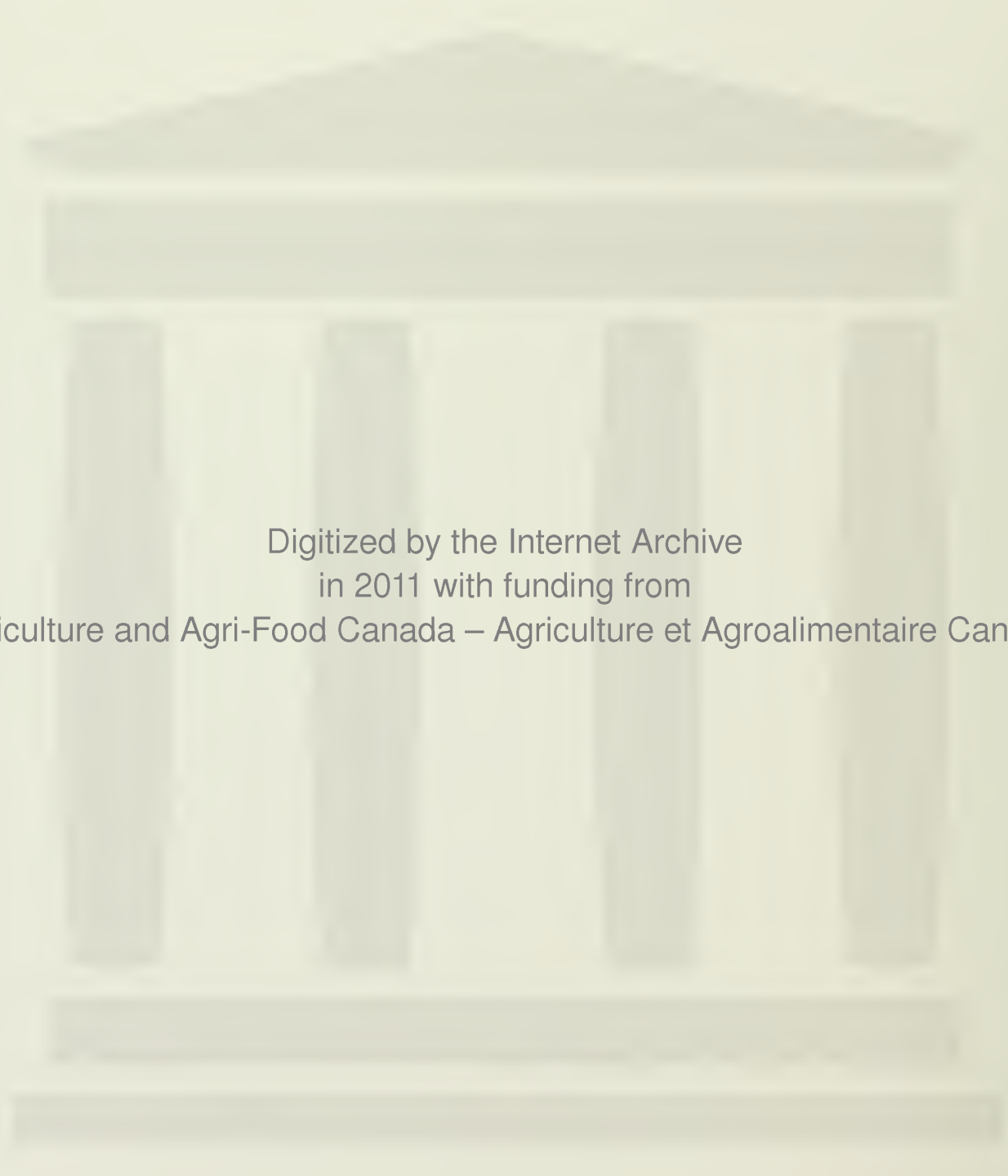


# HEALTH *of* ANIMALS

o v e r v i e w

sixth edition





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**Agriculture  
Canada**

Food Production and  
Inspection Branch

Health of Animals  
Directorate

Direction générale de la production  
et de l'inspection des aliments

Direction de l'hygiène  
vétérinaire

# HEALTH of ANIMALS

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sixth edition

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## Foreword



A year of great activity and continuing change brought new challenges to the Health of Animals Directorate in 1989.

