concentration and haematocrit values may be noted. The serum is deep red indicating haemolytic anaemia. The urine may be port-wine coloured. Maximal red blood cell destruction is observed during 7-12 days after exposure.
2.5.3 COMPLICATIONS

Abortion, stillbirths, and the chronic carrier state have all been reported in sheep with leptospirosis.

2.5.4 DIAGNOSIS

2.5.4.1 Clinical diagnosis. The typical clinical picture may not always be apparent because some serovars cause mild infections. Laboratory diagnosis is essential (see section B 3.).

Post-mortem findings. The animals that die during the acute phase of the disease are usually in good condition but show a variable degree of icterus (especially noticeable on the sclera, visible parts of mucous membrane, and in the body fat). The blood may appear watery. The bladder may contain pink-coloured urine. The kidneys are enlarged and petechial haemorrhages may be observed in the kidneys and lungs. Abortions may be encountered in pregnant animals. Leptospires occasionally penetrate the placental barrier at a time which probably precedes the appearance of maternal antibody. Fetal deaths occur owing to direct infection with the leptospires.

In subacute and chronic cases, the gross changes are confined to the kidneys and are characterized by the development of white spots (1-3 mm in diameter), which may extend for a variable distance into the cortex. Microscopically, the lesions are characterized by focal or diffuse infiltration by mononuclear cells, especially plasma cells and lymphocytes, in the interstitium, periglomerular and perivascular areas. Glomerular atrophy, fibrosis and proteinaceous tubular casts may be observed. Perivascular haemorrhages and perivascular cuffing in the brain have been reported in experimentally infected goats. In experimentally infected sheep, endometrial changes characterized by moderate vacuolization of the surface epithelium have been reported.

2.5.4.2 Laboratory. Culture of blood in the acute stages will allow identification of the infecting serovar (section B 3.3, p. 70 and C 3.2.2, p. 126). Tissues obtained at autopsy, especially fetal tissues and membranes (if fresh), liver and kidneys (in acute cases), and kidneys (in chronic carriers), may be examined for leptospires by culture (section B 3.2, p. 68) or immunofluorescence (section B 3.4.2, p. 80) or silver staining. Serological tests (agglutination, ELISA) will detect antibodies if the infecting serovar is known (section B 3.4, p. 76.). During the infection, as the antibody is produced, the leptospires disappear from the blood and are confined to the proximal convoluted tubules of the kidneys. Leptospirosis is demonstrable in 2 weeks and may be detectable after up to 9 weeks in sheep, although in most cases it persists for about 6 weeks. Antibodies detectable as early as 6 days after infection reach a maximum by 2-3 weeks. The antibody titre persists for variable periods and occasionally for as long as 3 months or longer.

2.5.5 TREATMENT, MANAGEMENT and PREVENTION

Little has been published about the chemotherapy of acute infections in sheep or goats. The same regimens as are used for cattle or pigs may be employed (see sections B 2.1.3, p. 56 and B 2.2.3, p. 38). For management and prevention of spread of infection, please see the relevant sections under B 2.1.6, p. 37; B 2.1.7, p. 37; and B 2.2.4, p. 39 and B 2.2.5, p. 39.

2.6 DOMESTIC CATS

Although the domestic cat is apparently not an important host for leptospires, these organisms have been isolated from both natural and experimental infections. Serological surveys have reported low reactor rates in cats located in various regions throughout the world. The isolations have included at least six serovars and the cats involved have not exhibited any apparent signs of infection. However, histological examinations of the kidneys of several infected cats demonstrated subacute and chronic lesions compatible with tissue changes observed in other hosts.
2.7 NON-HUMAN PRIMATES

The use of monkeys as pets and the extensive use of primates other than man in medical research make it important that some consideration be given to leptospirosis in these animals. Although leptospirosis generally does not cause serious signs in monkeys, some reports of severe signs and death have been observed in some species. Serological studies have indicated reactor rates which vary from 1% to 21% depending on the geographical regions and species.

Signs of leptospirosis in primates other than man are usually mild and limited to a febrile response, malaise and conjunctivitis. Often the mild signs go unnoticed in natural infections. However, in experimental infections utilizing several serovars, the signs varied from only febrile responses to very severe infections with high fever, marked depression, pronounced icterus, and in a few cases death.

Lesions vary with the clinical findings in leptospiral infections in these species. In some animals, no gross lesions are evident; in others, kidney and liver lesions are present.

Diagnosis of leptospirosis in the non-human primates is more difficult than in some other animal species because the clinical signs and lesions are less consistent and the antibody responses are detectable for only relatively short periods. "Paradoxical" serological reactions also occur (see section B 3.4.1.1.3,(p.78)), as in man, further increasing the difficulty in evaluating the results of the tests.

2.8 LIKELIHOOD OF DIAGNOSIS OF ACUTE INFECTION IN ANIMALS

Although the clinical features of acute leptospirosis are not pathognomonic, the presence of characteristic symptoms lends weight to the likelihood that an acute illness is leptospirosis, as set out in Table 4, p. 64. The clinical and serological features are scored, to assist in deciding whether or not leptospirosis is a likely diagnosis. The features listed also help as a checklist for clinical and laboratory investigation.

2.9 CARRIER STATE IN FARM AND DOMESTIC ANIMALS

2.9.1 RECOGNITION

The carrier state in domestic animals is characterized by the shedding of leptospires in the urine following the acute phase of the disease. This shedding may persist for many weeks or months in animals which appear perfectly normal, either following the acute phase or after an inapparent infection. As an example, both the onset of infection and persistent shedding may occur without any outward signs of ill health in L. interrogans serovar hardjo in cattle, where shedding can occur for 16 months or more.

The detection of leptospires in the urine is the only way in which individual carrier animals can be identified but even this requires careful search. Intermittent shedding makes it difficult to be sure that negative laboratory results mean that the animal is not a carrier. Although acute leptospirosis in animals may be accompanied by easily recognizable clinical signs, the carrier state usually is not. In cases where one of the acute signs of infection is the abortion of the fetus, leptospires are often shed in the urine at the time.

2.9.2 DIAGNOSIS

The diagnosis of the carrier state in live animals is based almost entirely on the detection of leptospires in their urine. However, within an infected flock or herd, some animals may be leptospiraemic at the time of an investigation; for this reason blood samples should always be taken for culture, along with urine samples. A distinction can also be drawn between the methods used for determining the carrier state in animals within herds or flocks and for the diagnosis of such a state in individual animals, especially those kept away from contact with others of their own
Table 4. LIKELIHOOD OF THE DIAGNOSIS OF ACUTE LEPTOSPIROSIS IN ANIMALS

This checklist is designed for use by those attending to sick animals. To use the list, note
the main clinical features listed, mark the box "Yes" or "No", and write the appropriate
score in the right-hand column. A total score of up to 20 makes the diagnosis of leptospirosis
unlikely; between 21 and 29 it is a possibility; at 30 and above, a presumptive diagnosis may
be made with confidence.

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Total score for Part A
B. Bacteriological laboratory findings

Isolation of leptospires in culture - diagnosis certain

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</table>

Total

species, such as companion animals (e.g., cats and dogs, ponies, etc.). Animals kept in groups have close enough contact with each other to produce an epidemic of leptospirosis which is generally more easily detected by serological and cultural means.

If a particular serovar is known to set up a carrier state in a given species of animal, it is only necessary to detect active infection in the group to know that a proportion of the members of that group will be carrier animals. A presumptive diagnosis of infection in these cases can therefore often be made by serology alone, although a positive culture from their urine would give a greater degree of confidence in the diagnosis. On the other hand, while a positive urine culture in individual animals leaves no doubt about the carrier state of the animal at the time of sampling, a negative urine culture in the presence of a positive serum antibody titre is more difficult to interpret. In large groups of animals it may be possible to slaughter a sample of animals to detect the presence of leptospires in their kidneys or other tissues by culture, histopathology, or immunofluorescence to give added evidence that active leptospiral infection is present in the group.

2.9.2.1 Clinical diagnosis. The term "carrier state" is usually given to that phase of the disease in animals where the acute phase signs have largely returned to normal, and for this reason specific clinical signs are usually not present. Reliance must therefore be placed on laboratory tests to detect "shedder" animals.

2.9.2.2 Laboratory Specimens. The material for inoculation into culture media must be taken using aseptic techniques. The cultures may take up to 4-10 weeks to yield positive results so that any contaminating organisms are likely to overgrow the leptospires. Urine samples (midstream) are collected into a sterile container. Blood is best collected using a sterile vacuum tube and needle. Cerebrospinal fluid (CSF) is also best collected into a vacuum tube. Kidneys and any other material collected from animals at necropsy must be removed with great care using sterile instruments (see section C 1.2.6 p. 104).
Urine can be cultured directly or examined by direct dark-field microscopy before centrifugation. After centrifugation both the supernatant and sediment are examined. Selective agents (see section C 3.2.1.4. (1), p. 125) may aid urine culture. One drop of urine is added to 5 ml of culture medium and at least 2 tubes of the medium should be used; one containing 200 µg/ml and the other 400 µg/ml of fluorouracil. Alternatively, attempts may be made to select leptospires from contaminated specimens by adding 9 ml of urine to 1 ml of a sterile suspension containing 25 µg/ml of each of neomycin and fluorouracil. After mixing and standing at room temperature for 1 h, the culture media may be inoculated from the mixture (see section C 3.2.1.4. (1), p. 123).

Kidneys. After careful removal from the cadaver and removal of the kidney capsule, a 10-20 g portion is doused with alcohol and ignited to sterilize the outer surface. This portion of kidney is then ground up with 50 ml of Stuart's basal medium and 1 ml of the "slurry" is inoculated into 5 ml of culture medium. A 0.1 ml aliquot of this inoculated medium is then transferred into two more 5 ml tubes of medium and incubated at 27°C for up to 10 weeks. Direct examination of this "slurry" made after light centrifugation at 2000 g.

Blood may be cultured (see section C 3.2.2.1, p. 125) but the success rate is not likely to be high, because the majority of carrier animals will no longer be in the leptospiraemic phase. Blood is therefore best examined by serological tests. Serum, drawn from the blood sample after clotting, is tested against a series of antigens using the MAT, CF or other serological tests which have been found to be appropriate. These serological tests should start with a dilution no greater than 1:24. The number of antigens used routinely largely depends on local knowledge of the prevalent serovars (see sections B 3.4, p. 76 and C 3.2.1, p. 123).

In order to determine the likelihood of the carrier state in a group of animals by the use of serology alone, it is necessary to bleed a "stratified" random sample of approximately 20% of the group. The stratification is done according to the age structure of the group.

In the case of an endemic infection, the young animals will have high titres; and titres in the other age groups will decline in relation to their age. The degree of persistence of titres varies according to the infecting serovar and the species of animal under study. Where an endemic infection occurs, persistent "shedders" are inevitable.

Following an epidemic of leptospirosis, the animals in all age groups are likely to have titres, whose level will depend on (a) the degree of stimulation of antibodies by the infecting serovar, and (b) how long ago the epidemic occurred. Whether "shedders" are still present will depend on how long ago the epidemic occurred and for how long it is usual for these species of animals to remain "shedders" of the particular serovar.

Liver and brain can be treated in much the same way as the kidney.

2.9.3 TREATMENT

Dihydrostreptomycin has been found to be the most useful antibiotic and must be given in a dose of 25 mg/kg body weight every second day for three doses (see section B 4.2.5.1, p. 94). Treatment of clinical cases is warranted but is of limited use as a means of curing the "shedding" state in asymptomatic carriers. It is too expensive a treatment to use in large groups of animals and it is unlikely to be completely effective. For these reasons, unsuccessfully treated animals will remain as a source of infection for young and susceptible stock which are introduced into the group.

2.9.4 PREVENTION OF SPREAD

Where appropriate vaccines are available, they may be used to protect susceptible animals but they are unlikely to shorten the length of "shedding" time in already infected animals. The degree of protection afforded to susceptible animals by vaccination is very dependent on the amount of exposure to infecting organisms (see section B 4.2, p. 90 and B 4.2.3, p. 93).
3. LABORATORY METHODS

3.1 METHODS FOR MICROSCOPY

3.1.1 EXAMINATION BY DARK-FIELD MICROSCOPY

The use of microscopic methods to detect leptospires in fluids or tissues may frequently provide a rapid diagnosis (see section C 3.2.3, p. 126). However, the concentration of organisms may be too low, $2 \times 10^4$/ml or less, to allow ready detection, especially in blood obtained during the leptospiroaemic phase of natural infections. Also artefacts such as fibrils and extrusions from cellular elements are easily mistaken for leptospires by the inexperienced. Consequently, microscopic examination of tissues or fluids is not recommended as a single diagnostic procedure.

Direct dark-field microscopy, however, can be used to advantage for specimens in which profuse numbers of organisms may be present, as is frequently the case for urine or suspensions of kidneys (from naturally infected wildlife, dogs, swine, and other domestic animals) and for blood, peritoneal fluid, liver, or kidney (from hamsters or guinea pigs infected with clinical material) (see section B 3.6, p. 83 and C 3.3, p. 150).

Phase-contrast microscopy is useful for visualizing leptospires in the laboratory but, because of its technical limitations in thick suspensions and its optical characteristics, has no place for practical purposes where dark-field microscopy is available.

