Animal health: Global support for diagnosing infectious diseases

Researchers around the world are using diagnostic kits developed at the IAEA's Seibersdorf Laboratories

by Peter F. Wright

What do domesticated animals contribute to the well being of mankind? Perhaps the first thing that comes to mind is food: meat, poultry, and dairy products. Animals also provide other materials such as wool and leather. In some areas of the world, draught animals still play a major and vital role in farming and transportation.

A number of factors, however, may adversely affect the stability and health of animal populations. Environmental stresses and poor nutrition place serious constraints on animal productivity, especially in the developing world. Animals which are in weakened condition are all the more vulnerable to disease.

The threat of infectious or communicable diseases is of global concern. Underscoring this is the fact that the Food and Agriculture Organization (FAO), the International Office of Epizootics (OIE), and the World Health Organization (WHO) jointly compile and publish an annual compendium of disease occurrence throughout their member states. The *Animal Health Yearbook* lists over 140 communicable diseases having varying degrees of socio-economic, public health, and/or zoo-sanitary consequences. It covers the major diseases of cattle, sheep, goats, horses, pigs, poultry, rabbits, fish, molluscs, crustaceans, and bees.

Infectious diseases

Infectious diseases are caused by microorganisms. The majority of diseases of veterinary importance are caused by viral and bacterial agents and helminths. Fewer, but nonetheless important, diseases are caused by mycoplasmal, rickettsial, protozoal and other microbial agents.

There are a number of factors which contribute to the impact of any given infectious disease. Obviously those diseases which cause widespread death in adult and/or young animals are very serious. Some diseases severely affect natural reproduction by causing infertility or abortion. Other diseases may cause heavy losses in productivity in terms of the quality and quantity of food, materials, or work produced. Zoonotic diseases pose a threat to human health when the infectious agent may be transmitted from animals to man. The importance ascribed to a given disease may be related to a combination of the above factors.

Infectious diseases may be transmitted by various routes. Some organisms are present in high numbers in the bodily secretions and waste of infected animals and they can spread through communal or sexual contact. Some organisms may travel long distances on air currents or in moving water before encountering a susceptible animal. Other organisms may be transmitted from mother to offspring during pregnancy. Still others may be transmitted by insect bites. Some organisms may also infect other hosts, such as wild animals, which act as a reservoir for potential transmission to domesticated animals. Depending on the route of transmission and reservoirs, some diseases are very difficult to contain and eliminate.

Definitive diagnosis

Many infectious diseases present similar clinical signs and this makes accurate diagnosis very difficult in the absence of laboratory tests. Definitive diagnosis of any infectious disease requires direct demonstration and identification of the causative agent found in infected tissues or fluids.

Classical laboratory techniques used to isolate, culture, and identify organisms are labour

Mr Wright is Head of the Animal Production Unit at the IAEA's Seibersdorf Laboratories

intensive and expensive, and they require considerable expertise and equipment. Although useful for characterizing and typing micro-organisms, these techniques are not applicable as routine and large-scale diagnostic procedures in any laboratory.

More recent advances in biotechnology, such as nucleic acid probe techniques, will no doubt allow for rapid and economical identification in the future, but at present they are still considered as experimental techniques.

Diagnostic serology

In immunological terms, infectious agents are composed of large, organic molecules which are recognized by an animal as being foreign or "non-self". One aspect of the development of an immune response to these organic molecules or antigens is the production of antibodies to the multitude of antigens which characterize any given organism. This repertoire of antibodies represents the first line of defense in protective immunity.

Antibodies have the capacity to actually bind to antigens which are unique to specific micro-organisms. Because antibodies are only produced after an encounter with a foreign antigen, the presence of specific antibodies is indicative of exposure to, if not infection with, a certain micro-organism. Detection of specific antibodies in blood and other body fluids forms the basis of diagnostic serology.

