An overview of the roles and structure of international high-security veterinary laboratories for infectious animal diseases

P.K. Murray
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Animal Health, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong, Victoria 3220, Australia

Summary
The unique structure, role and operations of government high-security (HS) laboratories which work on animal diseases are described, with particular reference to the laboratories of nine countries. High-security laboratories provide cost-effective insurance against catastrophic losses which could occur following exotic disease outbreaks. The importance of these laboratories is reflected in the fact that several new laboratories have recently been constructed at considerable expense and older facilities have undergone major renovations. Biosecurity is fundamental to the operation of high-security laboratories, so good facility design and microbiological security practices are very important. High-security laboratories conduct exotic disease diagnosis, certification and surveillance, and also perform research into virology, disease pathogenesis and improvements to diagnostic tests and vaccines. The mandate of these laboratories includes the training of veterinarians in the recognition of exotic diseases. One extremely important role is the provision of expert advice on exotic diseases and participation (both nationally and internationally) in policy decisions regarding animal disease issues.

Keywords
Animal diseases — Biocontainment — Emerging diseases — Exotic diseases — High-security laboratories — Microbiological security.

Overview
High-security (HS) laboratories are specialised facilities where work with infectious agents is conducted without risk of their escape to the environment, and without health risk to the staff who work with them.

The HS laboratories discussed in this review are national facilities which have been commissioned by governments (Table I and Fig. 1). Such laboratories play an important part in the response to exotic animal diseases, and represent a cost-effective insurance against the catastrophic loss which could occur in the event of a serious outbreak. They also play an increasingly important role in supporting international trade in animals or animal products by providing scientific evidence of freedom from disease.

What, when, where and why exotic disease outbreaks happen is unpredictable, but it is certain that they will occur and so continual preparedness is important. In the United States of America (USA), the occurrence of foot and mouth disease (FMD) could result in outbreaks with an estimated cost of up to US$12 billion over a fifteen-year period (28). In Australia, a major exporter of red meat and wool, FMD could cause losses of up to Australian (AU)$6 billion (46). In 1997, the Netherlands suffered a serious outbreak of classical swine fever (hog cholera) which caused an estimated loss of US$2 billion to the economy of the Netherlands and necessitated the slaughter of fourteen million pigs. Taiwan experienced massive economic loss because of an outbreak of FMD in pigs.

Besides the real risk, the perception of risk of exotic diseases is an important consideration since this can also affect trade. Public concern about disease has undoubtedly been
magnified in recent years, with emerging infections widely reported in newspapers and on television, film and radio.

Increasingly, the work of HS laboratories is directed to providing support for trade in livestock and livestock products. There has been a decided shift in emphasis over the past decade towards more comprehensive scientific underpinning of import/export decisions and better technical standardisation of approaches. These international initiatives have dictated the need for greater effort in the areas of national disease surveillance, disease-free certification, quality standards, national health information systems and risk assessment. HS laboratories, by virtue of their expertise in exotic and emerging diseases, have therefore a very important role to play in support of trade.

The need for HS facilities is clearly recognised by governments, as reflected by the fact that four new national facilities around the world have been commissioned in recent years. The Australian Animal Health Laboratory (AAHL) in Geelong was opened in 1985 at a cost of AU$160 million. The Swiss Institute of Virology and Immunoprophylaxis (IVI) in Mittelhausern was opened in 1992 at a cost of US$40 million. The Spanish Centre for Animal Health Investigation (CISA) near Madrid opened in 1993 at a cost of more than US$40 million and the Canadian National Centre for Foreign Animal Disease (NCFAD) in Winnipeg, which is part of a combined human/animal facility, will be fully commissioned in 1998 at a cost of Canadian (CD)$142 million. In Germany, high-security work is in a state of transition at this time since plans are being developed to establish a new central veterinary research facility on the Isle of Riems. This new institute will include laboratories for work at biosafety levels 2, 3 and 4. In addition, important new building and upgrading work has taken place in longer-established laboratories, for example, at the Institute for Animal Health, Pirbright Laboratory (IAH) in
the United Kingdom (UK), at the Plum Island Animal Disease Center (PIADC) in the USA (12) and at the Danish Veterinary Institute for Virus Research (DVIV).

HS laboratories do not directly prevent disease incursions; that is the province of quarantine services. They do, however, conduct work that centres around early and accurate disease diagnosis. A high priority is placed on this since detection of disease at the earliest possible time is the key to minimising evolution of the outbreak from both epidemiological and economic standpoints. The early recognition of an unusual disease event in the field, and its early reporting to veterinary authorities, is a central concept, so HS laboratories actively promote this through exotic disease training programmes for veterinarians and by raising overall exotic disease awareness in farming circles. Shortening the time taken for diagnosis after suspect specimens reach the laboratory is also a key, so diagnostic teams and technologies are expressly committed to this. This work is central to minimising economic loss during an outbreak and quickly regaining disease-free status afterwards so that export trade can recommence. Besides the known exotic diseases, authorities must deal with the worrying phenomenon of new, emerging and re-emerging diseases (25, 35, 48). While some of these may cause severe animal or human health problems, others can have a adverse effect on trade simply because of perception. Importing countries must be very conservative about sourcing livestock produce from any area where an unusual disease has occurred. So, increasingly, scientific expertise sufficient to deal with new diseases is expected from HS laboratories.

Usually, HS laboratories also provide other essential services. For example, the testing of imported animals or animal products for disease-free certification, development of improved diagnostic tests and vaccines, scientific research on disease pathogenesis and transmission (essential for effective disease control) and the provision of scientific advice to national and international regulators and policy-makers.

All HS laboratories make substantial investments in infectious disease research. Besides resulting in breakthroughs of direct benefit to livestock industries, conducting research is vital for the continued capability of the laboratory since it sustains their capacity for cutting-edge disease diagnosis and permits skills to be sustained at the highest level. Research staff represent deployable expertise in the event of emergencies and can provide the best scientific advice to authorities.