Examination of blood, CSF, urine or peritoneal fluid is conducted on a drop (about 10-20 μl) which is spread out in a thin layer between a glass slide and a coverslip. The material is examined by dark-field microscopy, at first by low magnification (i.e., 100 to 150×) and then by high dry magnification. The use of a dry dark-field condenser, of n.a. about 0.6-0.9, is strongly recommended because it is easier and safer to use, and gives a wider field of view with lower magnifications (see section C 3.2.3, p. 126).

The chances of demonstrating leptospires in blood may be increased by first centrifuging the specimen, treated with an anticoagulant, at low speed (i.e., 1000 g for 10 min) to remove cellular elements, and then at high speed (i.e., 3000-4000 g for 20-30 min) to concentrate the leptospires. The double-centrifugation procedure could be used for other fluids or tissue suspensions. A presumptive diagnosis is made only if the typical morphology and motility are demonstrable. The failure to detect leptospires does not rule out their presence. Dark-field microscopic diagnoses should be confirmed by cultural or serological methods.

Technical details of the examination of kidneys and other tissues are described in sections B 3.2, p. 68 and C 1.2.6, p. 104.

3.1.2 IMMUNOFLUORESCENT TECHNIQUES

3.1.2.1 Direct Fluorescent Antibody (FA) Test. This is conducted with fluorescein-conjugated immunoglobulins from rabbits immunized with specific serovars. Generally, in direct FA tests, reactions are more pronounced with homologous or serologically closely-related serovars and are less intense or negative with heterologous serovars. The extent and degree of cross-reactivity are apparently related to the dilution and titre of the conjugated serum and to the degree of labelling. Serovar-specific tests may be useful for epidemiological purposes in providing clues to the identity of the infecting serotype but are laborious if multiple leptospiral serovars are considered. From the point of view of the clinician and diagnostic laboratory whose primary interests are in the diagnosis of leptospirosis, a genus-specific test with a single antigen would be more desirable.

3.1.2.2 Indirect Immunofluorescence (IF) Tests. Many of the technical difficulties of FA are overcome by using IF procedures—see section C 3.1.4.2, p. 118, where they are described (1) both as methods for diagnosis and identification, and (2) as applied to staining smears from tissues and to tissue sections.
3.1.2.3 Staining Methods. Various silver-deposition procedures have been proposed for detecting leptospires in tissues and body fluids. These have the same limitations as dark-field procedures regarding the detection of small numbers of organisms in tissue sections and the presence of artefacts which may be mistaken for spirochaetes. Therefore, they are not recommended for routine direct diagnosis of fluids and tissue specimens. Silver-staining and immunofluorescent methods are, however, particularly useful for demonstrating leptospires in specimens such as fixed tissues, contaminated aborted fetuses, and formalized or contaminated urine which cannot be examined by other methods. Technical details are given in section C 3.2.5.1, p. 128.

Silver-deposition techniques. Leptospires in smears of tissues or fluids on slides can be stained using silver-deposition methods. Technically satisfactory results in the presence of tissue fluids and debris are difficult to obtain, so this method is not recommended.

However, silver-staining methods for fixed tissue sections may be particularly useful. The variously described procedures may be primarily modifications of the Warthin-Starry method for staining slides of thin sections cut from paraffin-impregnated blocks of tissues, or for staining in blocks before cutting (see section C 3.2.3.1, p. 128). The former is recommended. The stain is based on the chemically-reducing surface properties of leptospires and other spirochaetes, but the test is not specific because other tissue elements are argentophilic. Staining conditions may be adjusted to obtain the best differential staining of spirochaetes. Well-stained preparations show black spirochaetes in a pale yellow or colourless background which may be counterstained by HE stain. Establishment of a presumptive diagnosis is contingent on observation of the characteristic morphology of the organisms. If possible, positive findings should be verified by cultural or microscopic methods. Silver-deposition procedures have principally been used for epidemiological surveys of wildlife, and for retrospective demonstration of leptospires in fixed pathological specimens.

Other staining methods. Conventional aniline-dye staining techniques for demonstrating leptospires in clinical specimens or cultures are rarely utilized because the organisms are more easily detected by dark-field examinations or by silver deposition techniques. When stained with aniline dyes by the usual bacteriological methods, leptospires are faintly coloured and difficult to discern. More satisfactorily stained preparations may be obtained by a prolonged (i.e., 30 min) Giemsa stain.

3.2 Isolation Procedures

3.2.1 CULTIVATION

The sources of leptospires to be cultivated may be (a) primary isolation from blood, e.g., blood cultures (section C 3.2.2.1, p. 125), urine (section B 2.9.2.2, p. 65), water, (section C 3.4.1, p. 132) autopsy tissues (sections C 1.1.6, p. 103 and C 1.2.6, p. 104), or other clinical specimens such as cerebrospinal, anterior chamber, amniotic, or peritoneal fluids (section C 3.3.2.2, p. 130); or (b) existing cultures, either stock laboratory cultures or recently isolated from specimens. The techniques for primary isolation are described in detail in the sections referred to.

Stock cultures and isolates, in the course of investigation and identification, are maintained by regular subculture into fresh medium, usually at intervals of not more than one month. In an adequate medium, viability is easily maintained for this period; cultures have been viable after years of storage. Cultures may be stored at room temperature below 40°C. Stock cultures may be preserved reliably by storage in liquid nitrogen containers (see section C 3.2.4.1, p. 127). While TA media are excellent for primary isolation and routine maintenance of strains, rabbit serum media are preferred for cultures to be used for immunizing rabbits to produce antisera, because repeated injection of bovine albumin in TA media into a rabbit may produce anaphylactic reactions including death.

3.2.1.1 Subculture Techniques. Cultures are usually incubated at 28°C or 30°C. The size and age of the inoculum influence the time taken to reach maximum density of cultures. A volume of inoculum, comprising 1-10% of the volume of the medium to be incubated, is added by graduated or dropping pipette, giving a starting density of 10⁵-10⁷ leptospires per ml in conventional laboratory conditions. (WARNING: Never pipette by mouth; always use a bulb or pipetting device). A well-grown unshaken culture may reach about 1-3 × 10⁸ leptospires per ml in 3-10 days, in containers
filled to not more than one quarter full, and is barely opaque, with a low optical density (OD) scarcely measurable in a 1 cm cell in a spectrophotometer. The growth is characteristically bi-refrangent in liquid media. Cultures grow to greater densities if aerated. Shaking to increase aeration can increase the final density dramatically by approximately ten times. Further increases may be obtained by adding extra tween or tweens as a carbon source, in the late logarithmic phase of growth. Heavier growth (10^9/ml or more) is opaque, and may resemble a culture of staphylococci in broth, in opacity and OD.

Industrial-scale production of large volumes of cultures of high density for vaccine purposes can be achieved economically by attention to the medium (protein-free for preference), aeration and supplementation with extra nutrients. The greatest care must be taken to avoid contamination of cultures with airborne bacteria and moulds. Perfect aseptic technique is essential whenever containers are opened for microscopic observation or for subculture.

Growth on solid media (section C 3.2.1, p. 124) is not utilized in diagnostic laboratories at present. If required, the colonies may be recognized and characterized and subcultured by puncturing the agar over the edge of a colony with a sterile Pasteur pipette, into which the leptospires and agar are sucked gently and then expelled into a tube of sterile medium or buffer. This fluid suspension is then used as the next subculture, or as a basis for inoculation of other plates of solid medium or experimental animals.

3.2.2.2 Counting of Leptospires. See section C 3.2.6, p. 129.

3.2.2 ANIMAL INOCULATION

Experimental infections are produced in young guinea pigs (under approx. 150 g), hamsters (approx. 50 g), and gerbils in order to test and maintain virulence and to isolate or purify cultures. A variety of other animals (deer-mice, Australian hopping mice, 11-day chick embryos, laboratory mice) have been used or been recommended for similar purposes. Hamsters are the most widely used animals, where they are available, because they are susceptible to a number of different serovars, such as 

**pomona,icterohaemorrhagiae and copenhageni, hardjo (carrier infection), and canicola.** They are also the standard reference animal for laboratory testing of vaccine preparations (2, 3). Guinea pigs are generally susceptible to lethal infection by a much narrower range of serovars, mainly **icterohaemorrhagiae and copenhageni, and occasionally pomona.** Naturally resistant species, such as adult laboratory mice, may be made susceptible by immunosuppression with cyclophosphamide or irradiation.

In all animals other than the 11-day chick embryo (which may be infected by dropping the culture on the exposed chorio-allantoic membrane), the route of infection is intraperitoneal. It is an advantage to use white (albino) animals, if jaundice is to be observed. Likewise, if the carrier state is to be sought, it is advantageous to use female animals from which urine is more easily obtained (see sections C 1.2.4, p. 104 and C 3.3.2.3, p. 130).

The clinical appearance of infected animals is variable, including no observable change from normal, or with loss of appetite, reluctance to move, ruffled fur and arched back, conjunctival suffusion, pallor, jaundice, and bloody diarrhoea; death can occur suddenly and sometimes unexpectedly. Death will usually occur between the third and twelfth days. The temperature taken rectally may be raised within the first 3 days; it often drops to below normal shortly before death. Urinary output is often low during the acute illness.

Following an intraperitoneal injection with a virulent culture, leptospires will be visible by dark-field microscopy in the peritoneal fluid in 3-5 days after injection, reflecting a leptospiraemia which is less easily observed in the blood at that time. Leptospires may be cultured, usually from heart blood (see section C 3.3.2.1, p. 130) throughout the acute infection, especially just before death when large numbers of leptospires may be expected in the blood in animals dying early (3-6 days) after infection. Animals dying later may have cleared the leptospires from their tissues, but die nevertheless because of irreversible lesions (renal or hepatic or brain damage, or haemorrhage).
Animals which survive may become carriers in 10-28 days after infection, and should be kept up to 28 days. Leptospires may be found in the urine (sections B 3.1, p. 67 and C 1.2.4, p. 104), although it may be necessary to alkalinate it first by dietary or other means. Leptospires may also be found in the kidneys by direct observation (section C 3.2.3.1, p. 126), or cultivation (section B 3.2.1, p. 68 and C 3.3.3, p. 131), or by staining with silver (section C 3.2.3.1, p. 128) or immunofluorescent (section C 3.1.4.2, p. 119) or immunoperoxidase stains. Cultivation is the most reliable method, and allows eventual identification of the isolate, if required.

All animals, whether successfully infected or not, should be subjected to a routine autopsy, including taking blood for serology and culture for leptospires, if required. Sometimes, where the cause of death is not clear, especially in the first day or two after infection, cultures should be taken in order to detect other bacteria, particularly coliforms and salmonellae, which may have been the cause of death.

3.2.3 PURIFICATION OF CULTURES

Cultures of leptospires are usually maintained in fluid media, and thus, once contaminated, are likely to be lost through overgrowth of the contaminants. Occasionally, the contaminant may be another leptospire, even a saprophytic (biflexa) leptospire, through carelessness or accident in the laboratory; or through making up culture media using water which has not been heat-treated, but only filtered; or through keeping media before sterilization in containers in which there is residual tap water.

Attempts may be made to salvage contaminated cultures by:

3.2.3.1 Plating on solid media. (see section C 3.2.1.2 (3), p. 124). The leptospiral colonies spread horizontally through media containing 1% of the agar, away from the immobile colonies of the contaminating bacteria. (section C 3.1.4.2, p. 119) or immunoperoxidase stains or other inhibitors to kill the leptospires or prevent leptospiral growth by nutritional competition. If the leptospiral growth can be picked free of contamination from the colonies of the contaminant bacteria, the leptospires can be subcultured and may grow (see section B 3.2.1, p. 68).

3.2.3.2 Filtration. Leptospires can pass a cellulose membrane filter of average pore diameter (APD) 0.22 μm. The contaminated fluid culture may be diluted and filtered through a suitable filter arrangement, in the hope that some leptospires will pass through the filter membrane and can be cultured from the filtrate.

3.2.3.3 Selective Media. Lightly contaminated cultures may be subcultured into a medium containing fluorouracil, in anticipation that other bacteria will be inhibited (see section C 3.2.1.4, p. 125). A further subculture should be carried out after 3 days since, after this time, some contaminating bacteria may be selected which are able to grow in fluorouracil.

3.2.3.4 Animal Inoculation. Laboratory animals (hamsters, guinea-pigs, mice) have been used to purify leptospiral cultures, by making use of the knowledge that after intraperitoneal injection, the leptospires invade the bloodstream almost immediately, while other bacteria are trapped by host defences in the peritoneal cavity and invade later, if at all. Thus 1 ml of the contaminated culture is injected intraperitoneally and the animal bled aseptically by heart puncture 10-60 min later. The blood is inoculated directly into the culture medium, approximately 1-10% (i.e., 0.1 ml in 1-10 ml of medium). It is useful to inoculate several tubes of medium from the heart blood to lessen the risks of further contamination from the heart puncture procedure, or of failure of a culture to grow leptospires because of very small numbers in the blood.