Serological techniques have been used for well over half a century for the presumptive diagnosis of infectious diseases. Many types of tests have been developed, some being far more complex than others. In the most general of terms, serological tests may be divided into two groups. Classical serological tests rely on antibody-dependent phenomena occurring as a consequence of the binding of antibody to a specific antigen. These phenomena include the precipitation of soluble antigens, the agglutination of bacteria or red blood cells, the lysis of red blood cells, or the neutralization of virus infectivity. Many of these classical techniques are still in diagnostic use today but are gradually being replaced by a second generation of assays known as primary binding assays.

Primary binding assays are not exactly new, in fact they have been in existence for over 30 years. The binding of antibody to antigen at the molecular level is not readily discernible. However, when one component of a test system is "tagged", or labelled with a molecule which produces a signal or product, then antigen-antibody binding becomes detectable and measurable. The first tags used were radioisotopes and fluorescent dyes which could be chemically bound to either purified antibodies or antigens. Both radio- and fluoro-immunoassays are still in use today in some laboratories. On the other hand, enzyme immunoassays have come into widespread use. This is because enzyme-labelled reagents are inherently more stable and are not associated with health or disposal problems. By choosing substrates which produce a coloured product after enzymatic degradation, the antigen-antibody reaction occurring in the test may be detected visually or measured photometrically. Enzyme immunoassays are commonly referred to as ELISA tests. The term is based on an acronym for enzyme-linked immunosorbent assay and was coined over 20 years ago when the technique was first introduced.

Enzyme immunoassays

Serological diagnosis is not always as straightforward as it would seem. Some antigens present on different organisms are similar enough on a molecular basis to cause the production of antibodies which will cross-react and cause false positive reactions in any serological test. It is also known that certain types of antibody are more likely to cause false positive reactions than others. The widespread use of vaccines for certain diseases also complicates serological diagnosis because vaccine antigens also will induce antibody production which cannot readily be distinguished from antibodies produced due to infection.

Most major veterinary research laboratories worldwide are actively engaged in the development and improvement of reagents for use in diagnostic ELISA techniques; improvements are possible in ELISA but not in classical serological techniques. Advances in biotechnology have had and will continue to have great impact on ELISA techniques. The use of highly specific monoclonal antibodies and highly defined recombinant antigens has already improved the diagnostic performance of many ELISAs.

Animal health programmes

Serological techniques are the backbone of animal health programmes throughout the world. They are used in sero-epidemological surveys to determine the presence and/or extent of a particular infectious disease in an animal population. They further are used to monitor disease control programmes which may involve the test and slaughter of infected animals. Vaccination

Laboratories collaborating on ELISA development

Fifteen laboratories in nine countries are collaborating on ELISA development with the IAEA's Agricultural Laboratory at Seibersdorf: Their disease areas of specialization are in parentheses.

- **United Kingdom** — Institute for Animal Health, Pirbright (Rinderpest, Peste des petits ruminant, Bluetongue, Foot and Mouth Disease); Centre for Tropical Veterinary Medicine, Edinburgh (Trypanosomiasis); Central Veterinary Laboratory, Weybridge (Brucellosis, Infectious Bovine Rhinotracheitis)
- **France** — Institut national de la recherche agronomique, Nouzilly (Brucellosis); Institut d'élevage et de médecine vétérinaire des Pays Tropicaux, Maisons-Alfort (Rinderpest, Peste des petits ruminant, Contagious Bovine Pleuropneumonia)
- **Canada** — Animal Diseases Research Institute, Nepean (Brucellosis, Bluetongue, Aujeszky's Disease)
- **Australia** — Regional Veterinary Laboratory, Benalla (Brucellosis, Hemorrhagic Septicemia); Long Pocket Laboratories, Indooroopilly (Babesiosis); Australian Animal Health Laboratory, Geelong (Bluetongue, Newcastle Disease)
- **Denmark** — National Veterinary Institute, Copenhagen (Enzootic Bovine Leukosis)
- **Brazil** — Pan-American Foot and Mouth Disease Centre, Rio de Janeiro (Foot and Mouth Disease)
- **United States** — New York State College of Veterinary Medicine, Ithaca (Infectious Bovine Rhinotracheitis); National Veterinary Service Laboratory, Ames, Iowa (Brucellosis, Bluetongue)
- **Kenya** — International Laboratory for Research on Animal Diseases, Nairobi (Trypanosomiasis)
- **Sweden** — National Veterinary Institute, Uppsala (Aujeszky's Disease, Enzootic Bovine Leukosis)

programmes are also monitored serologically to determine how many animals have been vaccinated or whether they are responding properly. Serological techniques are used to prevent the spread of disease through the movement of infected animals within or between countries.