Appropriate procedures were developed and implemented for the safe importation of

large shipments of cattle, sheep and deer from New Zealand. Bovine embryos were imported from Austria, the first such importation from Continental Europe. Discussions were held concerning the importation of pigs from France and llamas from Chile. The decentralization of the issuance of import permits to the regional offices was implemented.

On the export side, there was a 26 per cent increase in the number of consignments and a total of 115 protocols were developed or amended.

During the year, delegations were received from the United Kingdom, France and India for discussions on import and export trade.

A major effort was directed at reducing the level of salmonella contamination of food.

On the production side, control programs were developed and pilot programs initiated at the breeder and hatchery levels. A survey of layer and broiler producer premises was carried out, with a particular interest in *Salmonella enteritidis*.

Research efforts were directed towards the development of rapid tests for the detection of the presence of salmonella in food products with a significant measure of success.

An outbreak of brucellosis in a large purebred beef herd in western Canada led to an extensive investigation in order to trace and remove all at-risk movements from the herd, and to identify the source of the infection. "Stamping out" measures were applied.

A number of meetings and review sessions were held related to the establishment of the Federal Environmental Assessment Review Panel to consider possible solutions to the problem of the northern bison infected with bovine brucellosis and tuberculosis. The

formal hearings under this panel are to take place early in 1990.

Surveys of apiaries along the Canada/U.S. border for the presence of the varroa mite resulted in the detection of the infestation in a small apiary in eastern Canada. The bees in this apiary were destroyed and more intensive surveys in the immediate area have not detected additional infestations. The total ban on the importation of honey bees from the United States, including Hawaii, has been extended.

Work continued throughout the year on preparing the Bill to amend the Animal Disease and Protection Act. A model set of regulatory guidelines and a biotechnology "road-map" were developed to address public concern with the control of biological products arising from the application of biotechnology. A practical risk assessment concept was developed and implemented to establish a formal process for dealing with issues.

While a considerable portion of the research effort was directed towards salmonella, work continued in a number of other areas: Two of our researchers uncovered further evidence of the identity of the scrapie agent, and work continued exploring the risk of disease transmission during embryo transfer. A study on the incidence and economic significance of Johne's disease in dairy cattle was completed.

An intensive, highly focused research coordination team was put together to define the overall direction for the research program. Its mandate includes rationalizing the management of the research program and enhancing its visibility and achievements.

Laboratory amalgamation (under which various laboratories within the Branch are being co-located for increased efficiency) continued smoothly throughout the year. The Health of Animals Laboratories in Winnipeg, Manitoba and Richmond, British Columbia were closed and the responsibilities and staff transferred.

A major modification to the Animal Diseases Research Institute (Nepean) was developed to accommodate a plant health component.

Planning is continuing towards establishing a new Animal Plant Health Laboratory at Charlottetown, Prince Edward Island. A team was put together to help develop a joint High Security Virus Laboratory (with Health and Welfare Canada) in Winnipeg, Manitoba.

The implementation of the Laboratory Information Management System is underway and a draft manual for the quality assurance program has been developed. The accreditation of non-federal laboratories to carry out various tests was initiated with eleven laboratories being accredited for equine infectious anemia and ten for enzootic bovine leucosis. Additional laboratories are in the process of approval.

Staff of the Directorate continue to play a bigger role in various international activities.

One of our officers acted as a process leader for the Office International des Epizooties Regional Workshops, held last year.

A considerable amount of time and effort was directed towards developing Directorate strategies, with extensive input from the staff at each of the locations.