3.3 CLASSIFICATION AND IDENTIFICATION OF ISOLATES

The basic taxon for *Leptospira* is the serovar. The main emphasis in identification and classification is therefore on serological methods. Cultural methods are mentioned below, but find limited application at present.
3.3.1 CULTURAL CHARACTERISTICS

All serovars of *L. interrogans* have essentially the same cultural properties (see section A 2.2, p. 17). Colonial and morphologic characteristics and growth requirements are not characteristic of any serovar or group of serovars, and have not been used to classify or attempt to identify leptospires because there is no constant correlation of these properties with serological classifications. *L. biflexa* serovars may be differentiated provisionally from *L. interrogans* by the ability of *L. biflexa* to grow at 13°C, and in media containing 225 µg/ml of 8-azaguanine (see section C 3.2.1.4 (2), p. 125). The final differentiation is serological, and by DNA taxonomy (section A 2.3, p. 29).

3.3.2 SEROLOGICAL CHARACTERISTICS (see section B 3.4.6).

Leptospires possess relatively stable specific antigens, many of which are recognized by agglutination of leptospires by homologous specific antisera. This property permits a classification of the leptospires according to their serovar, which is currently the basic taxon of *Leptospira*. Each serovar is represented by a reference strain to which the description of the serovar is attached. The differences in agglutinogens between strains are determined by cross-agglutination tests and cross-agglutinin-absorption tests. For practical purposes, those serovars that have a close serological relationship and yet show individual antigenic differences have been grouped together in serogroups (Table 3, p.72). The Taxonomic Subcommittee on *Leptospira*, which met in Munich in 1978 (7), confirmed the 1967 WHO report (18) and accepted the following definition of a serovar: "two strains are considered to belong to different serotypes if after cross-absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titre regularly remains in at least one of the two antisera". The 10% limit of residual antibodies in absorbed sera, when compared with the homologous titre, was chosen arbitrarily. According to the definition, strains of the same serovar are allowed a certain degree of serological difference. Two strains are considered to belong to the same serovar, if less than 10% of the homologous antibodies remain in both sera after absorption.

3.3.2.1 Identification of Serogroup and Serovar Status by Serological Typing: (18, 19). The identification of leptospires is a step-by-step process. First the serogroup to which a strain belongs is determined. Then the serovar status within the serogroup is defined.

Determination of SEROGROUP STATUS (see section C 3.1.4.1. (6), p. 116) is a relatively easy procedure provided that the suggested battery of "group sera" is used (Table 6, p.74) (see section B 3.4.6, p.82). A "group serum" is a selected rabbit antiserum which agglutinates optimally all serovars within a given serogroup and which gives the least cross-agglutination with strains of other serogroups.

Based on epidemiological criteria where there is a known high prevalence of some serovars, some modifications in the suggested battery of "group sera" may be warranted. In practice a well grown culture of the strain under investigation is tested by MAT against the 20 suggested "group sera". The "group serum" giving the highest titre usually indicates the serogroup of the unknown strain. Alternatively, the group sera may be diluted to e.g., 1:3200, in which case only the antiserum representing the serogroup of the unknown strain will usually react.

Determination of SEROVAR STATUS (see section C 3.1.4.1.7, p. 116) is much more complicated. Two typing methods can be applied: the standard classical method, based on the agglutinin-absorption technique, and the factor serum method. Both methods should lead to the same result.

(1) Standard Classical Method, which has been approved by the TSCL (18,19). By definition, serovars are characterized by their serological differences. This means, in principle, that the investigated strain must be compared with all reference strains. In practice, to simplify the procedure, the strain under investigation is compared, by agglutination tests and cross-agglutinin-absorption tests, only with the reference strains of all known serovars within the determined serogroup to which the strain belongs (see section C 3.1.4.1.6, p. 116). Identification may be further expedited if the occurrence of a certain serovar within the serogroup is suspected with a high degree of probability on epidemiological grounds. Comparative absorptions may be started immediately with the reference strain of the suspected serovar.
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<td>Serogroup</td>
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<tr>
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<td>darien</td>
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1 Serogroup Louisiana
2 Included in serogroup Autumnalis
3 Serogroup Mini
4 Subgroup Borincana of serogroup Hebdomadis
5 Subgroup Hebdomadis of serogroup Hebdomadis
6 Classified as a member of serogroup Sejroe
7 Subgroup Sejroe of serogroup Sejroe
8 Subgroup Saxkoebing of serogroup Sejroe
9 Subgroup Wolffi of serogroup Sejroe
Table 6. LIST OF REPRESENTATIVE STRAINS OF LEPTOSPIRES WHOSE ANTISERA ARE USEFUL IN THE IDENTIFICATION OF ISOLATES. These antisera have been selected because they react with the representative antigens of their serogroup. They are listed according to the serogroup they represent. (Modified from 19).

<table>
<thead>
<tr>
<th>Serogroup (or subgroup)</th>
<th>Representative antigens found in antiserum to leptospires of</th>
<th>Serovar</th>
<th>Strain</th>
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<tr>
<td>Australis</td>
<td></td>
<td>bratislava*†</td>
<td>Je3-Bratislava</td>
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<td></td>
<td></td>
<td>lora*</td>
<td>Lora</td>
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<td>Autumnalis</td>
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<tr>
<td></td>
<td></td>
<td>bangkinang*</td>
<td>Bangkinang 1</td>
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<td></td>
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<td>Rachmat</td>
</tr>
<tr>
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<td></td>
<td>ballum</td>
<td>Mus 127</td>
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<td></td>
<td></td>
<td>castellonis†</td>
<td>Castellon 3</td>
</tr>
<tr>
<td>Bataviae</td>
<td></td>
<td>batavia†</td>
<td>van Tienen</td>
</tr>
<tr>
<td>Canicola</td>
<td></td>
<td>canicola†</td>
<td>Hond Utrecht IV</td>
</tr>
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<td>schueffneri†</td>
<td>Vleermuis 90 C</td>
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<td></td>
<td>cynopteri</td>
<td>3522 C</td>
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<td>Moskva V</td>
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</tr>
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<tr>
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<td></td>
<td>poi</td>
<td>Poi</td>
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<tr>
<td>Louisiana</td>
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<td>louisiana</td>
<td>LSU 1945</td>
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<td>Mini</td>
<td></td>
<td>szwajizak*</td>
<td>Swajizak</td>
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<tr>
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<td>TVRL 3214</td>
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<td>CZ 214K</td>
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<tr>
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<td></td>
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<td>Pomona</td>
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<tr>
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<td>pyrogenes†</td>
<td>Salinem</td>
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<td>robinsoni*</td>
<td>Robinson</td>
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<tr>
<td>Sejroe</td>
<td></td>
<td>hardjo*</td>
<td>Hardjo-prajtino</td>
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<tr>
<td>Wolfii</td>
<td></td>
<td>medanensis*</td>
<td>Hond H.C.</td>
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<tr>
<td></td>
<td></td>
<td>wolfii†</td>
<td>3055</td>
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<td>Shermani</td>
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<td>LT 821</td>
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<tr>
<td>Tarassovi</td>
<td></td>
<td>tarassovi†</td>
<td>Mitis Johnson</td>
</tr>
</tbody>
</table>

* alternatives within a serogroup
† recommended as one of 11 basic serogroup antisera
Comparison with other serovars in the serogroup is necessary only if the unknown and reference strains do not appear to be identical.

(2) Factor Sera Method (19, 20). Many different agglutinogens are present in leptospires, forming a mosaic which is different for each serotype. One agglutinin (or a combination of agglutinogens) forms a "factor". A "factor serum" is prepared by repeated absorption of an antiserum to remove all antibodies apart from those directed against a specific factor (see section C 3.1.4.1, p. 118). The factors of the serovars belonging to a given serogroup are assembled and tabulated to form a scheme, which shows the specific arrangement of factors of each serovar (19, 20). The incubation of a strain with a battery of "factor sera" will reveal which factors are present, i.e., corresponding to those in the "factor sera" that cause agglutination. Comparison with the typing scheme allows the rapid provisional determination of the serovar status of the strain under investigation. Results may be available within a few hours if well-grown cultures are used. This method is used in a few laboratories and single factor antisera are not generally available. Final verification must be made by the standard method of cross-absorption (see (1) above).

3.3.2.2 Serovar Varieties. Most leptospiral agglutinogens are thermostable. However, some thermostable agglutinogens have been reported, giving rise to the recognition of a thermostable variety of a serovar (e.g., strain "RGA" is the thermostable variety and "Ictero No. 1" is the thermostable variety of serovar icterohaemorrhagiae, which is the only serovar in which these antigens have been noted). Thermostable antigen can be detected by absorbing an antiserum of the strain under investigation with a heated (at 36°C for 30 min) culture of the homologous strain. The detection of residual antibodies by agglutination of a living culture of the homologous strain indicates the presence of thermostable agglutininogen. The control agglutination test with a heat-killed culture should remain negative (see section B 3.4.6, p. 82 and C 3.1.4.1, p. 115).

3.3.3 OTHER TESTS

Investigations have been reported on chemically defined serovar-specific antigens, including those on antigens of flagellar origin, using precipitin reactions in gels. They have not yet been evaluated as routine procedures.

Several biological groups of leptospires have been distinguished by different biological properties. In genetic studies, using DNA annealing affinities, six distinct genetic groups of leptospires have been described (21); the strains in these groups were not necessarily antigenically related. Classification by DNA similarities (revealed by gel electrophoresis of fragments of DNA released by restriction endonucleases) also awaits evaluation (22).

Further progress in this field could have an impact on the present classification of Leptospira.

3.3.4 INTERPRETATION AND IDENTIFICATION

The taxonomic status of a leptospira-like organism can be defined by a combination of its various characteristics, bearing in mind that some variation in the expression of a characteristic may occur. Therefore different markers should be considered or tests should be repeated. For classification and identification of leptospires, the following scheme can be used:

<table>
<thead>
<tr>
<th>Taxonomic status</th>
<th>Main characteristic or test used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Genus</td>
<td>morphology, movement</td>
</tr>
<tr>
<td>2 Species</td>
<td>growth with 8-azaguanine; growth at 13°C</td>
</tr>
<tr>
<td>3 Serogroup</td>
<td>agglutination with &quot;group sera&quot;</td>
</tr>
<tr>
<td>4 Serovar</td>
<td>serological tests: (a) agglutination and agglutinin-absorption tests or (b) factor analysis with factor sera.</td>
</tr>
</tbody>
</table>
3.4 SEROLOGICAL METHODS

3.4.1 AGGLUTINATION OF LEPTOSPIRES

This is the most widely used serological procedure. Agglutination of leptospires can be observed by dark-field microscopy in the MAT (the microscopic agglutination test) or by the naked eye using a slide (or similar) test with a concentrated antigen (the macroscopic agglutination test).

3.4.1.1 The Microscopic Agglutination Test (MAT) (see section C 3.1.4.1, p. 115).

The microscopic agglutination test is the basis of serological diagnosis and classification. The usual method is to mix equal volumes of a series of serum dilutions and leptospiral culture in the wells of a plastic haemagglutination plate, a microtiter plate or in glass tubes. The serum antigen mixtures are allowed to react during a period of 14-4 h at room temperature (20-30°C), after which time the degree of agglutination and end-point titre are determined by examining a sample of the mixture by dark-field microscopy. The amount of agglutination may be hard to evaluate; therefore the test is read subjectively by determining the number of free non-agglutinated leptospires (see section C 3.1.4.1 (3), p.115 ). The age and the density of the antigen are further variables which may affect the reproducibility of the results. Antigens derived from an old culture and those with a high concentration of leptospires may lack sensitivity, which is enhanced by antigens with a low density of leptospires.

It is recommended that a 4-14-day-old fluid culture be used that has been incubated at 30-32°C and contains a density of 1-2 × 10^8 leptospiral organisms/ml. The antigen should be free from "breeding nests", which are conglomerates of leptospires. The density can be determined by direct counting (see section C 3.2.6, p. 129) or by nephelometry. Living cultures or cultures killed with formalin may be used (see below).

(1) Antigens

(a) Living antigens. In comparison with formalin-killed antigens, living antigens tend to be more sensitive and often reactions one titre step higher are obtained. Contamination of the suspension occurs readily. When live cultures are used, great care must be exercised to prevent laboratory infection (section C 3.5, p.133 ).

(b) Formalin-killed antigen. Killed antigen is usually prepared by adding neutralized formalin (to give a final 0.2% concentration) to a well grown live culture containing approximately 1-2 × 10^8 leptospires/ml. Formalin is a 40% aqueous solution of formaldehyde. This type of antigen has the advantages of stability for a long period (for as long as 2 months in serum-enriched medium) and relative freedom from contamination. It is also safer for staff to use. Compared with living antigen, more cross-reactions with heterologous serovars tend to occur in the early stages of infection, and lower titres may be observed.

(2) Techniques

(a) Preliminary tests. To detect leptospiral antibody, the serum under investigation is screened in low dilutions (1:40 or 1:50). Urine and CSF are examined undiluted. A battery of antigens is used, covering the range of serovars that are expected and are likely to be present locally. The battery should contain locally isolated strains if possible, because they may give a higher titre reaction than reference laboratory strains. No more than one representative strain per serogroup should (or need) be included.