Because serological information is so vital to animal health programmes, it is important that test results be as diagnostically accurate as possible. In order for a test to be reliable, the biological reagents should be stable and the technique should be robust. To be applied on a large scale, the test should be economical and rapid. For these reasons, ELISA techniques have become the primary focus of research and development. ELISA techniques are well-suited for diagnostic use at laboratories in developed and developing countries.

Laboratory research and support

Diagnosis of infectious disease is one of the major animal health activities within the programme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. At the IAEA's Seibersdorf Laboratories, the Animal Production Unit in the Agriculture Laboratory provides support to this programme through the development and transfer of specially developed diagnostic kits.

For the diagnosis of infectious disease, ELISA kits are provided to laboratories where serology is an integral component of research on particular infectious diseases. (Additionally, regarding other problems of animal health and productivity, the Laboratory distributes radioimmunoassay (RIA) kits for the measurement of the reproductive hormone, progesterone. This hormone is used to study and monitor reproductive cycles in cattle, sheep, goats, buffalo, and camelids as an aid to improving breeding success. In 1991 alone, more than 300 RIA kits (about 125 000 analytical determinations) were provided to some 60 counterpart laboratories in Africa, Asia, and Latin America.)

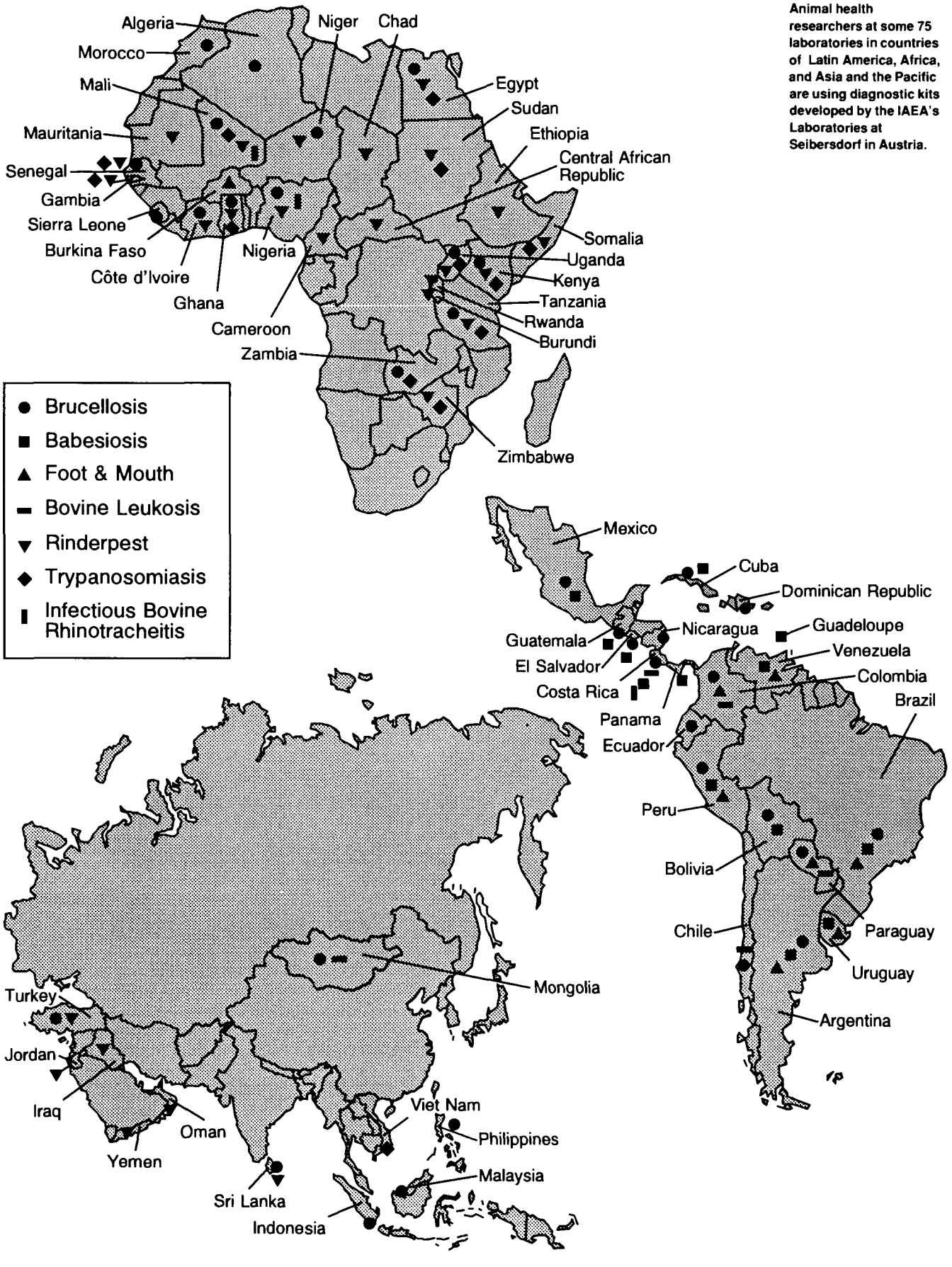
Over the past 6 years, priority has been given to infectious diseases of cattle. In the near future, it is expected that ELISA kits also will be developed for certain poultry and pig diseases.