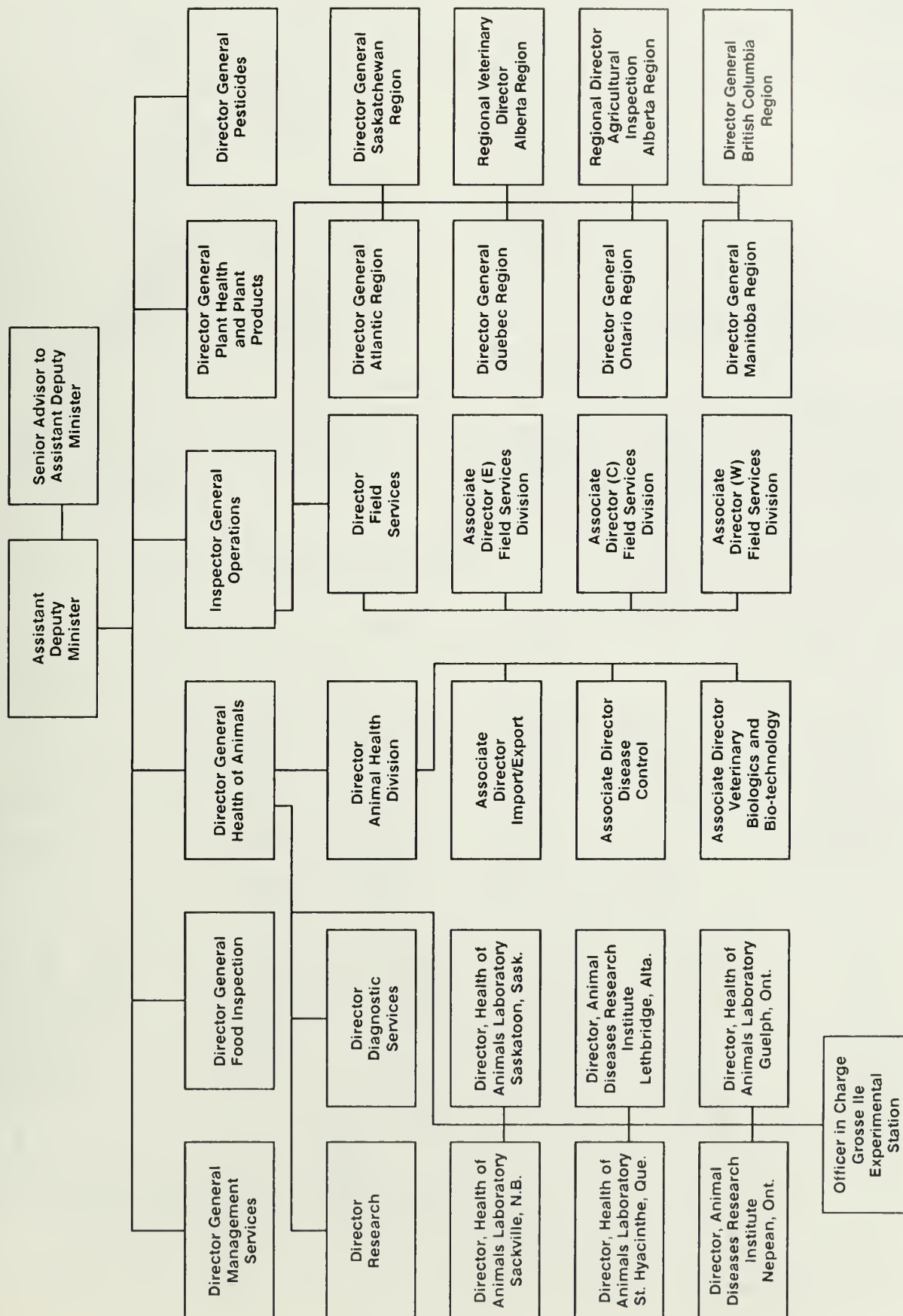
Mini-program reviews of the export, foreign animal disease control, rabies and humane animal transportation programs were carried out by the Animal Health Division.

For 1990, we see the major challenges ahead as the increasing concerns evolving around food safety issues, the management of the amalgamated laboratories and the refinement of our programs in order to achieve increased efficiency and effectiveness.