For human sera, one or more strains of saprophytic serovars, especially strain Patoc I of serovar patoc, should also be included (see section A 4.2.1). They tend to react as genus-specific antigens and to agglutinate with human antibodies produced by many infecting serovars, especially icterohaemorrhagiae and pomona. Thus, they may detect infections caused by strains not yet known to exist in a specific region, or even those that indicate the existence of previously unknown serovars. In case of doubt as to which strains to use, the battery can be made up of one strain representing each known serogroup. Such
Table 7. LIST OF SEROVARS (WITH SEROGROUP) RECOMMENDED FOR USE AS ANTIGENS IN A BATTERY OF STRAINS IN MAT FOR DETECTING INFECTIONS BY AN UNKNOWN SEROVAR

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<th>Strain</th>
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<td>Ballico</td>
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<td>Jež Bratislava</td>
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<td>Akiyami A†</td>
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<td>Rachmat†</td>
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<td>ballum*</td>
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<td>Tarassovi</td>
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<td>Mitis Johnson†; Perepelicin†</td>
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* Alternative within a serogroup
† Alternative strains for the serovar
** Serovars of *L. biflexa* (see section A 4.2.1, p. 28).
representative strains should agglutinate optimally with antisera to all serovars of a given serogroup. A suggested list of serovars for this purpose is given in Table 7, p. 77. If many serum samples are to be examined and if the expected incidence of positive tests in the sera is low, five serum samples can be pooled together. Samples can be taken from each. The pooled sera are diluted so that for each sample, the final screening dilutions will be those indicated above.

(b) Confirmatory final tests. Positive-reacting sera are serially diluted in PBS to approximately 1:10 000, in tubes or in the wells of porcelain or plastic plates or of microtitration plates. Each serum dilution is then tested with the antigen or antigens which caused positive reactions in the screening tests, the test incubated as above, and the titre is determined. In the case of positive pooled serum, the individual reactive serum (or sera) within the pool is first identified by testing against the reacting strains. The above procedure is then followed.

(3) Reading, evaluation and recording of results.

(a) Reading. The final result is based on the end-point reaction, which is defined as the highest serum dilution in which the serum-antigen mixture shows approximately 50% agglutinated leptospires. This means that 50% free leptospires are present. It is helpful, in the subjective microscopic assessment, to compare the density of non-agglutinated leptospires in consecutive serum dilution-antigen mixtures, with control dilutions of the antigen suspension used, diluted to 100, 50 and 25% of the starting density. These controls are made up by making 1:2, 1:4 and 1:8 dilutions of the culture used originally.

(b) Evaluation. The MAT is only relatively serovar-specific. Often cross-reactions between different parasitic serovars belonging to separate serogroups are observed following infection with only one strain. The cross-reacting antibody may appear first. This "paradoxical" reaction is seen especially in patients' sera obtained during the second or third week after the onset of the disease. However, such paradoxical reactions tend to drop in titre during the following weeks and usually the homologous titre finally predominates, indicating the serogroup to which the causative organism belongs. The reactions may be harder to interpret if the serovar of the strain causing the illness is not included in the battery of antigens. A continuous lower titre reaction with one of the strains of the battery during the first 4 weeks after onset of the disease may indicate this. It may also indicate residual antibodies due to a previous infection, in which case the patient's serum should be tested against strains representing all serovars belonging to the serogroup indicated. Strains that then give high-titre reactions are considered to be related to the infecting strain. The serovar of the infecting strain may be revealed eventually by means of absorption tests with those strains and the serum under investigation. If the strains of 2 serovars both continue to react strongly during the first 4 weeks, a double infection might be considered.

Occasionally, a low titre or no detectable agglutination titre can be demonstrated for weeks after an infection. Unless the causative organism is isolated in blood culture during the first week of the illness or from the urine from the second week onwards, the diagnosis of leptospirosis cannot be made with certainty and will have to be based on clinical evidence alone.

(c) Recording the results. The results can be recorded in several equally effective ways. It is therefore unnecessary to recommend a method, provided that the recording is efficient, easy to read and surveyable. Before the serological diagnosis of a suspected case of leptospirosis can be undertaken, it is useful for the laboratory to have details of the date of onset of the illness and the date on which the specimen was taken, as well as the clinical signs and symptoms and epidemiological data. Such records are indispensable for a correct interpretation of the results and for retrospective epidemiological surveys, for detecting endemic areas, and for gathering clinical details (see section B 4.1.1.1, p. 84).
3.4.1.2 Macroscopic (slide) Agglutination Test. (23-27)

In principle, the test is performed in a similar manner to other well known slide tests (such as those used for salmonellosis and brucellosis, and the VDRL cardiolipin test for syphilis). A certain amount of concentrated killed antigen and patient’s serum are mixed on a plate, slide or card and allowed to react for a specified period, after which the presence of agglutination is determined by naked eye. This method has proved quick and simple. Slide-test antigens are usually suspensions of leptospires that have been killed by formalin, but antigens killed by thiomersal or by heating in a boiling water bath have also been used. The antigen can be stained (e.g., red by Ponceau S) for better contrast.

Many laboratories use the slide test as described by Galton and colleagues (24), and consider it to be a reliable screening test for the detection of acute and recent infections. The test is less sensitive than MAT, i.e., it gives an earlier positive reaction, but the titres are lower and tend to decrease sooner.

The Mazzonelli-Mailoux slide test (24), modified by Coghlan (see section C 3.1.3.1.1, p. 107), uses a heat-killed concentrated Patoc 1 antigen as the sole reagent. Some workers have found this test useful as a quick, reliable means of determining whether a patient is likely to be suffering from acute leptospirosis, no matter what the serovar of the infecting strain. It soon becomes negative or weak on recovery from infection. It is not suitable for surveys or screening for evidence of previous infection (see below).

1) Antigens. A satisfactory antigen should react well with antiserum that is within a 4-fold dilution below the known titre determined by MAT. Antigens which do not meet these criteria or which show clumps or auto-agglutination should be discarded. The antigens are usually stable for at least one year, but should be tested for stability and sensitivity before use. For rapid screening, the batteries of 12-21 antigens (24, 26, 27) are combined into 4-7 pools, each with 3 antigens and representing one serogroup. One or more of these pools will react with most antibodies induced by the infecting strains of leptospires. Locally prevalent strains should be included. Reactive sera should be tested by MAT to determine the antibody titre and the presumptive serogroup of the infecting strain.

(a) Cross-reactivity. The antigens used in the macroscopic (slide) agglutination test appear to react with a wider range of different antisera than do the antigens in the MAT so that sera, when positive, usually react with several slide-test antigens of different serogroups.

Antigens prepared from strains of the L. biflexa species, such as Patoc I and Sao Paulo, show a broad genus-specific coverage with human sera (18, 27). It can therefore be an advantage to add Patoc I antigen to the existing pools of slide-test antigens when human sera are examined (25, 27) (see section B 3.4.1.1.2(a), p. 76).

(b) Specificity. The specificity of the original slide test appeared to be influenced by the species of animal from which the serum under investigation was taken and by the preparation of the antigen used. With human and rabbit sera a broad cross-reactivity with many different antigens within the genus was demonstrated (24), whereas with bovine sera cross-reactivity seemed to be limited. A high percentage of apparently false-positive reactors was obtained (24), possibly owing to the lack of standardization and quality control of the antigen preparations by different workers. The number of reported so-called false-negative reactions has been small. One must bear in mind that the slide test reveals antibodies in the acute phase up to 1-2 years after an infection, whereas the MAT will react to residual antibodies for many months (and longer, up to over 20 years) after contracting the disease.

3.4.2 IMMUNOFLUORESCENT METHODS

Immunofluorescent methods are fast and reliable means of determining the levels of antibodies to leptospires (28) (see section A 4.2). They may be used for animal sera, but have mostly been developed for use with human sera. The technique is the same in both cases, but different antigens
are recommended (see sections C 3.1.4.2, p. 118, and C 3.2.5.3, p. 129). Indirect fluorescent (IF) methods are used, with an antiserum to the globulins of the animal species under investigation, conjugated usually with fluorescein isothiocyanate. Such conjugates may be further refined as anti-IgM or anti-IgG. In all cases, they may be prepared by laboratories where special immunochemical skills are available, or they may be purchased commercially.

Various satisfactory techniques have been described, differing in such details as the strains and their preparation for use as antigens, the time and method of fixation, and conditions of staining. The procedures described here are suitable for general use. The final choice of a method may depend on individual experience, equipment, skill and costs.

3.4.2.1 Preparation of Antigens. Antigen suspensions may be prepared from any strain of a serovar known to react (in MAT) with homologous animal or human antisera. In practice, this means that locally prevalent serovars should be used for animal sera, which react relatively specifically with the infecting serovar. For human sera, which react much more broadly, especially in the early stages of infection, a cross-reacting strain such as L. biflexa serovar patoc strain Patoc 1, or L. interrogans serovar copenhageni strain Wijnberg, may be used.

The method of preparation of the antigen, and other technical details of performance of the test, are described in sections C 3.1.4.2, p. 118 and C 3.2.5.3, p. 129.

3.4.2.2 Performance of the Test (see section C 3.1.4.2, p. 118).

(1) Serum dilution. Serial twofold dilutions are made, using small volumes (e.g., 0.1 ml) and starting with an initial 1:10 dilution. Each serum dilution is placed over a drop of dried, fixed antigen on a prepared slide. The mixture is incubated at room temperature for 30 min. The slide is washed and the antigen-antibody spots are overlaid with a fluorescein-conjugated antiserum to the immunoglobulins of the animal species being studied. Specific anti-IgM or IgG conjugates may be used.

(2) Microscopic methods. Various illumination systems are satisfactory. Incident light illumination, if available, gives the best results, but good easy readings may be made using transmitted light from a quartz-halogen lamp or a mercury vapour lamp, and a dark-field condenser. While the reading is facilitated by specially designed equipment, the results can be obtained by adapting any suitable simple microscope using simple lamps and filters.

(3) Evaluation of results. The immunofluorescent method is suitable for determining recent infections, especially in human sera. In these, the reactions considered to be "non-specific" occur with low dilutions, so that it is recommended that only titres of over 1:40 should be considered "significant". Sera tend to give positive results at about the same time as the MAT, i.e., about 1 week after the onset of symptoms, or earlier. Maximum titres are reached 10 days after the onset, remain high for about 5 weeks, and then fall until the antibody level may be undetectable by about 5 months. A titre of 1:80 or more is considered "positive", as is a rising titre from 1:40 to 1:80. Stable low titres between 1:40 and 1:80 are difficult to interpret. It may be helpful in these and other cases to determine the titres of IgM and IgG (section B 3.4.3, p. 81). Usually the IgM titres predominate in the early stages, although the reverse may occur. An IF titre ratio of IgM/IgG = 1 or greater indicates recent infection.

3.4.3 COMPLEMENT FIXATION (CF) TESTS (see sections A 4.2, p. 28 and C 3.1.3.2, p. 110).

Complement fixation tests, using as antigen a suspension of leptospires, or a preparation from leptospiral cultures, have been used as screening tests, especially for human sera. In the latter case, an antigen derived from L. biflexa, patoc Patoc 1 (see section C 3.1.3.2.1, p. 110) has been used. The advantages of CF are the ability to process large numbers of sera and to read endpoints objectively and quantitatively, using equipment (including instrumented and automated systems), techniques and skills that are available in most microbiological (including viral) serology laboratories. The disadvantages are the instability and short shelf-life of the reagents, the anticomplementariness
of the sera, especially animal sera contaminated with bacteria during bleeding or transport, and the technical complexity on account of the considerable standardization of the reagents required. The latter point precludes (for practical purposes) the use of CF as a quick test on a single or small number of sera, unless a battery of similar tests is being processed. It is thus of more value as an epidemiological tool than as an aid to rapid diagnosis in individual sick patients or animals.

For the preparation and standardization of antigens, see sections C 3.1.3.2(1), p. 110 and (2), p. 110; for the preparation and standardization of other reagents and for the performance of tests, see section C 3.1.3.2, p. 110.

3.4.4. **HAEMAGGLUTINATION AND RELATED TESTS (HA and HL) AND THE ELISA TECHNIQUE**

Sheep or human group O erythrocytes, coated with leptospiral polysaccharides, are useful antigens for detecting IgM antibodies to leptospires. Although the haemagglutination tests have been available for many years and been recently modified for use in microtiter plates, they have not been as widely used as MAT except when used in parallel with it. The results do not always correlate with the MAT results, probably because different antigen-antibody systems may be involved. Consequently, there has been a tendency to discount the value of the HA test and to accept the results of the reference standard test, the MAT. Nevertheless it is valuable for screening and for the diagnosis of recent infection. The erythrocytes may be coated freshly for each test, or may be preserved. The sera to be tested may need to be absorbed to remove heterophil antibodies. Different antigens are required for human sera and animal sera (see section A 4.2).

The haemolytic test (HL) (see section C 3.1.3.4, p. 113) is essentially similar to HA with respect to both antigen and antibodies reacting, except that the addition of guinea pig complement at 37°C leads to haemolysis of the sensitized erythrocytes in the presence of antibody. The end-point is haemolysis instead of haemagglutination, and can be read by automated equipment which will detect and measure the haemolysis. The test is somewhat more sensitive than HA.

For the preparation of HA and HL antigens, coating of erythrocytes, standardization, and performance, reading and interpretation of results, see sections C 3.1.3.3, p. 111 and C 3.1.3.4, p. 113; for the ELISA technique (29, 30), see section C 3.1.4.3, p. 120.