The importance of an animal industry to its national economy is usually reflected by the size of effort on exotic disease work. However, the standard of an HS facility and its microbiological practices directly equate to the risk level of the infectious agents being handled. That is, agents which are highly infectious and pose a serious threat to animals and man, particularly where no treatment or prevention exists, are those that demand the most rigorous facility design and operating procedures. Progressively less stringent approaches are acceptable for agents of lower risk. Therefore, microbiological laboratories around the world structure their facilities and establish their procedures according to the biosafety levels which are deemed necessary to work with the infectious agents with which they must deal. HS laboratories operate at the upper end of the microbial risk spectrum (Levels 3 and 4) and work mostly on agents which are exotic and cause very serious disease.

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<thead>
<tr>
<th>Country</th>
<th>Institute name</th>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Australia</td>
<td>Commonwealth Scientific and Industrial Research Organisation Division of Animal Health, Australian Animal Health Laboratory</td>
<td>AAHL</td>
<td>Geelong, Victoria 3220</td>
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<tr>
<td>Canada</td>
<td>National Centre for Foreign Animal Diseases / Centre national sur les maladies exotiques</td>
<td>NCFAD</td>
<td>Winnipeg, Manitoba RBE 3M4</td>
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<tr>
<td>Denmark</td>
<td>Statens Veterinaere Institut for Virusforsking / Danish Veterinary Institute for Virus Research</td>
<td>DVIV</td>
<td>Lindholm, DK-4771, Kalvehave</td>
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<tr>
<td>Germany</td>
<td>Bundesforschungsanstalt für Viruskrankheiten der Tiere / Federal Research Centre for Virus Diseases of Animals</td>
<td>FRCVDA</td>
<td>Paul-Ehrlich-Strasse 28, D-72276 Tübingen</td>
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<td></td>
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<tr>
<td>Netherlands</td>
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<td>CVI</td>
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<td>CISA</td>
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<td>Switzerland</td>
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<tr>
<td>United Kingdom</td>
<td>Institute for Animal Health, Pirbright Laboratory</td>
<td>IAH</td>
<td>Pirbright, Woking, Surrey, GU24 ONU</td>
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<tr>
<td>United States of</td>
<td>Plum Island Animal Disease Center</td>
<td>PIADC</td>
<td>Greenport, New York 11944</td>
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Table I
Some major animal disease high-security laboratories...
Each country has its own established repertoire of endemic diseases and so, by corollary, each country has its own individual list of exotic diseases. For example, in Australia (which is recognised to be free from most major animal diseases) the list of exotic agents is lengthy and covers all Office International des Epizooties (OIE) List A diseases. The work of the major HS animal disease laboratories listed in this review is therefore highly individual and reflects the assessment made by each country of their main exotic disease concerns. All laboratories, however, have a core interest in the major OIE List A diseases (39) such as foot and mouth disease, African swine fever, classical swine fever and sheep and goat pox.

Each HS laboratory is unique in many regards and the differences between HS facilities and other comparable biological research laboratories deserve to be emphasised. They are more expensive to operate, since biocontainment and microbiological safety are mandatory and can only be achieved at a price. Extra cost is incurred in both the construction and the ongoing operation of these facilities. For example, air handling and filtration, sewerage treatment, construction and the ongoing operation of these facilities. For example, air handling and filtration, sewerage treatment, equipment/facilities testing, energy requirements and specialist staffing are necessary additional costs and laboratories must be continuously operated.

In other biological research facilities, routine maintenance is often given a low priority, with the result that their physical structure can become seriously dilapidated over a period of years without adversely affecting the operations of the laboratory. With HS laboratories, however, this approach has to be avoided. Peak biocontainment mandates continuous, high-level maintenance, otherwise the risk of escape of infectious agents increases.

A further clear difference between HS laboratories and conventional microbiological laboratories is the critical nature of adherence to thorough work procedures. For scientists, this means strict observance of microbiological security practices and procedures. For support staff, it means learning and applying microbiological awareness. For management, it means assuming a very serious responsibility and accountability to ensure that practices and procedures are thorough so that staff, the public and livestock industries are protected from disease. Together, these requirements result in a significant additional level of complexity and responsibility for HS laboratory staff.

This review provides an overview of the structure, functions and operations of HS laboratories with particular reference to the activities of some of the main facilities around the world (Table I and Fig. 1). To achieve this, basic information is provided on secure laboratory design and operations, on microbiobiological risk and biosafety levels and on the main ‘products’ that these facilities provide to their livestock industries, their governments and the international community.

### Work of high-security laboratories

The work of HS laboratories in animal health covers diagnosis, research, training and advice (Table II). Most laboratories also have additional special functions which make them unique. For example, PIADC, IAH and DVIV house vaccine banks for foot and mouth disease, CISA works with fish diseases and environmental toxicology, AAHL also works with fish diseases and NCFAD has a special policy and epidemiology unit. The areas of scientific expertise in HS laboratories include virology, pathology, serology, molecular biology, electron microscopy, bacteriology, immunology and epidemiology.

#### Table II

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<thead>
<tr>
<th>Category</th>
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<td>Diagnosis</td>
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<td>Import/export testing of livestock</td>
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<td>Diagnostic test development</td>
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<td>Production/provision of diagnostic materials</td>
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<td>Research</td>
<td>Virus characterisation</td>
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<td>Disease pathogenesis/pathology/transmission</td>
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<td>Development and testing of vaccines</td>
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<td>Immunology</td>
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<td>Epidemiology</td>
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<td>Exotic disease diagnosis for laboratory professionals</td>
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<td>Technical, microbiological training</td>
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<td>Advice</td>
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<td>Advice for field services, regulators, policy-makers, quarantine services</td>
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<td></td>
<td>Fish diseases</td>
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<td>Toxicology</td>
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### Role in supporting trade in livestock produce

Over the years, and continuing as a process, trade negotiations have resulted in a General Agreement on Tariffs and Trade (GATT), and the formation of the World Trade Organisation (WTO) which superseded GATT as the umbrella organisation for international trade. The purpose of the WTO is to remove unjustified technical and non-technical barriers to trade by implementing an agreement on ‘Sanitary and Phytosanitary (SPS) measures’ which addresses agricultural products and quarantine matters (5).
This SPS Agreement emphasises international standards and the importance of countries developing and applying scientific approaches to prevent the entry of animal and other diseases. Its basis is risk assessment, and this requires sound quantitative and qualitative information on the disease situation in both importing and exporting countries; however, particular emphasis is now being placed on quantitative data.