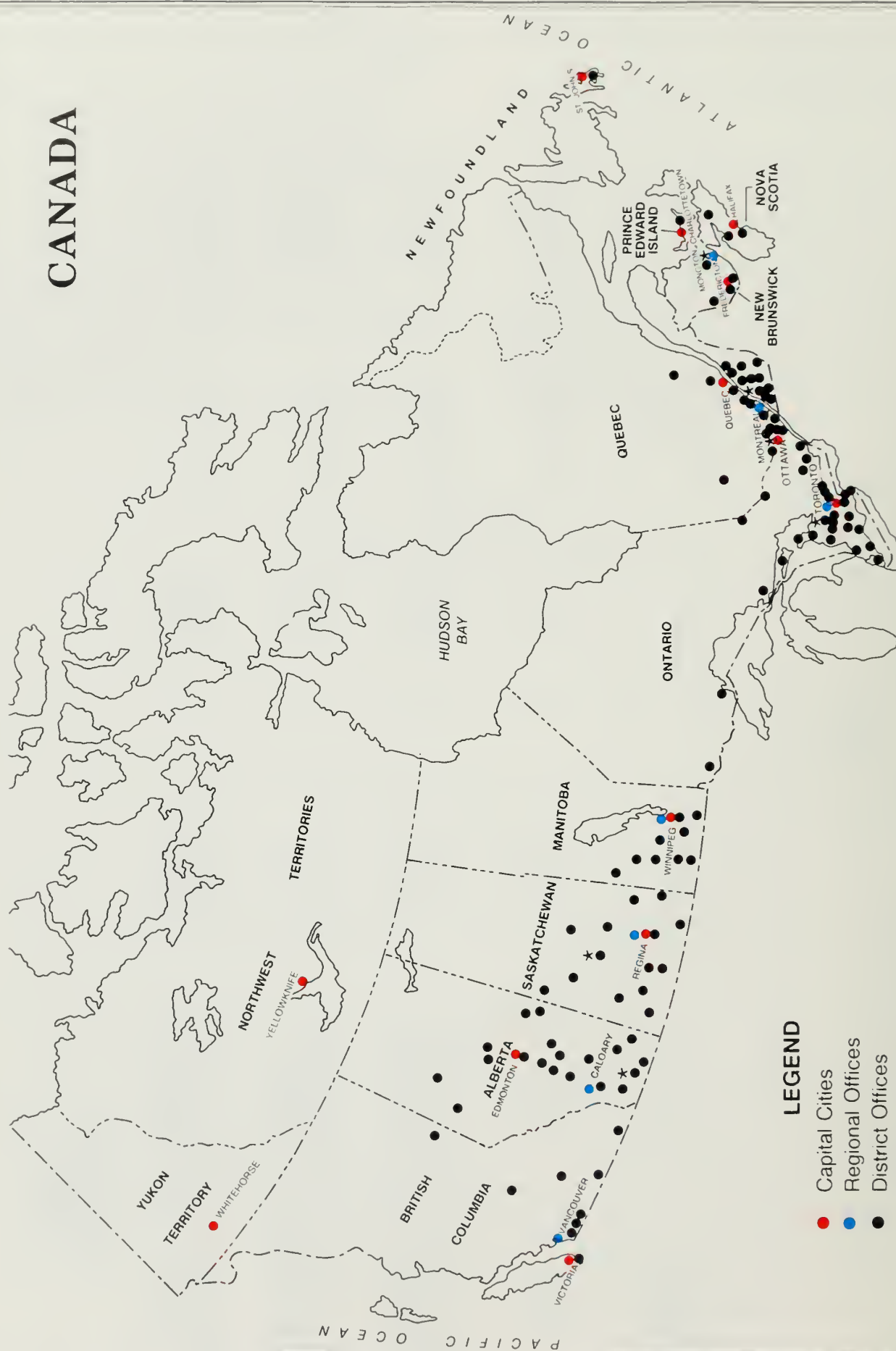
N.G. Willis  
Chief Veterinary Officer and Director General  
Health of Animals Directorate