3.4.5 **IMMUNOGLOBULIN FRACTIONS (IgM and IgG)**

Antibody activities in IgM and IgG classes of immunoglobulins have been demonstrated in many animal species after an infection or immunization, revealing distinct and important differences. All human patients produce IgM agglutinating antibody following infection, but not all produce IgG.

The reasons for individual differences are unknown, but they are related to the patient rather than to the leptospires. IgG antibody has been detected by ELISA in those in whom it could not be demonstrated by MAT (29), but it is not clear whether it reacts with the same antigen in both tests. IgM antibodies appear first, and it may be several weeks before IgG antibodies can be demonstrated. Antibodies of both immunoglobulin classes, derived from either human, rabbit or cattle sera, have been shown to be protective in passive protection tests. Antibodies to polysaccharide antigens have been shown to be solely IgM in man and rabbits after intravenous immunization. In rabbits, immunized subcutaneously using Freund adjuvant, the antibodies were IgG as well as IgM. The IgG antibodies from rabbit and human sera have shown greater specificity (than do the IgM antibodies) to those leptospiral antigens with which they were tested.

The class of immunoglobulin reacting with leptospires or their antigens may be determined by indirect methods without the preparation of separate fractions of the antiserum. These methods include immunofluorescence and immuno-electrophoresis. With immunofluorescence, the antiserum is allowed to react with the leptospires or antigen, and washed to remove excess uncoupled antibody. The preparation is next treated with a fluorescein-labelled antiserum to the immunoglobulin of the animal species used, specific for either IgG or IgM; it is then washed and viewed microscopically as described for immunofluorescence. Antiserum labelled with other fluorochromes or with enzymes (e.g., peroxidase) may also be used. Sera from patients or animals may also be studied by immuno-electrophoresis. The serum is electrophoresed, and the leptospiral antigens are placed in a trough.
alongside the electrophoresed serum. Anti-IgG, anti-IgM or anti-whole serum (each of these corresponding to the appropriate animal species) is placed in a similar trough on the other side of the electrophoresed serum. The lines of precipitation are compared, on either side, after incubation. At present, this approach has been more useful as a research than a diagnostic method, although counterimmunoelectrophoresis has been used for diagnosis.

Radial immunodiffusion has been used to measure the IgM or IgG in sera, but the technique does not ensure that only specific, i.e., antileptospiral antibody activity is measured.

3.4.5.1 Preparation of Fractions (see section C 3.1.1.3.(1), p. 106). The most useful methods are sucrose gradient density centrifugation, selective immunabsorption, or gel filtration.

3.4.5.2 Standardization and Use in Serological Tests (see section C 3.1.1.3.(2), p. 107).

3.4.5.3 Interpretation of Results. It is clear that all animals produce IgM antibody, but the rate of production and the stage of illness or immunization at which the antibody is detectable, are variable. IgM antibodies are produced which react in MAT, in the agglutination of erythrocytes coated with polysaccharide antigens (HA), or in ELISA using sonicated leptospires. IgG antibody is produced regularly by rabbits; only sometimes (late in the infection) by man; and variably in other animal species. The antibody produced may depend on the nature of the antigen and the route of immunization. IgG antibodies have proved to be more specific for their antigens, in general, than IgM. Further research is required to understand the significance and value of the responses of the various immunoglobulin classes during and after infection or immunization.

3.4.6 SEROLOGICAL METHODS FOR THE IDENTIFICATION OF ISOLATES (4, 19)

Typing of newly isolated strains is a laborious procedure suitable only for well equipped laboratories with adequately trained and experienced staff and a battery of reference strains and immune sera. However, serogrouping and sometimes identification to serovar level may be practicable in laboratories performing MAT.

3.4.6.1 Production of antisera (see sections C 3.1.5, p. 121 and C 3.1.6, p. 122). Rabbit immune sera are used for serotyping.

Two agglutinogenic systems in leptospires were described in 1971. One, based on thermostable antigens (TSA), is considered to be the fundamental system. The second, based on thermostabile antigens (TLA), may be used for the subdivision of serovars (sharing this antigen) into "varieties". Two types of immune sera are therefore required in typing procedures. Immune sera, free of agglutinins against TLA which may be present (i.e., containing anti-TSA alone), are used for grouping and serotyping. Sera prepared with living cultures, containing anti-TLA as well as anti-TSA, are necessary for the recognition of varieties containing a TLA (see section C 3.1.5.2, p. 121).

For immunization procedures, see sections B 3.1.5.2, p. 121, C 3.1.5.2, p. 121.

3.5 PREPARATION OF ANTISERA

3.5.1 SELECTION OF ANIMALS (see section C 3.1.5, p. 121).

3.5.2 PREPARATION OF ANTIGENS (see section C 3.1.5, p. 121).
3.5.3 IMMUNIZATION SCHEDULES and TECHNIQUES (Method recommended by TSCL).

Rabbits should be inoculated intravenously into the marginal ear veins with successive doses of 5-7 day-old live culture containing approximately $2 \times 10^8$ organisms per ml. Five injections of 1 ml, 2 ml, 4 ml and 6 ml should be given at 7-day intervals (see section C 3.1.5.2, p. 121). A different protocol for preparing antisera to fresh isolates to be identified, using TLA as well as TSA, is described in section C 3.1.5.2, p. 121. Similar results are produced with either method.

3.5.4 BLEEDING SCHEDULE AND TECHNIQUES

Fourteen days after the last injection, a small sample of blood should be taken from the ear vein for testing.

3.5.5 TESTING OF ANTISERA

To determine the titre of the antiserum, serial 2-fold dilutions are prepared in buffered saline beginning at a dilution of 1:25. Equal volumes of antigen are added to the diluted antiserum, and the mixture incubated at 30°C for 90 min. A drop of each mixture is examined by dark-field microscopy using x10 objective and x10 oculars. The titre of the antiserum is the last dilution showing 50% agglutination (see section C 3.1.4.1, p. 115).

3.5.6 MAINTENANCE OF STOCKS OF ANTISERA (see section C 3.1.5.4, p. 122).

3.6 ANIMAL EXPERIMENTATION

3.6.1 INOCULATION FOR ISOLATION OF STRAINS (see section B 3.2.2, p. 69).

3.6.2 INOCULATION FOR MAINTENANCE OF VIRULENCE

Virulence may be restored to, or maintained in, a strain of leptospires by passage through a susceptible experimental animal, depending on the serovar (see section C 3.3, p. 130). The principle is that avirulent mutant leptospires grow in laboratory conditions, gradually replacing the virulent leptospires in a culture. Animal passage selectively allows the survival of virulent organisms, while the avirulent ones die in the animal. Some cultures, in which only avirulent leptospires exist, are irretrievably avirulent, (i.e., they cannot be made virulent by animal passage. Others, however, in order to regain virulence, must be injected (usually intraperitoneally) into an animal and the leptospires are isolated from the blood or organs just before death (see section C 3.3.2, p. 130), or at autopsy (see section C 3.3.3, p. 131). The former is preferable in order to reduce the risk of contamination from other bacteria which may invade the carcass post-mortem. Best results are obtained from the injection of a dose of 1 ml of about $10^8$ leptospires/ml of a late log-phase culture (see section B 3.2.2, p. 69). The strain is recovered for further culture by heart puncture or at autopsy (see section C 3.3.3, p. 131). This culture, after it has grown, may be inoculated again into another animal, and so on, to maintain maximum virulence; or the blood, or an emulsion of the liver containing large numbers of leptospires, may be injected directly into another animal. In the case of direct transmission to another animal, it is desirable not to use too large a volume of blood or tissue, especially from animals 5-7 days after injection, because they may have sufficient antibody to protect the recipient.

Observations at autopsy and autopsy techniques are described in section C 3.3.3 p. 131.

3.6.3 INOCULATION FOR PURIFICATION OF CULTURES (see section B 3.2.3 p.70).
4. CONTROL OF LEPTOSPIROSIS

The control of leptospirosis is difficult (1, 3, 31, 32). The disease may not be obvious in either animal or human populations. Diagnosis is usually serological, and complicated by the large number of serovars which can cause infections indistinguishable from one another in practice. Each of these serovars is often, but not always, associated with certain carrier animals, but even within a particular region many types of animals may be carriers. It is not possible to compare conditions between regions, without factual current information, because the epidemiological pictures may be modified by differences or changes in agriculture, geography, economics and social factors (see section A 3). Thus, in addition to the need for accurate clinical and laboratory diagnosis (sections B 1.1 and B 2.9), adequate surveillance of potential sources of infection in domesticated animals or in wild animal populations, especially rodents, is necessary as a basis for a comprehensive epidemiological reporting and statistical service.

4.1 EPIDEMIOLOGICAL INVESTIGATIONS

Clinical and laboratory methods that are useful for identifying human leptospirosis have been described (see sections B 1.1, p. 43 and B 3.1, p. 67). Epidemiological investigation builds on a foundation of sound clinical and laboratory diagnosis and surveillance data, so that the infection is recognized whenever and wherever it occurs. The information must be reported, collated and assessed as a guide to preventive action (see sections below). A coordinating administrative structure is essential for collecting, assessing and distributing information (see sections B 4.4, p. 97 and D 1.3, p. 145). Central to every programme for control is the requirement that leptospirosis must be a "notifiable" ("reportable") disease in man and animals (see section B 4.3, p. 96, and Fig. 1).

Thus the principles in epidemiological investigation involve:

1. Identification of cases - initially clinically, followed by appropriate laboratory confirmation.
2. Reporting and collation of surveillance data.
3. Search for the source of individual infections, and of epidemic and epizootic waves.
4. Based on the above, the establishment of a level of endemic or enzootic infection.
5. Advice on the implementation of control action.

4.1.1 IDENTIFICATION OF HUMAN CASES

4.1.1.1 Utilization of Information Available. Simply reporting the cases diagnosed and collating the information will provide statistics of incidence, which may be refined by further classification. The date of infection, places of residence and work, age, sex, occupation, and contact with animals and infecting serovar are the minimum necessary data. Information, if available, on whether the diagnosis was made by culture, or serology, or both is also important on account of cross-reactions and the relatively low diagnostic reliability of serological diagnosis in an individual.

The above data, if collated from several patients, will provide information about when an epidemic has occurred, over what period of time, in what age and occupational groups, and in what geographical confines. A common source may then be sought, if indicated. If no epidemic is apparent, the sporadic cases can be followed up individually (see section B 4.1.3, p. 87). The importance of occupational and geographical factors has been referred to in sections A 3.2, p. 21 and A 3.3, p. 23.

4.1.1.2 Serological Surveys. Sometimes clinical information is not available, or diagnoses of leptospirosis are not made, or suspicious cases are not reported. There may not even be a reporting system. In these situations, a screening serological survey can give useful information about whether or not leptospirosis exists. The tests used employ the antigens of L. biflexa (Patoc I) in MAT, CF or ELISA. The survey may be general, covering sera from all age and occupational groups,
or selective for certain groups known to be at risk, such as farm workers, meat workers and rice planters. In either case, suitable statistical planning is necessary in advance of the survey, in order to ensure that the results can be analysed and will be statistically meaningful. Randomness of the samples is important whether in a general or a selective survey, unless the whole of a population is to be tested. The sample size will depend on the size of the total population to be tested, and on the likely incidence of reactive sera amongst the population (33) (see section C 4, p. 135).

4.1.1.3 Information from Hospital, Medical and Laboratory Records. In areas where leptospirosis is not known to occur and where laboratory facilities are not available, initial information should be sought through personal enquiries from hospitals, medical practitioners or the health authorities. Information should be centred on typical cases of Weil's disease, undiagnosed cases of "aseptic" meningitis, and especially on the epidemiological characteristics of epidemics of undiagnosed febrile illness, so that at least a suspicion of a diagnosis of leptospirosis might be considered. These illnesses may sometimes have a local designation, such as "water-fever", "cane-fever" and "ricefield-fever". Additional serological investigations are essential to confirm or negate the possible leptospiral etiology of these diseases. In regions believed to be free from leptospirosis, or if no documented information is available, serological surveys using a broad battery of antigens are indicated. Surveys of sera submitted for undiagnosed "viral meningitis", fever of unknown origin, hepatitis, or for blood transfusion cross-matching are useful in order to establish serological evidence of the background levels of sero-reactivity to leptospiral antigens in either selected occupational groups or the population in general.
Laboratory records of "positive" reactions of sera, and of isolates from cultures sent for diagnosis, are also useful in ascertaining whether leptospirosis exists in a region. Unless all cases are similarly investigated, and the laboratory serves a cross-section of the occupational, social and geographical distribution of the population, such records cannot be used for generalizations about incidence or prevalences.

4.1.1.4 Statistical Information. Statistical information is of no value unless it deals with accurate diagnosis, based on uniform and acceptable methods, coupled with reliable reporting. The epidemiological likelihood in diagnosis will be influenced by a knowledge of the likely incidence, and the statistics of incidence in turn depend on accurate diagnosis and reporting.

4.1.1.5 Delimitation of Areas. The statistics may show a high incidence of sporadic, apparently unrelated cases, or whether an epidemic is in progress. They may also indicate whether there is an occupational or geographical risk. In these cases there is a good chance of finding the source of infection (see section B 4.1.3, p. 87). At least, attention can be focused on a more defined problem than on cases that are sporadic and diffuse.