The WTO has adopted the OIE *International Animal Health Code* (39) and *International Aquatic Animal Health Code* (40), which are therefore now considered as the norms for import requirements for animal products. It is in this context that the priorities of the OIE are very important and they relate directly to the work of HS laboratories. The OIE has identified the evaluation of the Veterinary Services of a country to be very important, particularly its disease intervention activities, disease surveillance and history of meeting international reporting obligations (36). So, from the perspective of the importing country, its import risk analysis processes should take these issues into account and should be scientifically based.

When these policy issues are formulated into operational approaches, some of the requirements which emerge as being essential in support of livestock trade are as follows:
- early detection and reporting of exotic animal diseases
- assurance of freedom from diseases or infection
- detection of new diseases
- accurate knowledge of the patterns of endemic diseases
- comprehensive assimilation and communication of national animal disease status.

Clearly, because of their particular focus, national HS laboratories play a major role in many of these activities and in doing so they underpin national and international trade in livestock commodities.

**Diagnosis**

Diagnosis is a central theme of HS laboratories and many of their other activities stem from this base. Diagnosis underpins the following:

1. **Disease investigation**
2. **Import and export certification of animals and animal products**
3. **National disease surveillance**

**Disease investigation**

Most targets of diagnostic work are major exotic diseases included among OIE Lists A and B (39). Each laboratory has its own, country-specific targets within these lists. Awareness of new, emerging and re-emerging diseases has increased greatly in recent years and so, in addition to exotic diseases, HS laboratories generally play an important role in investigating these as well. The involvement of the Institute for Animal Science and Health, Central Veterinary Institute (CVI), in the Netherlands, in porcine reproductive and respiratory syndrome (PRRS) detection (49), of DVIV in potential PRRS live vaccine problems (11) and of AAHL in diagnosing equine morbillivirus (33) and a new rabies-related virus (22) are recent good examples of this role.

Undoubtedly, the preferred result from a diagnostic investigation is to be able to exclude the involvement of exotic disease, rather than to diagnose one. Early exclusion is extremely important since in serious outbreaks it is usual to impose animal quarantine and movement controls immediately. Quarantine may only be lifted if there is clear evidence of lack of involvement of exotics and/or the disease comes under control and does not spread.

Diagnosis and exclusion is a complex business, involving much more than the simple application of routine tests. The need is for both speed and accuracy, so a battery of different tests is applied simultaneously and experimental tests are often applied along with conventional approaches. In HS laboratories the traditional repertoire of serology, pathology, virology and bacteriology is continuously being enhanced by the application of advanced electronmicroscopy techniques, molecular biology techniques for the detection of viral genomes and improved knowledge of, and reagents to detect, agent-specific epitopes.

Reaching a diagnostic conclusion, which always occurs in close association with field and government veterinary authorities, means taking into account the results of these various tests which may sometimes be quite disparate, especially early in outbreaks, along with information on epidemiology, clinical and pathological signs seen in the field. Usually within hours, the earliest results come from direct tests of tissues from affected animals, such as immunofluorescence or antigen detection enzyme-linked immunosorbent assay. Within days, virus may be seen to grow in tissue culture and it can then be harvested for further characterisation. Within weeks (although this depends on the nature and duration of the outbreak) serological evidence of disease patterns emerge. Thereafter, even greater effort is sometimes needed for surveillance until control/eradication programmes are successful. Throughout these processes, HS laboratories are involved in providing accurate results, expert advice, leadership, support to regional laboratories and to national veterinary authorities and, not infrequently, to public health authorities when zoonotic diseases are involved.

Since speedy and accurate diagnosis is the key to effective disease control, keeping diagnostic skills and technology at the cutting edge is important so laboratories are heavily involved in the development of new or improved tests. All produce diagnostic reagents such as monoclonal antibodies, immune sera, viral antigens and recombinant proteins as the base for their tests. These tests and materials may be
transferred to regional laboratories to enable a broader diagnostic competence across the country; they are also widely exchanged among laboratories in different countries.

**Disease certification**
Testing samples from animals for exotic diseases, particularly before they are imported and are held in quarantine, is also part of the diagnostic activity of HS laboratories. Pre-importation testing is a critical part of import risk management. The 'List of Tests for International Trade' in the OIE Manual of Standards for Diagnostic Tests and Vaccines includes tests for the important OIE Lists A and B diseases (37). Vesicular diseases in ruminants, significant arbovirus infections in animals, epidemic poultry viral diseases, pox virus infections and swine fevers, among many others, are diseases from which imported animal products must be shown to be free.

Less frequent, but also important, is providing certification for animals and products for export, although this work is generally done by other laboratory facilities since this testing is for endemic diseases. However, there are circumstances, for example with horses in Australia, where an importing country requires pre-export certification for freedom from the new equine morbillivirus.

For HS laboratories, however, the main activity is import testing and in recent years two significant developments have resulted in increased demand for this. One of these has been the development of 'new' livestock industries, such as alpaca and ostrich farming. Where these have been established in non-traditional areas, large and costly importation programmes for livestock have been initiated, bringing with them the need for comprehensive pre-importation disease testing and certification. Another factor which undoubtedly influences the level of testing is international commercial competition in the animal industries. To optimise production it is necessary to work with preferred genetic lines and so the global trade in genetic material, such as embryos and embryonated eggs, is increasing markedly and these, again, must be thoroughly tested.