# Food Production and Inspection Branch – Organization Chart



# CANADA



## LEGEND

- Capital Cities
- Regional Offices
- District Offices
- \* Health of Animals Laboratories



# The Canadian Animal Health Program

## History

Animal health has been vitally important in Canada since 1865 when the legislature of Upper and Lower Canada established an "Act to Prevent the Introduction and Spread of Disorders in Animals." After Confederation, an "Act Respecting Contagious Diseases in Animals" was the first agricultural bill that the fledgling country passed.

High animal health standards were crucial to expanding and improving Canada's early livestock industry. Top quality cattle, sheep and swine were imported to lay the foundation for a disease-free livestock population. Good stock was imported from England and Scotland and, to a smaller extent, from other European countries.

To protect this growing industry, quarantine stations were established at Halifax, Nova Scotia, Saint John, New Brunswick and Levis, Quebec to intercept imports.

Early control measures were stiff. An 1868 report indicated that livestock were inspected before unloading, quarantined for 90 days and slaughtered if infected with a listed disease. Incoming animal products, packing material and equipment used to handle animals were also inspected. Because of Canada's strict import practices and disease control measures, Canadian livestock were permitted free access to English markets, even while there was contagious bovine pleuropneumonia in the United States.

Agriculture Canada's present policies on preventing and eradicating exotic diseases were established many years ago. The department recognized the need to study exotic diseases in other countries and to train veterinarians in diagnosing and controlling them. Training courses have been held for many years at Grosse Ile, an island in the St. Lawrence River.

In 1977, when the Animal Disease and Protection Act replaced the Animal Contagious Diseases Act, Canada boasted an impressive record of disease eradication (see table of diseases eradicated in Canada).

## LIVESTOCK POPULATION AND FARMS IN CANADA

	Cattle	Swine	Sheep	Horses and ponies *	Poultry
<b>Livestock population</b>					
Atlantic	336 200	380 500	60 200	9 277	8 252 000
Quebec	1 504 000	3 080 000	111 000	25 481	2 604 100
Ontario	2 285 000	3 370 000	201 000	74 961	38 064 000
Manitoba	1 075 000	1 200 000	22 000	40 691	7 650 000
Saskatchewan	2 150 000	845 000	51 000	67 484	4 528 000
Alberta	4 015 000	1 735 000	198 000	135 025	10 500 000
British Columbia	695 000	236 000	53 500	42 034	11 679 000
<b>TOTAL</b>	<b>12 060 200</b>	<b>10 846 500</b>	<b>696 700</b>	<b>394 953</b>	<b>83 277 100</b>
<b>Farms</b>					
Atlantic	7 148	1 555	792	2 576	2 734
Quebec	26 062	4 706	1 261	6 607	6 495
Ontario	39 647	12 933	3 708	13 523	18 622
Manitoba	13 868	3 563	473	5 540	7 019
Saskatchewan	26 389	5 778	1 021	13 420	18 125
Alberta	33 498	6 538	2 148	20 921	17 563
British Columbia	9 333	1 399	1 536	6 561	7 690
<b>TOTAL</b>	<b>155 945</b>	<b>36 472</b>	<b>10 939</b>	<b>69 148</b>	<b>78 248</b>

No reportable data for the Yukon and the Northwest Territories

SOURCE: 1986 Census of Agriculture (\*) Statistics Canada update June 1988.

## The Program Today

Agriculture Canada's Food Production and Inspection (FPI) Branch is headed by the assistant deputy minister. As Chief Veterinary Officer, the Director General of the Health of Animals Directorate, through the Animal Health Division and the Health of Animals Laboratory Division, is responsible for the negotiation and establishment of sanitary conditions for the import and export of animals and animal products, program planning and evaluation, operational management of laboratories and program coordination.