4.1.2 IDENTIFICATION OF ANIMAL INFECTION

The first steps are the recognition of leptospirosis (see section B 1.2, p. 43) and reporting, collating and circulation of information (section B 4.1.1, p. 84). Accurate diagnosis of infection in animals is essential. The sources of infection and of epizootics can be traced more easily if the infecting serovars are known (sections B 3.3, p. 70 and B 3.4, p. 76). Isolation of leptospires in culture is required in addition to serodiagnosis.

4.1.2.1 Information from Farmers, Veterinarians and their Records. Infection in animals will be recognized first by their attendants (see section B 2.1.2, p. 55). If it is severe enough, they will seek veterinary assistance, if available. The local, personal knowledge of illness resembling leptospirosis in domestic and farm animals can be collected into local statistics, but the information is suspect unless it is documented by proper diagnosis, confirmed in a reliable laboratory. Laboratory statistics form a satisfactory basis for epizootiological data, amplified by records from local veterinary practices. When leptospirosis is well recognized, and enzootic in either farm or wild animals or both, a knowledge of locally prevalent serovars and their hosts is valuable in determining the sources and controlling spread.

Where leptospirosis is not known to occur, enquiries should be directed to seeking cases of typical leptospiral presentation (Table 1, p. 16), such as haemoglobinuria or agalactia in cattle or other ruminants; etiologically unclear abortions, mainly in swine; "moonblindness" in horses; or Stuttgart disease in dogs. Information about the findings in slaughtered animals may be helpful. An observed high infestation with rats and other rodents is also very indicative.

4.1.2.2 Serological Surveys. In herds suspected to be infected, serological surveys cannot be avoided if the extent of the problem, including information about the number of different serovars involved, is to be assessed. The results of such surveys frequently also provide indications of infections to be expected in free-living animals that are endemic in the area. The size of the sample will depend on the size of the population of animals to be sampled, and on the expected incidence. Statistical planning in advance of the survey is essential (33, 34) (see section C 4, p. 135).

The methods to be used in the serological survey include MAT, CF and ELISA. The antigen chosen first should be the known serovars that are prevalent locally. However, serovars new to the area, or even not known before, may be prevalent. Representative sera of reactors should be tested with a wider range of serovars. The results of serological surveys of vaccinated farm animals are difficult to interpret.

4.1.2.3 Statistical Information. The data from isolations and serological diagnoses, together with survey data, provide the body of epizootiological information for control.
4.1.2.4 Delimitation of Points of Primary Interest. Geographical areas, types of animals, farming practices and climatic conditions, each representing special risks, can be deduced from a study of the statistics. It may be necessary to restrict ingress of farm animals into an infected area to allow an epizootic to subside, pending treatment or slaughter of the resident animals. Alternatively, fresh animals coming into an area free of leptospirosis may be screened before admission, to protect the resident animals (see sections A 3.2.3, p. 34 and B 4.1.3 below).

4.1.3 SOURCES OF INFECTION

Infection of humans, and of animals, derives ultimately from direct or indirect contact with other animals or at least their urine, or with water or soil contaminated with urine. Thus, in the search for a likely source of infection, it is necessary to know, if possible, (a) where the infection occurred - at work, at home or during recreation (see B 4.1.3.2 below), or in what barn, field or district, in the case of animals; (b) what serovars were involved; (c) what are the known local sources for that serovar (or serovars) at the place where the infection was believed to have been acquired; and (d) whether it was an isolated case, or endemic (or enzootic), or part of an epidemic or epizootic?

4.1.3.1 Information Concerning Domesticated Animals (see section B 4.1.2, p. 86).

4.1.3.2 Investigations into Suspected Foci. The investigations will depend on the answers to the questions (a) to (d) above. It is useful to start with the clinical case or sick animal. What serovar was involved? Where was the infection acquired? What contact was there with animals or water; and what animals (farm, household or wild), if any, are known to be carriers of that particular serovar, or any other serovar?

The quantitative relationships of the various animal species will determine the etiological structure of man-centred foci in farms. The predominant serovar in human infection will reflect that prevalent in the animals.

Seasonal incidence is related to water and immersion risks in cold climates (e.g., bathing in contaminated water), but not so much in warm climates. The seasonal incidence of occupational leptospirosis (see section A 3.2, p. 21) may be due to climatic conditions creating lush, moist feed, into which wild animals and rodents move; to seasonal farming activities, such as cropping, cutting fodder or planting in wet conditions; and to seasonal increases in slaughtering of animals for meat. Thus, many possible foci may need investigation even though initially the source of suspected infection may seem to be obvious.

Investigation of animals is described in section B 4.1.2, p. 86 and in the section below.

4.1.3.3 Laboratory Investigation of Animals in Suspected Foci in Risk Areas. (1) Farm and domestic animals. Every suspected or sick farm animal or dog should be tested serologically on two successive occasions, separated by 7-10 days, usually using MAT. Local strains should be used, if available, as the MAT antigens (sections B 3.4.1, p. 76 and C 3.1.4.1, p. 115).

Urine should be cultured (see sections B 3.2, p. 68 and C 1.2.4, p. 104), and examined microscopically if it is available when freshly voided (section C 3.2.3.1, p. 126).

Dead fetuses (following abortion), stillborn animals, and dead adult animals should be examined grossly at autopsy (section C 1.2.5, p. 104) and the tissues cultured for leptospires if they are fresh enough (section C 1.2.6, p. 104). Histopathological studies, including silver staining, should be included in the autopsy study (see sections C 1.2.6, p. 104; C 3.2.5.1, p. 128; and C 3.2.5.3, p. 129).

In large herds, when there is no clinical evidence of leptospirosis but a suspicion on epidemiological grounds, not less than 50 animals should be tested serologically, and not less than 100 should have their urine checked. On small farms, all the animals should be tested (section C 4, p. 130). An "unsatisfactory" herd test is given by detection of leptospires in any one animal; or by a single MAT 1:1000, in 25% of the animals tested; or by a 5-fold rise in titre in the MAT between tests. In pedigree breeding farms, the tests should be carried out
at least once a year on all sires, and on not less than 10% of the breeding female animals. All sires used as donors for artificial insemination should be tested by MAT twice a year, and pigs, cattle and horses should be tested before being moved in or out of a farm area. All animals should be tested in farms containing less than 25 animals, and 25% of those on larger farms (see section C 4, p. 135).

(2) **Free-living animals.** Free-living animals may be trapped in their natural habitats or in areas of exposure of domestic animals or people (section C 2.1.1, p. 104). Their species, age, sex and maturity are determined (section C 2.1.3, p. 105). This information is required to assist in the accumulation of data about host-leptospire relationships in different parts of the world. The animals are killed and autopsied and the organs are cultured for leptospires (section C 1.2.6, p. 104). Blood is taken for serology (sections C 1.2.3, p. 103 and C 3.3.2, p. 130), to be tested against isolates from that animal or other laboratory strains in the MAT (section C 3.1.3.1, p. 107). The cultivation of leptospires is important. Serological and histological information is valuable only to support statistics derived from successful cultures.

The presence of 3% or more carriers in the population is regarded as epidemiologically significant. If no cultural studies have been carried out, or if they are negative, an MAT of 1:100 in 3% or more of those animals studied is considered to be significant. The percentage of infected animals is also a good indicator of the epizootiological activity of a focus. In latent loci, the number of shedding animals is about 1% or less. In epizootiological waves, this rate rises 10-50-fold and involves, besides the main host, other animals inhabiting the same biocenosis.

### 4.1.4 TRANSMISSION OF INFECTION

**4.1.4.1 Defining Suspected Methods of Transmission.** Leptospirosis is transmitted between animals by contact (in one form or another) with infected urine or water contaminated with infected urine. Man can be infected by the same methods, or by direct contact with infected animals, as in abattoirs, or in veterinary practice, or very rarely from humans (see section A 3.2.2, p. 22). There are numerous ways by which transmission can occur in practice. They are determined by the animals involved, by the farming habits and social customs in the community in question, and by geographical and climatic considerations (section A 3.1, p. 20 to A 3.3, p. 23).

In the centre of the cycles of transmission are the carrier animals, whether wild or domestic. Amongst nesting animals, including rodents and other wild animals, infection may occur from the parents to the young soon after birth, before the young leave the nest. Congenital infection may also occur, both in nesting wild rodents and in farm animals. The control of carriers is discussed in section B 4.2.1, p. 90.

Insects and other arthropods have been shown experimentally to be capable of spreading leptospirosis. This is not considered to be a significant means of transmission, but further studies would be useful to clarify the point.

From the carriers, infection is almost always spread by water, whether in ground water or in free-flowing or stagnant surface water. Thus the major thrust in the investigation of transmission is to seek suspicious sources of infection and, having identified them, to look for the means by which spread has occurred. Alternatively, the method of transmission may become apparent, first from epidemiological or epizootiological studies, leading to identification of the sources of infection, as in epidemics due to a common source (see section B 4.1.3, p. 87). In all cases, it is easier to find a source of infection if there are several cases with a common source. Individual cases may be related to a particular incident of infection, but generally the sources are obscure (section A 3.1, p. 20).

The source of infection gives a clue to the method of transmission. In man, recognition of a common occupational source of leptospirosis can lead to information about how it was transmitted. The occupational source may be recognized by the occurrence of infection in epidemic or endemic or sporadic forms, in people who work in the same place, or in those who are engaged in similar tasks in the course of their jobs or daily farming or household duties. A common source of infection
during leisure activities (bathing, water-skiing, amateur farming) can more easily pinpoint the method of transmission (see section B 4.1.3.2, p. 87). A common place of infection, not related to occupation, may indicate a mode of transmission leading to the source.

4.1.4.2 Investigation of Surface Water and Drinking Water. (1) Direct evidence. Surface waters, contaminated by leptospires, can be sampled into sterile containers, which are then closed, cooled (not frozen) to below 30-35°C (if necessary), and sent to a leptospirosis laboratory. There, the water will be examined for the presence of pathogenic leptospires. If the latter are isolated, they can be typed to see if they are similar to the serovar responsible for the local clinical infections (see sections B 3.2, p. 68 and B 3.3, p. 70).

(2) Indirect evidence. Infecting sources (e.g., rodents or cattle, which contaminate water) may be found to carry the same serovars as those that infect other animals or people. The leptospires, however, may not have been detected in the water because of delays in sampling the area, changes in weather, use of fertilizer, etc. Nevertheless, the water may be presumed to be the vehicle for transmission if a likely source is found, and if evidence exists for the contact of patients or acutely sick animals with that water.

4.1.4.3 Investigation of Soil. Wet, contaminated soil has been shown to cause leptospirosis, even if it was only the soil round the root of a plant that was sent to a laboratory for agricultural studies. Thus soil is always suspect when there is a likely source of infection. The sources of infection of soil, mud, and rice-field water have been described (see sections A 3.2, p. 21 and A 3.3, p. 23). Best results are obtained by sampling wet soil early in the morning if nocturnal contamination is suspected. The soil is packaged into a glass or plastic, water-tight sealed container, shielded from the light, and sent to a leptospirosis laboratory. The methods for the isolation and identification of pathogenic leptospires from soil are similar to those used for the investigation of water. The leptospires are first washed from the soil with sterile water, allowing the mud to sediment. The supernatant is filtered coarsely. The filtrate is then treated as a water sample (section C 3.4, p. 132).

4.1.4.4 Investigation of Other Vehicles of Transmission. These may be animal carcasses or organs (see sections B 2, p. 34 and B 2.9, p. 63), or direct contact of man with an infective source during, for example, harvesting wet grass or vegetable produce (sections B 4.1.3, p. 87 and B 4.1.4, p. 88) or while milking (see sections B 4.1.5.2, p. 90 and B 4.1.5.3, p. 90). The methods of investigation of the sources and the methods of transmission are obvious from their contexts and techniques (sections B 3, p. 67 and C 1.2.6, p. 104).

4.1.5 ENVIRONMENTAL AND SOCIAL FACTORS

Environmental and social factors are of decisive importance for the spread of leptospirosis in a given region. Of utmost importance are the following:

(a) Geographical and climatic conditions. Leptospirosis flourishes in warm and humid areas, which are rich in surface waters, lakes and rivers, and are frequently flooded and inhabited by a broad range of different kinds of animals. Heavy rains increase the risk of spread of infections, apparently by washing leptospires out of the ground water. Outdoor occupational activity in these areas is always fraught with a high risk of infection (section A 3.2, p. 21). These conditions are in general beyond human control. When man-made projects cause flooding or increase the likelihood of water contamination, people and domestic animals are placed at risk. That risk can be recognized and steps taken in advance to prevent leptospirosis.

(b) The ecological relations in such areas lead to the formation of endemic foci of leptospirosis. Leptospires may circulate in these areas for many years. Factors influencing the dynamics of animal populations, together with climatic and other conditions, are considered to play a major role in the appearance of epizootic waves amongst wild animals, mainly small rodents (section A 3.1, p. 29). Epizootics are observed in varying time intervals and are often followed by outbreaks in domesticated animals and in man.
(c) Occupations of high risk, the most important of which are work in rice fields, cane fields, or involving other agricultural crops, animal husbandry, slaughter houses, sewers, mines, and military operations.

(d) Leisure activities, leading to outbreaks of leptospirosis, e.g., from swimming (see section A 3.2.3.2, p. 23).