**Disease surveillance**
Increasingly, HS laboratories are also required to produce national disease surveillance data; information which is essential for defining the animal disease status of a country and therefore for trade. Broadly speaking, disease surveillance may be considered as 'general or passive surveillance' and 'specific or active surveillance'. Facilities working on exotic and emerging diseases generate diagnostic test results and disease investigation results which are considered as general surveillance data and contribute to National Animal Health Information Systems, the basis for international reporting. Specific surveillance is a growing requirement arising from the OIE expectation of quantitative data on disease prevalence, so HS laboratories are becoming more involved in providing test results from structured surveillance studies to establish the absence of exotic animal diseases. One high-profile disease of current interest subject to specific surveillance requirements is bovine spongiform encephalopathy (41).

**Quality standards for tests**
Accuracy, reliability and reproducibility in diagnostic work is essential. It is also difficult to achieve because tests are usually complex, involving several procedural steps that depend on complex biological test materials. Comprehensive quality standards therefore need to be applied and test performance carefully monitored. Quality assurance is one increasingly important dimension of the work of HS laboratories in line with the potential economic importance of exotic disease testing and surveillance results. Approaches which are used to assure test performance include the use of standard diagnostic tests (14, 37), internal quality control procedures (10), external proficiency testing and laboratory accreditation to international standards (44). At this time the OIE is giving serious consideration to establishing quality assurance standards for veterinary laboratories and is working on guidelines based on ISO 9000 and ISO/IEC Guide 25, which gives 'General requirements for the competence of calibration and testing laboratories' (2, 4).

**Training**
The provision of training is a key part of HS laboratory activities. Due to the specialised nature of the diseases with which the staff at HS laboratories work, as well as their expertise, the training of veterinarians, laboratory workers and other professionals in exotic disease diagnosis is usually a major activity. Some laboratories provide intensive residential training courses and also provide training materials for broader distribution to a wider group of stakeholders. The PIADC exotic disease manual is an example of this, as are the series of broadcast-quality videos on exotic diseases prepared by AAHL (16) and videos in biosafety training prepared by CISA (13). Training also extends to other scientific activities. Most laboratories train students who are working towards Masters and PhD degrees, as well as post-doctoral students. Technology transfer is a feature of some laboratories. At AAHL, for example, training is provided to State veterinary and medical laboratories for specific diseases and training workshops are held for domestic and overseas participants.

**Advice and collaboration**
One of the main contributions of HS laboratories to animal health is the provision of expert advice and participation in policy development and implementation. These are tasks of importance at the national level and they may also extend to regional and international commitments. Expertise in exotic animal diseases is of direct help to policy-makers and regulators. High-security laboratories are participants in the development of national emergency disease management plans, together with disease control programme staff and regulatory and policy groups. This involvement comes at two levels: firstly, in preparing emergency response plans for individual diseases, and secondly, in contributing to
post-outbreak disease control/eradication planning and implementation. Close teamwork among these groups in developing emergency response plans, defining roles and responsibilities and ensuring excellent communication is essential.

Scientific linkages and the sharing of expertise is a particular feature of HS laboratories around the world and is of great mutual benefit. One recent initiative, for example, has been to set up annual workshops for veterinary biosafety specialists. Concepts are shared and agreements reached on microbiological security policies in HS laboratories, and procedures are published (3, 6). Collaborations on research are also common, and the exchange of diagnostic materials, procedures and knowledge is a continuous process.

High-security laboratories feature prominently in the global network of disease reference laboratories (38, 42). The IAH at Pirbright, UK, for example, houses the international reference laboratory for FMD, and CISA in Spain is the world reference centre for African swine fever and African horse sickness.

Research
Overall, major resources are committed by HS facilities to research, although some laboratories concentrate on applied work while others invest heavily in more fundamental work. A significant part of this work is supported by external funding sources, but all HS facilities are heavily dependent on direct government funding since the protection of livestock industries and trade is generally viewed as having a major 'public good' component and the national facilities which deliver this are very expensive to maintain. Contract research funding comes from animal industry bodies, research councils and the pharmaceutical industry.

Research covers the following four categories:

a) the assembly, structure and function of viruses
b) the pathogenesis, persistence and transmission of diseases
c) the diagnosis of diseases
d) the development of novel vaccines.

Appropriately, there is significant overlap among these and research forms a continuum from fundamental to applied. Quality scientific programmes and intellectual rigour are very important to ensure that laboratories operate optimally.

Some examples of recent high-security laboratory research in these four categories are shown below.

Assembly, structure and function of viruses
Work of a fundamental nature over the years in HS laboratories has greatly helped to illuminate the understanding of viruses of economic importance. A good example is the work undertaken at the Federal Research Centre for Virus Diseases of Animals (FRCVDA), Germany, on bovine viral diarrhoea (BVD) virus (32). For many years it was recognised that two viral biotypes, cytopathic (CP) and non-cytopathic (non-CP) which are closely related antigenically, were consistently found in animals that died of mucosal disease and it was thought that a CP virus developed from a non-CP virus by mutation. However, based on the comparison of gene sequences of CP and non-CP BVD isolates at FRCVDA, it appears that in the process of becoming pathogenic, a host cell gene sequence which encodes the cellular protein, ubiquitin, is inserted into the BVD virus genome. This insert is thought to provide an additional protease cleavage site in the virus polyprotein so that cellular ubiquitin proteases can generate the viral protein, NS3, which is associated with pathogenicity (32).

Breakthroughs like this often have important practical implications, such as opportunities for better diagnostics and vaccines, but most importantly they also provide the knowledge and conceptual framework on which science advances.

Pathogenesis, persistence and transmission
Good disease control depends on having knowledge of the infectious agent: how the agent infects an animal, replicates and causes disease, whether the agent may persist in an infectious form in the animal after recovery from clinical disease, and how infectious virus is excreted and transmitted is very important knowledge for designing disease control strategies.

Animal research on exotic viruses in HS laboratories has been pivotal in providing this information, and there are many examples of important contributions in this area. The work on pharyngeal persistence of FMD virus in cattle at IAH (23) has raised awareness of potential risk from FMD-recovered cattle. The work by CVI on 'Lelystad' virus, the cause of PRRS (49), was a key part of the definition of this 'new' disease. African swine fever animal studies at PIADC and CISA among others (18, 51) have been important in establishing the unusual pathogenesis of this important disease. Ongoing animal work at AAHL on equine morbillivirus (33, 34) and a new lyssavirus (22) closely related to rabies virus will be fundamental to the process of quantifying the threat from these new zoonotic agents.