The IAEA's Animal Production Unit does not conduct the initial research on the development of diagnostic reagents for use in the ELISA. Rather, it adapts existing ELISA reagents and techniques to a kit format which is suitable for use in counterpart laboratories in developing countries. In order to achieve this, the Unit is highly dependent on collaboration with major veterinary research laboratories around the world. At present, 15 laboratories are actively collaborating in ELISA kit development and supply of reagents. *(See table.)*

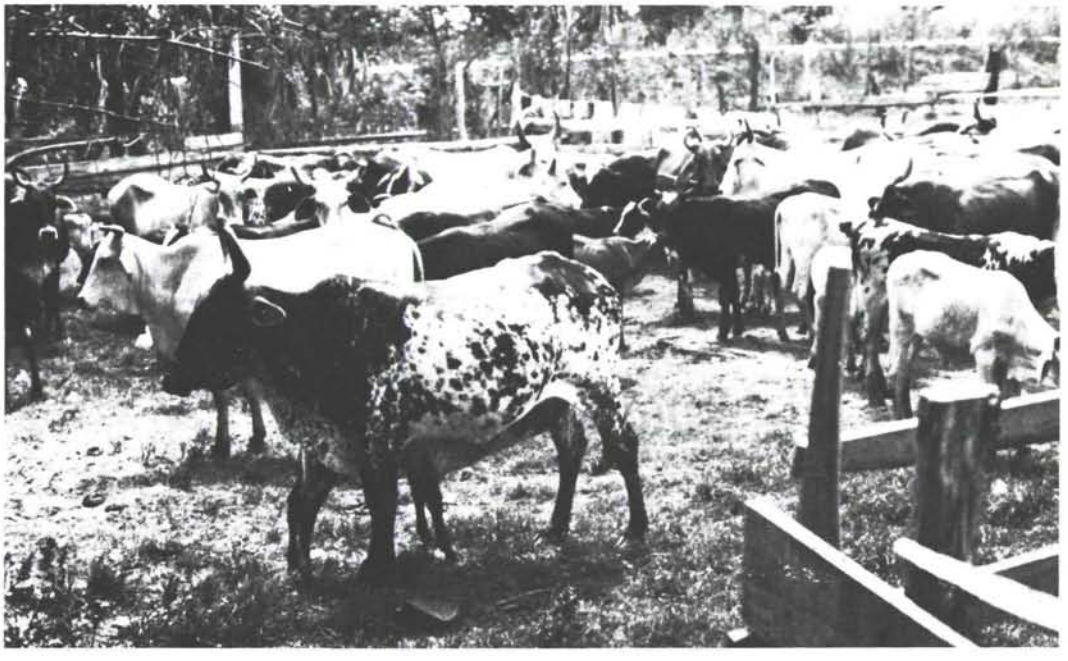
Standardization and quality control are key elements of kit development. They are essential for uniform diagnostic performance within and between laboratories. Detailed protocols are written which describe equipment requirements, preparation and storage of reagents, assay procedures, quality control activities, data acceptance criteria, interpretation of results, and troubleshooting.