Whenever there is a change in farming practice, the equilibrium changes. For example, with heavy stocking of cattle on farms, the soil becomes wetter (than it would otherwise be) and potentially more heavily infected with leptospires (see sections A 3.1, p. 20 and A 3.3, p. 23). The change to "herring-bone" milking sheds has also been associated with an increased incidence of leptospirosis. In a "herring-bone" milking shed, concrete-lined trenches (about 1.3-1.5 m deep) are set at an angle, on each side of a long central trench. The cows are placed in stalls at ground level, between the trenches, rear end to the central trench, and are machine-milked by operators who walk up and down the trenches; the udders of the cows are at about the level of the shoulders and face of the operator, who is easily splashed if the cows urinate during milking or when moved into the stalls. On the other hand, the burning of sugar-cane and mechanization of its cutting in some areas have led to the elimination of leptospirosis as a cane cutters' disease.

Similar occupations may present different pictures of leptospirosis in different countries, depending on their social organization and economies, e.g., compare the situation in a rice-growing village, in a European village, and in a dairy farm in New Zealand. A change from another type of agriculture to pig production is also often associated with increased leptospirosis. Changes in life-style that encourage leisure and water sports in fresh-water areas also increase the risk of exposure to leptospirosis. Improved working conditions in mines, however, have reduced exposure although the conditions for disease transmission remain.

4.1.5.1 Determination of High-risk Areas. This information must be gained from local statistics (sections B 4.1.1.1, p. 84 and B 4.1.2.1, p. 86). There are considerable local and social differences (section B 4.1.3, p. 90) which preclude generalizations. Once an area is defined, action can be taken to find the sources and the means of transmission and to control them. Conversely, without local statistics of value, it is impossible to make much progress except in very general terms. It is much more cost-effective to be able to identify risk areas and concentrate on them.

4.1.5.2 Determination of High-risk Occupations. The remarks in the previous section are relevant here also. A definition of "occupation" is required because what is seen as an occupation in some countries (e.g., tending pigs or cattle, or milking cows) is part of daily life in another and is sometimes carried out with other occupational or professional activities.

4.1.5.3 Determination of High-risk Environmental Factors. Similarly, a knowledge of local factors is required, in addition to general knowledge of the epidemiology of leptospirosis. The local knowledge must be based on current information which can be derived from the statistics available, following the previously delineated pattern of diagnosis of infection in people or animals, collation of the information and publication of the statistics derived from it.

4.2 PREVENTIVE MEASURES

4.2.1 CONTROL OF SOURCES OF INFECTION AMONG DOMESTIC ANIMALS

The following measures are important:

(1) Isolation. Domestic animals, once identified as either diseased or subclinically infected, should be separated from uninfected animals. The isolated animals are treated with chemotherapeutic drugs (see sections B 2.9.3, p. 66 and B 2.9.4, p. 66). Floors, soils, tools, etc., contaminated with the urine, fetus or fetal membranes of the animals, must be washed with
disinfectants (section B 4.2.3, p. 93). The isolated animals must be kept out of contact with rodents and wild animals. Pigs must be isolated from cattle, sheep or goats because infected pigs apparently shed leptospires in their urine in greater numbers and for longer periods of time than do cattle and other domestic animals. Infected dogs on farms should be isolated from other domestic animals for the same reason.

(2) Treatment—chemotherapy. Leptospiruric domestic animals, which shed enormous numbers of the organisms into the environment, present a danger to others and may be treated with dihydrostreptomycin, as described in section B 2.9.3, p. 66.

(3) Slaughter. Under certain circumstances, where the only source of enzootic infection is the continued presence of a carrier or carriers in a herd, the slaughter of these carrier animals may be contemplated. The economics of a "test and slaughter" policy must be assessed (see section B 4.3, p. 96). The advantages are potentially a leptospirosis-free herd, and the consequent freedom from clinical and subclinical leptospirosis (the latter is manifest as mastitis, loss of milk production and fetal loss), particularly if the herd can be kept free from further, introduced infection. The disadvantages are the economic burden, and the futility of the policy if the herd becomes reinfected from a fresh stock or wild animals or rodents.

Animals that have died of acute leptospirosis, or have been slaughtered during the acute infection, should be buried or burnt. When animals that are known to have converted to seropositive are slaughtered, their kidneys must be presumed to be infected with leptospires, and should not be used for food.

4.2.2 CONTROL OF SOURCES OF INFECTION AMONG FREE-LIVING ANIMALS

4.2.2.1 Environmental Control: Physical Measures. Agricultural practices frequently allow commensal and endemic rodent species to build up to high population densities either in growing crops and stockyards or in harvested products that are stored in bulk, and it is often accepted by farmers that this is inevitable. Such rodent populations not only act as sources of leptospirosis for animals and men working in the habitat, but also serve to disseminate infections when the populations disperse as a result of overpopulation or a change in the environment.

By far the most effective way to prevent the development of high rodent population densities is by controlling the physical environment, thus denying rodents access to their two principal needs of food and shelter. Once large rodent populations have developed, chemical rodent control is the only way to reduce their numbers.

Farm buildings should be made of concrete or brick, with solid foundations which prevent burrowing; doors should be tight-fitting, with metal "kicking plates" at the bottom. The basic principles of design and maintenance of premises, which will exclude rodents, have been explained by Jenson (9) and by Drummond & Taylor (10).

Animal feeding stuffs should be kept in rodent-free buildings and contained in metal or concrete silos which rodents cannot enter. Food spillage is unavoidable but should be cleaned up at once. The siting of piggeries in rodent-proof concrete pens is important because many cases of leptospirosis in the past have been due to badly housed, rodent-infested pig sties. As with pigs, food for poultry and cattle should be placed in adequate feeding troughs and spillage kept to a minimum.

Combine harvesting has been a valuable factor in reducing the numbers of rats and mice which formerly built up to high densities in cereal ricks and were released on threshing. Where cereal ricks are built, they should be surrounded with a rodent-proof barrier before threshing and all the rodents should be killed.

Stored, harvested cereals, if bagged, should be kept as in food depots (see section A 5.1.1, p.30), but storage in bulk containers is preferable because these are easier to keep rodent-proof. Even the premises of village stores in Africa, made from sticks and sun-baked mud, can be rat-proof when carefully constructed (39).
In both urban and rural environments, untidiness aids the settlement of rodents. The simple procedure of cleaning up urban blocks in Baltimore (USA) was shown to reduce the rat population by 36%. Heaps of untouched debris and machinery around a coal mine were shown to be a site for rat harbourage (37). Involvement of food in untidiness and waste benefits the rodents; even the sewers that serviced markets and canteens had high rat populations (38).

Demolition work to clear old buildings may release rats to the surface unless the sewer connexions are sealed. Where the water trap in the lavatory dries out in unused dwellings, rats may enter the building from the sewage pipe.

A major source of rat infestation is the municipal or communal rubbish tip where the food waste nourishes large rat populations. Tips should be sited well away from urban concentrations and the day’s tipping should be buried with soil at the end of each day, whenever possible. When this is not practicable, the tip should be surrounded by rat-proof barriers.

4.2.2.2 Chemical Control of Rodents. The use of poisons (rodenticides) is the most efficient means of urban and rural rodent control when conditions have allowed populations to build up. Such poisons are of two sorts: acute and quick-acting, relying on a single dose for killing; or chronic and slow-acting, requiring several doses.

Acute poisons, suitable for general use, are zinc phosphide (see section A 5.2.1.1, p. 35) and red squill. Others are effective but may be used only under strict supervision by specially trained staff (sodium monofluoracetate, thallium sulfate (section A 5.2.1.1, p. 35), arsenious oxide, fluoracetamide, and strychnine). Alpha-naphthyl thiourea (ANTU) is effective only for R. norvegicus, and causes extreme aversion and balt-shyness in survivors, limiting its usefulness to one attempt in 6 months in a given locality. Alpha-chloralose lowers the body temperature of rodents which consume it, and is effective only in cool climates. Chronic poisoning is carried out by using anticoagulants. These are used at low concentrations, their action being cumulative when eaten over several days. Warfarin, the best known and most frequently used anticoagulant, is a coumarin and was first used in 1950. More recently developed coumarins and indandiones are diphenadione, pival, coumatetralyl, chlorophacinone, brodifacoum and difencoum. Animals, poisoned by anticoagulants, die slowly from internal haemorrhages and rarely evidence poison-shyness which may occur after eating a sublethal dose of an acute poison. Unfortunately, resistance to warfarin is now known to occur in both species of rat and in the house mouse, and the brown rat and the mouse have developed resistance to other anticoagulants.

In field rodent control, acute poisons are most widely used, both on account of economy and effectiveness. They are not without their drawbacks, however, and because of their high toxicity, must be used so that neither human beings nor domestic animals can contact them during primary or secondary poisoning. Furthermore, indiscriminate use which might lead to the deaths of natural predators could in the long term make things worse.

Rodents living in burrows, away from buildings, can be gassed either by a powder that produces hydrogen cyanide gas or by aluminium pellets producing phosphine (39, 40, 41). This technique works best when the soil is damp and consolidated. Carbon monoxide may also be used and exhaust gases from trucks in the field have proved of value on more than one occasion.

Publications giving details of poisons in use today, their formulations, antidotes and techniques for field use are available (11, 12, 42-49).

4.2.2.3 Biological Control of Rodents. (1) Habitat manipulation. In agricultural systems, the tendency towards monocultures encourages a simple fauna with high population numbers in a few species. As a result of increasing complexity of the habitat, competing species might invade and affect the monopoly of the pest species. A landscape mosaic will increase the number of small rodent competitors and predators, as well as keep more buffer food for predators than in monocultures.

The balance of rodent species will be affected in various ways by measures such as cattle grazing, which will reduce small herbivorous rodents and increase the graminivorous species; soil disturbance by ploughing; or the use of herbicides and burning. An important carrier of leptospirosis may be excluded by such manipulation.
(2) **Habitat alteration.** In East Africa, in the years when heavy rain falls late in the season, there are periodic outbreaks ('plague') of rodents as a result of an abnormal growth of weeds and grasses during the following dry season. Outbreaks may therefore be predictable from rainfall data some months in advance. During the dry season, the rodents depend on the cover provided by weeds and grasses to protect them from predators, mainly birds, and on weed seeds and cereals left after the harvest for food. Gyromowing was found to cause a marked reduction in numbers, some being killed by the mowing but most by the markedly increased numbers of raptors in the area. When cover was removed, these found predation easier. Very little rodent migration from the area took place, most being killed on the spot. Similar or other measures to alter the habitat may be developed by studying the biology of endemic rodent populations.

(3) **Prospects for new methods of control.** Ecological control by competitive displacement, and the use of pheromones, chemosterilants and genetic control are experimental and unproved methods which cannot be recommended at present. They may prove to be useful if future research justifies their application.

### 4.2.3 CONTROL OF TRANSMISSION

#### 4.2.3.1 Disinfection of Drinking Water.** Standard hygienic methods for treatment of drinking water to render it microbiologically safe will kill leptospires. Drinking water for animals may be treated by similar methods. Raw stream or river water may be a source of infection for animals if used as drinking water. Treatment of the water will be justifiable only if it is shown to be the only method of transmission of infection, and no other simpler method of control (control of source, immunization of animals) will suffice.

#### 4.2.3.2 Disinfection of Surface Waters.** Attempts to disinfect surface waters (e.g., swimming pools) and soils have been generally unpromising (see section A 3.5.1, p. 35), and it is not generally practicable to attempt to prevent infection by these means. Control of the source of infection (see sections B 4.2.1, p. 90 and B 4.2.2, p. 91) or prevention of infection from the water (section B 4.2.3.4, below) is preferable.

#### 4.2.3.3 Physical Protection.** Protective clothing is indicated for those working in milking sheds and abattoirs; also, the wrapping of foodstuffs (e.g., meat packing) is necessary in certain circumstances (see section B 4.2.4.2, p. 94).

#### 4.2.3.4 Declaration and Closing of Areas of Risk.** Certain areas of ground may be heavily contaminated by infected urine from carrier rodents or farm animals. Geographical and ecological considerations may allow a field or a swamp to be a relatively localized source of infection (see sections A 3.1 to 3.3). Once such an area has been recognized (section B 4.1.3, p. 87), it is possible to declare it an "infected area". All livestock should then be removed, until evidence from laboratory tests (sections A 4.2.1, p. 28 and B 4.1.2, p. 86) shows that the soil and ground-water are free of pathogenic leptospires. This method of control is most applicable where the infection is clearly maintained from within the herd and from soil contaminated by it. The serovar in the water will be the prevalent serovar in the herd, but will be different from that in the local rodent carrier population. The method is not suitable where a rodent reservoir of infection continually replenishes the ground water with a serovar which infects the herd, and is not practicable (as a rule) to exclude field rodents and small animals from delimited areas.

### 4.2.4 HYGIENIC MEASURES

#### 4.2.4.1 Indications and Methods.** Hygienic measures must be based on an appreciation that the main routes of infection by leptospires to the body are through skin wounds and minor abrasions, as well as through intact mucous membranes, particularly the conjunctiva (see section A 3.2, p. 21).
The main sources of infection are infected animal kidneys, the urine of infected animals, and a wet environment which is heavily contaminated with animal urine (section A 3.3, p. 23). Introduction of hygienic measures for the control of leptospirosis is only appropriate for those working in an environment where there is a high rate of infection in animals or animal products in such an environment. For example, leptospirosis is seldom (if ever) an endemic disease of birds, so that it would be inappropriate to consider leptospirosis as a significant health hazard in a poultry processing plant, unless there was a concurrent rodent infestation problem. However, as leptospirosis is an endemic disease of rodents in most countries of the world, control of such infestation in an area where people live or work is a fundamental measure for controlling leptospirosis.