Diagnostic research
Research on diagnosis in HS laboratories is directed to the continuous improvement of existing tests, the development of novel tests and implementation of tests for 'new' diseases. The scientific skills applied are virology, serology, molecular biology, pathology and protein chemistry.

Improvements to existing tests are important. Although routine diagnosis is based on standardised, well-proven tests (14, 37), diagnosis is not static: continuous improvement is the norm and is very important. The quality of results and the decisions which are based on them are directly affected by test sensitivity, specificity, reproducibility and simplicity. For
example, research in HS laboratories has significantly advanced serological tests for FMD (30), bluetongue (1), African horse sickness (21, 29, 31) and African swine fever (7) among others.

In recent years, the polymerase chain reaction (PCR), a novel technology for hugely amplifying specific gene sequences, has revolutionised diagnosis (24). For example, over the past two decades, the time taken to diagnose bluetongue in sheep has dropped from several months (using traditional virus isolation and characterisation techniques) to a few days using PCR tests, which can also establish the geographic origin of the virus (43).

Vaccine research
The focus of vaccine research in HS laboratories is naturally on important exotic or emerging diseases. Recent work at PIADC has applied creative science to constructing a non-infectious FMD virus by deleting its cell-receptor binding sequence (45). This modified virus is capable of immunising, but not infecting, animals. However, having lost its receptor the virus cannot grow well in cell culture, an obvious limitation for the growth of the significant amounts of virus which would be necessary for vaccine production. Therefore, PIADC research focused on engineering a new receptor for FMDV into a tissue culture cell line so that the virus can enter cells and replicate. This completely artificial receptor is a membrane-bound FMD antibody and therefore it can specifically attach to the virus. Thus a new region of the virus surface now acts as the binding site for a newly engineered cell receptor. Remarkably, the engineered FMD virus grows in this new culture cell line but is non-infectious for animals, providing a prospect for novel vaccine development.

In addition to defining new epitopes for novel vaccine development, a major focus of effort on vaccine research in HS laboratories has been on better delivery vehicles for vaccines. Among these, the use of genetically engineered plants to produce animal virus antigen for use as a vaccine has presented a novel direction for new-generation vaccines (17). In this way, a protective peptide vaccine against parvovirus infection in mink, cats and dogs which incorporates a plant adjuvant has been developed by DVIV.

Although the biosafety classification developed by the World Health Organisation (WHO) for human infectious agents (50) is not strictly applicable to animal diseases, it has provided a framework for establishing four levels of risk, with Level 4 being the highest. However, incompatibility arises because the target of the risk is different in both cases. That is, in the assessment of human agents, only their ability to cause human disease is considered. Therefore, in the WHO system, animal agents which do not infect people rate low, even though they may cause serious epizootic disease and great economic loss. In veterinary laboratories working with infectious diseases, the needs are broader; facilities and procedures must protect animals (including fish), staff, the general public and the environment. For this reason, animal disease microbiological security experts have been discussing a more satisfactory international system for animal infectious agents in recent years (3), and the OIE has defined a classification of animal pathogens in the OIE International Animal Health Code (39).

There is broad agreement among experts on the principles of an international risk classification system (8). For the purposes of emphasising these similarities, Table III summarises the risk classification system and abbreviated definitions used by the WHO, those presently used in three HS animal disease facilities, a consensus proposed by animal biosafety experts and the system proposed by the OIE.

Microbiological security (biosafety and biocontainment)
The main elements that contribute to the safety of HS laboratories are listed in Tables IV and V. Broadly speaking, biosafety covers procedures and processes linked to the occupational health and safety of operators and animals, and biocontainment covers the physical/structural and procedural elements for ensuring that infectious agents do not escape into the environment. Together, biosafety and biocontainment constitute microbiological security. Microbiological security therefore has human, facility and procedural components, each of which is essential, and when integrated well, provides the highest level of assurance. The practices of laboratory safety, which are well described elsewhere (9, 19, 20, 26, 27, 47, 50), undergo serious ongoing consideration in animal disease HS laboratories.

All HS laboratories have comprehensive checks and balances in place to ensure safety. With the physical facility, a governing principle is that key systems are duplicated so that in the event of failure, back-ups become operational and integrity is maintained. Systematic testing and monitoring of equipment is a high priority, as is a high level of routine maintenance.

With personnel, a governing principle is that dangerous infectious agents are only handled by experts who have long experience in microbiology and microbiological security. Consequently, only a few individuals attain this status in each

Principles of biocontainment in high-security laboratories
Risk classification of infectious agents
The importance of having a well-based risk classification of animal infectious agents is very great, since this directly relates to the level of biosafety with which they must be handled. In turn, this dictates the costs and complexity of facilities, equipment, procedures and training and the safety of their operations.
A comparison of disease risk categories used by different organisations and a consensus for animal laboratories currently under consideration