All prototype ELISA kits must be diagnostically validated before they are considered ready for distribution to counterpart laboratories. Validation is accomplished by comparison of the kit performance to the present "gold standard" serological technique in the testing of defined sets of samples. To obtain an unbiased and critical assessment of kit performance, validation is done in collaboration with expert laboratories.

Once an ELISA kit has been standardized and validated, it is then ready for production and distribution to counterpart laboratories. The



Animal health researchers at some 75 laboratories in countries of Latin America, Africa, and Asia and the Pacific are using diagnostic kits developed by the IAEA's Laboratories at Seibersdorf in Austria.



Typical "dairy" animals in Peru.

transfer of kit technology requires the training of recipient laboratory personnel in the use of the kit and instrumentation, as well as the interpretation of quality control and test data. The principal function of the Unit in this respect is to technically assist the Joint FAO/IAEA Division in the delivery of training.

Often individual counterpart laboratories will require technical backstopping to solve local problems affecting kit performance. These problems may involve, among other things, equipment malfunction, reagent spoilage, or poor water quality. Water quality in particular is perhaps the most critical element, since all serological techniques require the use of aqueous buffers in reagent preparation and assay procedures. The Unit develops troubleshooting systems to help counterpart scientists resolve local problems independently. It also provides a repair service for equipment in collaboration with the Instrumentation Unit of the IAEA's Seibersdorf Laboratories.

The development of external quality-control systems is the last kit support function. Routine activities are supplemented by periodic testing of external check-sample panels, which are tested "blind" in counterpart laboratories (i.e., without prior knowledge of sample reactivity). This assures that they are an unbiased check of assay and technical performance. Success in testing this type of panel develops confidence in counterparts and provides a basis for international acceptance of data.

Antibody ELISA kits have been developed for the following major cattle diseases: Rinderpest, Brucellosis, Babesiosis, Bovine Leukosis, and Infectious Bovine Rhinotracheitis. Antigen ELISA kits have been developed for Foot and Mouth Disease, Trypanosomiasis, and Haemorrhagic Septicemia. These latter kits are for antigen, rather than antibody detection, and are used for definitive diagnosis. Nearing completion are antibody ELISA kits for Bluetongue and Contagious Bovine Pleuropneumonia. In the near future, kit development will begin for Aujeszky's Disease (pigs) and Newcastle Disease (poultry).

During 1992, more than 100 kits (about 0.6 million test doses) were dispatched to some 75 counterpart laboratories around the world. *(See maps.)*

International commitment

Because infectious diseases are a global concern, collaboration has been established with the FAO, WHO and OIE on the international standardization of ELISA techniques and reagents for use in control, eradication, and surveillance programmes throughout the world.

In 1992, the Animal Production Unit at the IAEA's Seibersdorf Laboratories was officially designated as the FAO/IAEA Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis and as the OIE Collaborating Centre for Immunoenzymatic and Molecular Diagnostic Methods.

These designations carry with them a commitment to international standardization which is of benefit to industrialized and developing countries alike. ❐