It is also axiomatic that people who do not need to come into contact with potentially infected animals should keep away from them.

In some areas, slow-moving water or ponds can become heavily contaminated with infected urine from domestic animals, thereby creating a hazard for people, particularly children who swim in such places. These areas of potential risk should be identified and publicized.

4.2.4.2 Protective Clothing and Sanitary Practices. Those working with animals likely to be infected with leptospirosis, especially pig farmers, dairy farmers and veterinarians, should take special precautions to avoid contact with infected urine and tissue fluids. Such measures should include keeping all cuts and abrasions covered with a waterproof dressing, and wearing boots or some other type of impervious footwear when working with animals. Urine contamination of the skin and particularly the face and eyes, should immediately be washed off with clean water. Similar precautions should be taken by abattoir workers, and particular attention should be paid to rodent control in food premises which can attract such infestation unless special precautions are taken.

Veterinarians and others handling live or dead animals that are suspected to be infected with clinical leptospirosis should wear full protective clothing including rubber gloves. As leptospires are able to survive in chilled or frozen kidney for several weeks, leptospirosis can be a hazard for food processors outside the abattoir. The wearing of disposable or easily cleaned gloves when handling kidneys would be a sensible measure, and rodent control programmes must be enforced.

This advice about protective clothing is often not followed because such clothing may be uncomfortable or may impair one's work capability by interfering with speed or skill, or even give a false sense of security if they contain holes (in the boots or gloves). It is sometimes necessary, nevertheless, to wear protective clothing because it is enforced by law, and workers may lose their entitlement to workers' compensation in cases where such clothing was not worn.

4.2.5 SPECIFIC MEASURES IN MAN AND ANIMALS

The majority of leptospiral serovars are adapted to specific hosts, which carry them but show few (if any) clinical signs of infection (see section A 3.1). While different serovars may be endemic in particular countries, they may also be maintained in different species of animals in other regions. The transmission of leptospires from one animal to another or to man in a particular area will therefore depend on the endemic serovars, the number and type of maintenance hosts, and the rate of infection in such maintenance or reservoir hosts. When free-living wild animals are the major maintenance hosts for a specific serovar, cross infection to domesticated animals is usually controlled by limiting all contact between domesticated animals and wildlife, whenever possible. This will depend on attempts to control the numbers or range of such wildlife reservoirs and on the rodent-proofing of buildings when mice and rats are involved.

4.2.5.1 Chemotherapy. No chemoprophylactic measures can be recommended as routine measures to prevent infection in domestic stock, but chemotherapy with dihydrostreptomycin, in a dose of 25 mg/kg body weight for 3 days, is recommended to eliminate infection in a leptospiuric animal. Some recommend the same dose every second day, for 3 doses (section B 2.9.3, p. 66). Such treatment would reduce the probability of cross infection within a herd and reduce the risk of human infection.
4.2.5.2 Immunization (see section A 5.2.3). Vaccines have been shown to be effective in preventing infection in susceptible animals, providing they have been prepared from serovars which are endemic in the area in which they are to be used. For example a vaccine prepared from serovars canicola and copenhageni in the United Kingdom would be quite inappropriate for use in cattle in New Zealand, which are most at risk to serovars hardjo and pomona. Polyvalent vaccines and bacterins are useful where more than one serovar is prevalent.

Vaccines should be administered when the levels of passive maternally-derived antibodies to leptospires are low or absent. In most species of domestic animals this would usually be between 3 and 4 months of age. As most vaccines are non-viable bacterins, the initial vaccination regimen should consist of 2 doses administered 4 weeks apart. It is accepted practice to revaccinate with one dose annually.

Vaccines have been used for the control of human infection (see section A 5.1.3, p.33). Most of them have had side effects ranging from local pain to fever of short duration. Further work on their standardization and efficacy is required. Immunization may be the only means of control in populations where environmental, social and climatic conditions make it impossible to control leptospirosis in any other way.

4.2.6 EDUCATION OF PROFESSIONAL GROUPS AND THE PUBLIC

The enzootic levels of leptospirosis in animals and the incidence of the disease in man are subject to considerable variation in different parts of the world. Also the serovars involved and the general epidemiology of leptospirosis will be quite different in one country compared with another. Without a detailed knowledge of the epidemiology of leptospirosis in the region, it would be impossible to introduce effective health education programmes.

As leptospirosis is a classical zoonosis, with animals as the major source of infection, any education programme must involve members of both the medical and veterinary professions and representatives of wildlife control administrations, if required. National departments of health will have, or must obtain, data on the annual incidence of human leptospirosis and comparative data on the incidence in different occupational groups (see section B 4.1.1, p. 84). Such information will allow objective assessment of the overall public health significance of the disease and will identify those groups of the population that are especially at risk. Government veterinary agencies should have a knowledge of the prevalence of the disease in animals and be able to indicate some of the likely sources of infection for those groups of the human population who appear to have the highest incidence of leptospirosis.

In countries which have a high endemic prevalence of leptospirosis and other zoonoses of public health importance in animals, it is important that every effort is made to carry out the recommendation of the Joint FAO/WHO Expert Committee on Veterinary Public Health in establishing within national ministries of health a veterinary public health unit (90).

The education of farmers and others with direct contact with animals will have to be carried out mainly by veterinarians. It is therefore important that veterinarians are kept up to date with knowledge of leptospirosis in their country. Apart from publications of relevant papers in professional journals, technical information leaflets should be prepared and distributed by government veterinary departments to all veterinarians. Leaflets with information, written clearly in non-technical language, should also be distributed directly to farmers and other animal owners who may be at risk. Talks to farmers and villagers by health officers, with the cooperation of local professional practitioners, will help to raise the level of awareness.

At village level, farmers and householders should be aware of the existence of leptospirosis as a cause of febrile illness in people and animals. Village practitioners of human and veterinary medicine should know the clinical presentation, methods of diagnosis at bedside or in the field, and courses of action to be taken.

There is a lack of awareness by the medical profession of the importance of certain zoonoses including leptospirosis. In countries where leptospirosis is a significant public health hazard, technical leaflets should also be distributed to members of the medical profession. Changed views about the
nature, presentation, incidence and diagnosis of leptospirosis are slow to reach medical practitioners. Unfortunately, in many countries there is still insufficient liaison between the medical and veterinary professions, thus further emphasizing the need for the formal establishment of departments of veterinary public health.

The education of other groups at risk, such as meat workers, sewage workers and those exposed to recreational hazards, will depend on other methods of approach. All those directly concerned with occupational health have an important role to play, by direct contact with workers, and the preparation of leaflets and posters. Government health departments must also consider disseminating knowledge on preventive measures by use of the mass media and preparation of simple leaflets designed for specific occupational groups.

Educational goals and special skills for professional workers in the clinic, field or laboratory are described in Section D.

4.3 ANALYSING THE COST-EFFECTIVENESS OF CONTROL PROGRAMMES: METHODOLOGY

A precise evaluation of the cost-effectiveness of different strategies for control is essential, because public health administrators need to ensure the most rational allocation of scarce resources (see section D 2.2.3, p.152). Complex as are the concepts underlying cost-effectiveness analysis in the health sector, the situation in leptospirosis is even more complicated because the disease causes health problems in domestic animals as well as among people, usually at the same time. It is thus necessary to conduct separate analyses of programmes for control among people and among animals.

4.3.1 INFORMATION REQUIRED

Establishment of the necessary data on cost-effectiveness of leptospirosis control programmes requires the following sets of information:

(1) Epidemiological information on leptospirosis (morbidity and mortality data by age and sex in a given population) under natural conditions (i.e., without control measures). One of the possibilities may be the retrospective analysis of leptospirosis in a given population or country (see sections B 4.1.1, p. 86; B 4.1.3, p. 87; B 4.1.5, p. 89; and D 2.1.1, p. 150).

(2) Information on costs and disability due to leptospirosis; cost of hospital and non-hospital treatment, etc. (Disability costs are defined as the direct economic costs of a person not being able to work, e.g., lost productivity, disability compensation).

(3) Calculations of the cost of various methods of prevention and control among people (see section B 4.2, p. 90). Economic aspects should be taken together with the operational aspects of putting the programme into effect. Several levels of effectiveness may be achieved at different cost levels (see Table 14, p.149).

(4) Epizootiological information on leptospirosis among domestic animal should be available. It is important to know the direct losses due to leptospirosis among animals under natural conditions (i.e., without any interference, or prevention or control measures) (see sections B 4.1.2, p. 86 to 4.1.4, p. 88).

(5) The cost of various preventive and control measures among given animals should be calculated (section B 4.2, p. 90). Again, different levels of effectiveness will be attainable at different costs (Table 14, p. 149).

4.3.2 CALCULATIONS

The cost-effectiveness of a leptospirosis control programme should be calculated in epidemiological and epizootiological terms and the following approaches may be considered:
(1) Economic losses due to leptospirosis in man in the population under natural conditions should be calculated (i.e., with no control programme; therefore no cost for the control programme).

(2) Economic losses due to leptospirosis in animals under natural conditions should be calculated (again, without a control programme; therefore no cost for this programme).

(3) The cost of the control programme should be calculated or estimated (see sections B 4.3.1.(3) and (5)). There are two ways to proceed. The first is to introduce a programme and calculate its costs as it is carried out. The second is to estimate the likely costs, as accurately as is feasible, of a programme which is planned for use in the future.

(4) A comparison can now be made of the economic losses due to leptospirosis in man under natural conditions, with the losses under the control programme, taking into account the costs of the control programme. In the case of programmes planned for future use, it will be necessary to estimate the expected amount by which the economic losses will be reduced after the introduction of the control programme, bearing in mind the affordable levels of control and benefit (see sections B 4.3.1.(3) and (5)).

(5) A similar calculation may be made for the losses due to leptospirosis in animals.

(6) The sum of the cost-effectiveness calculations in the preceding paragraphs gives the total cost-benefit. A control programme will be justified from an economic viewpoint if the cost of the programme and the losses from leptospirosis under the programme are less than the losses due to leptospirosis under natural conditions.

(7) In choosing the types and the levels of controls in relation to economic factors, one must bear in mind the social impact of the planned control measures. It is impossible to calculate the cost of social upheaval and changes in the way of life which people may regard as detrimental to their interests or traditions, regardless of the likely benefit to their physical health or economic status.

4.4 ADMINISTRATIVE COORDINATION AND INTERDISCIPLINARY COOPERATION

The main administrative plan is described in section D 1.3, p. 148. There is clearly a need for coordination between medical, veterinary and wildlife control authorities in the common purpose of control of leptospirosis. The means of achieving the coordination will depend on local governmental structure and policies. No formula can be provided for all situations.

4.4.1 LOCAL LEVEL

The information derived from veterinary and medical sources is fed to the control services (see section D 1, p. 140) for effective action as described in section D 2, p. 150. Thus cooperation is required in the preventive, as well as the diagnostic services. Rationalization of diagnostic facilities requires that the expertise for diagnosing leptospirosis should be localized in a leptospirosis laboratory, serving the needs of all three administrative authorities (section D 1.2.3, p. 143).

4.4.2 STATE OR REGIONAL LEVELS

Control beyond the local areas is required by the nature and transmission of the disease. Control of the movements of infected animals, immunization policies, wildlife and farm animal surveillance, and notifications of disease all require coordination on a wide basis, often across the boundaries of administrative responsibilities. Thus cooperation may be required between states or countries, in the interest of control in a geographical region.
4.4.3 NATIONAL LEVEL

National borders do not always follow geographical or social boundaries within which leptospirosis is delimited. It is therefore necessary to apply all the foregoing through the national administration. The regional leptospirosis laboratories would serve as foci for the coordination of national activities in leptospirosis (section D 1.2.3, p. 143), under the general guidance and overall supervision of the Reference Laboratory (section D 1.2.4, p. 143). Whenever leptospirosis is seen as an important health hazard, it is the responsibility of the national government health authorities to see that the measures in section D are available and carried out, in the interest of human and veterinary health. The national leptospirosis administration is also then in a position to communicate and cooperate with counterparts in other countries.

4.4.4 INTERNATIONAL LEVEL

The World Health Organization offers the opportunity for cooperation between national administrations and for the dissemination of knowledge, provision of expert advice, coordination of international activities, and integration and support for surveillance and research programmes. Reference Laboratories, which are Collaborating Centres, can take advantage of the facilities provided and act as international centres of expertise for their geographical and administrative regions.

4.5 ACTION IN EPIDEMICS OR EPIZOOTICS

Beginning with case-finding and diagnosis, the first steps are notifications to the health authorities, definition of the problem, and the initiation of preventive measures (see Fig. 3, p. 151).

The problem is defined mainly by making use of laboratory data (see section D 2.1, p. 150), the procedures to find the source of infection are outlined in section D 2.1.1.2, p. 150, and the selection and implementation of preventive measures in sections D 2.2, p. 152 and D 2.3, p. 152.