Table III

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Risk Group I</td>
<td>Risk Group I</td>
<td>Class 1</td>
<td>Risk Group 1</td>
<td>Risk Level 1</td>
<td>Group 1 Animal Pathogens</td>
</tr>
<tr>
<td>Low individual and community risk</td>
<td>A biological agent unlikely to cause disease in healthy workers or animals</td>
<td>Not known to cause disease in healthy human adults</td>
<td>Low risk, found in healthy animals</td>
<td>Unlikely to cause disease in animals, no deleterious impact on the environment, no hazard to personnel</td>
<td>Enzootic disease organisms. No official control</td>
</tr>
<tr>
<td>Risk Group II</td>
<td>Risk Group 2</td>
<td>Class 2</td>
<td>Risk Group 2</td>
<td>Risk Level 2</td>
<td>Group 2 Animal Pathogens</td>
</tr>
<tr>
<td>Moderate individual and limited community risk</td>
<td>Moderate individual risk, limited community risk to humans or animals</td>
<td>Associated with human disease</td>
<td>Causes disease in animals but limited community risk to animals (most OIE List B agents)</td>
<td>Causes mild disease, modest economic impact, limited survival in the environment, limited hazard to personnel, readily treated and prevented, endemic</td>
<td>Exotic or enzootic organisms. Low risk of spread. Official control. Not vectorable, species-specific, limited economic significance</td>
</tr>
<tr>
<td>Risk Group III</td>
<td>Risk Group 3</td>
<td>Class 3</td>
<td>Risk Group 3</td>
<td>Risk Level 3</td>
<td>Group 3 Animal Pathogens</td>
</tr>
<tr>
<td>High individual risk and limited community risk</td>
<td>High individual risk, low community risk to humans or animals, can be treated</td>
<td>Indigenous or exotic agents with potential for aerosol transmission, disease may have serious health effects</td>
<td>Cause serious disease in animals and man, readily transmissible</td>
<td>May cause serious disease, may be lethal, significant economic impact, easily transmitted by aerosol, medical treatment and vaccines readily available, endemic or exotic</td>
<td>Exotic or enzootic organisms. Moderate risk of spread. Official control. May be vectorable, quarantine applied, severe economic significance</td>
</tr>
<tr>
<td>Risk Group IV</td>
<td>Risk Group 4</td>
<td>Class 4</td>
<td>Risk Group 4</td>
<td>Risk Level 4</td>
<td>Group 4 Animal Pathogens</td>
</tr>
<tr>
<td>High individual and high community risk</td>
<td>High individual and high community risk, serious disease, often untreatable, readily transmitted</td>
<td>Dangerous/exotic agents which pose a high risk of life-threatening disease; aerosol transmission or related agents with unknown risk of transmission</td>
<td>Causes serious disease in individual animals, can cause serious economic loss, readily transmissible</td>
<td>Causes serious disease, high mortality, high risk to personnel, readily transmitted by aerosol or insects or transmission unknown, limited or no medical treatment, endemic or exotic</td>
<td>Exotic or enzootic organisms. High risk of spread. Official control. May be vectorable, quarantine applied, movement controls, extremely severe economic significance</td>
</tr>
</tbody>
</table>

USA : United States of America
OIE : Office International des Epizooties

institute and they operate under the strictest guidelines and overview. At the AAHL, for example, only six individuals out of 250 staff are permitted to work with a new Level 4 zoonotic agent, the equine morbillivirus (33) in animals.

Procedural aspects of microbiological security

Table III lists important procedural components of biorcontainment in HS laboratories. A view shared by all is that microbiological security is the primary responsibility of each individual within the facility; it is not the singular responsibility of management or of microbiological security staff. The role of microbiological security staff is to provide expert advice, training, auditing and testing, but not policing.

Before being allowed to work with infectious agents, staff are thoroughly briefed and trained in microbiological security, emergency and spills procedures and are made familiar with the facility. A ‘buddy’ system is common in these laboratories where a new staff member is accompanied and mentored by an experienced staff member until such time as induction and training is complete.

As an extra level of ‘insurance’, HS laboratory staff have specific restrictions imposed on them and are expected to comply rigidly with these. The details vary from facility to facility but the principles are the same. Usually, staff may not keep cloven-hoofed animals at home since they are
susceptible to FMD, among other infections. After working in the secure area they may not visit farms, zoos or agricultural premises for a period of days (three to eight, depending on the institute). Some laboratories also prohibit ownership and contact with horses, fish and birds. Together with the isolation of working in 'secure' areas, the need for showering and rigid compliance with procedures, staff of HS laboratories face significant additional challenges in their work. Visitors to HS facilities are also highly regulated and must sign affidavits to testify that they will comply with regulations.

Fundamental to HS work are well-documented microbiological security policies and procedures. The IAH Pirbright Laboratory, for example, has an 18-section manual of 'Disease Security Regulations' which, together with appendices and other documentation on the 'Importation and Keeping of Animal Pathogens', provides comprehensive advice and instruction to staff. All institutes use these as 'living' documents which are modified, updated and corrected frequently. Overall, the approach taken by most laboratories in this area is one of active and continuous improvement.

Leadership from the top, a high level of ongoing commitment and effective management are recognised to be very important for good biocontainment and safe practices. The challenge faced in HS laboratories is to keep the profile of this aspect of the work very high on a continuing basis. Constantly keeping microbiological security issues on the management agenda is one approach; rigorous incident reporting and analysis is another; and frequent communication and regular debate of issues is a third. Coupled with refresher training, these can be highly effective. These approaches are greatly enhanced where the institute has systematic and rigorous auditing processes in place. The concept of imposing an additional level of external auditing of microbiological security is gaining increasing support in order to provide independent assurance of compliance. In Australia, the AAHL Security Assessment Group (ASAG) provides a good model for external auditing of HS laboratories. ASAG members include microbiologists, biocontainment experts and major stakeholder representatives and they conduct entirely independent assessments of all aspects of microbiological security; reporting not to the laboratory itself, but to the parent organisation, the Commonwealth Scientific and Industrial Research Organisation.

**Physical aspects of microbiological security**

Important physical components of biocontainment in HS laboratories are shown in Table IV. Undoubtedly the most striking feature of HS laboratories for visitors is their structure and design. However, this represents only one of three levels of physical barrier designed to ensure safety (Table V).

Primary containment barriers are designed to protect staff and include such things as gloves and gowns and extend to more complicated devices such as respiratory protection and positive-pressure air supply suits when working at Level 4 (Fig. 2). Primary barriers also include biosafety cabinets, designed to protect operators from exposure when working with live agents by capturing and controlling aerosols. These devices are also a critical part of protecting the environment in the laboratory, where cross-contamination can lead to false positive results and opportunities for occult transfer or escape of agents. For scientific purposes, with the widespread
Work with horses at Level 4 at the Australian Animal Health Laboratory on a recently discovered virus, the equine morbillivirus. Positive-pressure air supply full body suits are used to protect operators.

Application of PCR tests for diagnosis, which are extraordinarily sensitive, prevention of cross-contamination is even more essential.

Secondary barriers, discussed later, are principally those of facility design.

Tertiary barriers are those which are put in place around facilities to keep susceptible animals away as a potential source of infection. These include fencing, walls, physical security and often an exclusion zone in which, by decree, no domestic livestock and poultry may be kept. When facilities were constructed in the early days, the main idea was that safety could be assured by building them remotely on islands. The DVIV on the island of Lindholm in Denmark and PIADC in the USA are the epitome of this idea. There are, of course, significant overhead costs to operating such facilities. However, there is no doubt that this provides a strong positive perception of safety.

Over time, thinking has changed along with the growth of knowledge of biocontainment principles. It is now recognised that the combination of primary barriers (safety equipment) and secondary barriers (facility design) really provides the best security.

Facility design, the focus of most attention on physical barrier containment, the secondary level of biocontainment, has undergone radical rethinking over the past two decades and the newer HS laboratories show outstanding innovation and utility. Superb engineering, architectural and microbiological expertise and creativity have gone into their planning and the free international exchange of design concepts and technical advice has been exemplary.

A principal design element for biocontainment has been the ‘box within a box’ principle of laboratory construction (Fig. 3). The concept, seen in AAHL, NCFAD, IVI and CISA, is of a sealed building within which airtight rooms are partitioned at differential air pressures so that, as doors are opened, air flows to lower pressure zones. That is, the innermost ‘box’ is at the lowest pressure. The most hazardous infectious disease work is performed at the lowest air pressure. Should a leak occur in any of the rooms, air flow moves inwards towards the low pressure, thus effectively preventing the escape of micro-organisms since exhaust air is thoroughly filtered to remove infectious agents. Well-controlled and routinely monitored air-handling is central to this concept, so HS facilities have major plant and equipment devoted to air handling.

Air-handling systems are designed to maintain well-regulated air pressure differentials. At AAHL, there are seven different pressure zones. The most hazardous work is considered to be where infected animals are held since they may excrete large amounts of virus, so this area (the ‘innermost’ box) is maintained at −300 Pascal (Pa) relative to atmospheric pressure. One other conspicuous feature of the newer HS laboratories is air locks. Entry between rooms and suites within the secure area requires staff and materials to pass through an air lock in which rapid air exchange and filtration takes place to ensure no net air movement occurs between zones.
Air filtration is also a very important feature of secure laboratory design; particularly exhaust air, since this has to be scrubbed clean of viruses. All air is exhausted through high-efficiency particle air (HEPA) filters which have a minimum standard of removing 99.97% of particulate material of 0.3μm or greater. Exhaust air is usually passed through two such filters in sequence so that, should one fail, there is automatic backup (Fig. 4a).

Sewerage collection, treatment and disposal is a major undertaking in HS laboratories since everything must be collected and thoroughly decontaminated before disposal. In the newer laboratories, the processes used are similar in principle (Fig. 4b). Waste water from the whole containment area is collected by gravity and reticulates to the sewage treatment plant. Pumps may be used for special circumstances. The sewage collection system provides complete containment of micro-organisms and is designed to prevent cross-contamination between rooms, collection systems and different hazard levels. Once collected, sewage is stored in large storage containers until treated. Different treatment regimes may be applied, depending on the nature of the effluent: however, regardless of whether the process is continuous flow treatment or batch treatment, energy is recovered after treatment by heat exchange units.

All other materials leaving containment areas are sterilised by autoclaving, gamma radiation or chemical immersion, and animal carcasses are sterilised by autoclaving and/or incineration.

**Testing and maintenance**

Microbiological security depends heavily on the integrity of engineering plant and the attitude of staff who maintain and operate it. Monitoring of plant and equipment is most important and some HS facilities have elaborate systems in place which provide real-time monitoring of events. Functions which are monitored include the integrity of physical biocontainment systems described earlier, engineering plant operations, physical security and site access protocols. Regular testing of containment processes usually will include room pressure, air-handling systems, HEPA filters, autoclaves, incinerators, sewerage treatments systems, biological safety cabinets, chemical decontamination system showers and dunk tanks.
In earlier periods, all of the HS laboratories were physically and financially independent, with their own research programmes, culture and focus. Very marked change has taken place in this arrangement over the past decade, and a variety of new organisational models now exist which deserve to be followed and studied with interest. These changes have mostly been driven by financial imperatives. Pressures to increase efficiency and cost-effectiveness have led to reductions in animal health laboratory numbers and consolidation of overall resources. Generally, there has been a strong move towards integrating broad animal health programmes with exotic disease activities. The IAH in the UK began these initiatives in the 1980s, Lelystad in the Netherlands followed a similar course and the Division of Animal Health at AAHL in Geelong, Australia, has recently completed a major reorganisation as a result of which all animal health activities, including exotic diseases, take place at one facility.

In Canada, the initiative taken has been to co-locate both HS animal and human disease activities in Winnipeg. In the USA, the tactical activities of the Animal and Plant Health Inspection Service and the research activities of the Agricultural Research Service were brought much closer together in 1994 by the construction of a joint facility and the
appointment of a director overseeing the exotic disease work of both agencies at PIADC.

Only CISA in Spain, IVI in Switzerland and DVIV in Denmark appear to have retained their institutional independence for work on exotic diseases.

Staffing in HS laboratories has one common characteristic. That is, the level of support staff generally exceeds that seen in conventional microbiological laboratories. The reason, discussed earlier, is that biosecurity requires physical, microbiological security and engineering staff as well as the usual administrative, management and laboratory service groups. Overall, staff levels in the HS facilities listed in Table 1 range from approximately 50 to around 180 members, with a median close to 130. Broadly speaking, PhD-level scientists account for up to 20% of these; scientific technical staff for up to 30%, and support staff, including management, account for the remaining 50%. A recent trend, seen in PIADC, CISA and NCFAD, for example, has been the contracting out of some support services. Given the nature of the work, there are surprisingly small numbers of veterinary scientists overall in these facilities, illustrating the importance of the commitment of other scientific disciplines to the art and science of exotic disease work in HS laboratories.

**Discussion**

High-security laboratories have moved with the times and now play an even more important part in supporting livestock trade through their work on exotic disease research and diagnosis. Given the frequent emergence of 'new' diseases and the regular re-emergence of others, this role will grow. The contribution of HS laboratories to national economies and public good appears to be increasingly recognised, as evidenced by the construction and upgrading of laboratories around the world.

Against this positive background, the pressure on resources for animal health research and development around the world has increased and HS laboratories now have to be more efficient and effective than ever. Choices have to be made and priorities set. In HS laboratories, support costs are high, often 50%-60% of the total, and these may not be reducible because of the demands of microbiological security. So the remaining component, the 'science' element of the budget, is therefore under constant pressure.

Under these circumstances, focusing on tactical, short-term projects may appear to be attractive. However, all practitioners of HS laboratory work agree that tactical outcomes must be underpinned by quality strategic science, and that it is very important to maintain this aspect of the work.

Among the considerations arising from such fiscal pressure, three deserve to be discussed briefly: firstly, the concept that exotic animal diseases may be diagnosed using modern technology without the need for live viruses and therefore biocontainment; secondly, the further integration and rationalisation of national laboratories; and thirdly, the opportunity for more active collaboration and specialisation across the global network of HS laboratories.

Undoubtedly, with modern technology some laboratory results can be generated without access to live viruses. However, it is diagnosis in both the specific and the broad sense that is required of HS laboratories and this requires intimate familiarity with the diseases and considerable experience, especially in outbreak situations. This is only obtained by working with live agents. Usually diagnosis requires the application of a range of tests, some of which require these live viruses. Undoubtedly, the option of having an exotic disease diagnosis made by normal diagnostic laboratories using non-infectious test systems could be progressed much further than it currently is. However, given the economic significance of these diseases and the complexities of rapid, accurate diagnosis, an expert facility with access to all diagnostic options, including viruses, will remain essential for the foreseeable future.

Consolidation of national laboratories has been a recent trend and, in most instances, a highly appropriate one to minimise infrastructure costs and maximise investment in science. Special consideration has to be given to microbiological security, however, since the absolute integrity of the high containment unit remains critical.

Finally, one option which deserves to be explored further in efforts to improve cost-effectiveness is to build on the existing excellent links between HS laboratories around the world. Greater sharing of specialist expertise, training and research would reduce duplication and enhance capabilities.

**Acknowledgements**

The author gratefully acknowledges the information provided by colleagues in high-security laboratories mentioned in the text. N. Willis, S. Alexanderson, A. Torres, J.M. Sanchez-Vizcaino, M. Arias, C. Griot, P. Manni, J. van Oirschott and P. Wilkinson and their staff have been particularly helpful. The help from colleagues at AAHL in reviewing and typing the manuscript is greatly appreciated, as is that of the AAHL library and photography services.
Vue d’ensemble sur le rôle et la structure des laboratoires vétérinaires internationaux de haute sécurité pour les maladies infectieuses des animaux

P.K. Murray

Résumé
L’auteur décrit la structure, le rôle et le fonctionnement, uniques en leur genre, des laboratoires vétérinaires d’État de haute sécurité, en prenant pour exemple ceux de neuf pays. Les laboratoires de haute sécurité offrent la garantie d’un bon rapport coût-efficacité contre les risques de pertes catastrophiques qu’entraînent les maladies exotiques. Ces établissements revêtent une grande importance comme en témoignent les budgets considérables récemment affectés à la construction de nouveaux laboratoires et les travaux importants de rénovation réalisés dans les installations plus anciennes.

La biosécurité est un élément essentiel du fonctionnement des laboratoires de haute sécurité ; aussi est-il important de bien concevoir ces établissements et de veiller au respect des bonnes pratiques de sécurité microbiologique.

Les laboratoires de haute sécurité assurent le diagnostic de maladies exotiques, la certification et l’épidémiosurveillance ; ils mènent également des travaux de recherche en virologie et en pathogenèse, ainsi que sur l’amélioration des épreuves de diagnostic et des vaccins. La mission de ces laboratoires comprend la formation de vétérinaires à l’identification des maladies exotiques. Ils ont également l’importante responsabilité d’offrir leur expertise en cas de maladies exotiques et de participer (aux plans national et international) aux décisions en matière de santé animale.

Mots-clés

Visión de conjunto acerca de las funciones y estructura de los laboratorios veterinarios internacionales de alta seguridad para las enfermedades infecciosas de los animales

P.K. Murray

Resumen
El autor describe las singularidades de estructura, función y métodos de trabajo de los laboratorios públicos de alta seguridad que trabajan en el campo de la sanidad animal, haciendo especial referencia a los laboratorios de nueve países.

Los laboratorios de alta seguridad proporcionan un seguro de coeficiente costo/eficacia positivo, contra la eventualidad de pérdidas catastróficas causadas por brotes de enfermedades exóticas. La reciente construcción de varios de tales laboratorios, a costo de cuantiosas inversiones, así como la profunda renovación de instalaciones obsoletas, atestiguan la gran importancia de este tipo de establecimientos.

Considerando que la seguridad biológica es un elemento capital para el funcionamiento de los laboratorios de alta seguridad, la adecuada concepción de las instalaciones y su aplicación de buenas prácticas de seguridad microbiológica son factores de suma importancia.
Los laboratorios de alta seguridad llevan a cabo tareas de diagnóstico, certificación y vigilancia de enfermedades exóticas, además de investigaciones en virología, patogénesis de enfermedades y perfeccionamiento de pruebas de diagnóstico y vacunas. Entre los deberes de tales laboratorios figura también la capacitación de veterinarios, para prepararlos a reconocer enfermedades exóticas. Otra función de suma importancia es el asesoramiento sobre enfermedades exóticas y la participación (a nivel tanto nacional como internacional) en los procesos de decisión relacionados con temas zoosanitarios.

**Palabras clave**


**References**