Six facilities offer diagnostic services and conduct animal disease research. The Animal Health, Food Inspection, Agriculture Inspection and Plant Health programs are delivered across Canada by the Inspector General of the Operations Directorate through seven regional organizations (one for the Atlantic provinces and one for each of the six other provinces).

The unification of the Veterinary Inspection and Agricultural Inspection Directorates' Regional Offices into the Operations Directorate was completed in all regions, with the exception of Alberta, as staffing actions created the opportunity.

The National Animal Health Program (NAHP) employs approximately 1000 persons, including 307 veterinarians and 35 research scientists. The remainder are technical and support staff.

There are 110 field offices, three quarantine stations for offshore imports of livestock and four quarantine stations on the Canada-United States border.

In 1987 the NAHP and the Diagnostic Services Program which supports it, were subjected to intensive review. The review established a strategy to help NAHP meet the needs of a changing clientele into the 1990s. Although NAHP has traditionally tried to balance productivity and marketability, its focus has shifted toward marketability support, with an emphasis on human health and safety.

NAHP is focusing attention on zoonotic diseases, animal identification, residues, voluntary herd certification plans and animal welfare.

The NAHP is renewing and assuming the lead in Agriculture Canada's ten year effort aimed at Salmonella control throughout the poultry industry. After successfully eradicating brucellosis and virtually eradicating tuberculosis from the cattle population, these eradication programs are focusing on farmed game species.

	Atlantic Provinces	Que	Ont	Man	Sask	Alta	B.C.	Yukon*	Total
Regional offices	1	1	1	1	1	1	1	-	7
District offices	10	23	29	11	11	17	9	-	110
Veterinary colleges	1	1	1	-	1	-	-	-	4
Inspection ports	17	20	27	5	6	5	16	-	96
Health of Animals labs	1	1	2	0	1	1	0	-	6
Diagnostic laboratories (provincial)	4	7	6	1	1	5	1	-	25
Federal abattoirs	11	60	46	9	7	19	20	-	172
Domestic abattoirs	-	21	270	37	10	-	6	-	344
Auction markets	8	22	56	18	28	42	11	-	185
Embryo transfer centres	-	4	10	2	6	11	2	-	35
Artificial insemination centres	0	3	6	2	1	4	1	-	17
Registered dairies	26	121	181	29	11	28	21	-	417

\* Includes Northwest Territories



The diseased wild bison of the Northwest Territories and northern Alberta are a disease threat to the domestic cattle population and are being studied by a multiple-agency task force co-chaired by Agriculture Canada and an Environmental Assessment Panel.

To better protect Canada's livestock, several safeguards are being strengthened. These include domestic and international disease intelligence, foreign animal disease preparedness and import controls.

The success of these initiatives depends on restructuring programs, cross-utilization of staff with associated programs, provincial cooperation, and more industry self-regulation and cost-recovery. Fundamental changes to the Animal Disease and Protection Act now in progress will facilitate these initiatives, as will the restructuring, in 1989, of the Animal Health Division from the three sections, Disease Control, Import and Export, into Disease Control, Import/Export and Biologics/Biotechnology. This allows more efficient and effective international negotiations and liaison by the responsible Import/Export Associate Director.

At the same time, it recognized the important and rapidly growing biotechnology sector and its unique role in the NAHP.

Many changes highlighted this year take their initiative and direction from the 1987 NAHP review.

## **Disease Control**

### **Foreign Animal Diseases Emergency Response Program**

The Foreign Animal Disease Eradication Organization is an emergency response network that responds immediately and effectively when foreign diseases are introduced to Canada.

The network includes a national headquarters response team and seven regional alert teams (one for each administrative region in Canada). It is staffed by departmental employees normally engaged in other duties. The network also has operational links with emergency-response officials in provincial and other federal agencies.

Teams are kept ready through yearly simulation exercises, seminars and other training activities at national and local levels. A procedures manual for responding to foreign animal disease outbreaks has been completed, and official policies on specific foreign animal diseases have been developed.

The response organization also monitors the evolution of outbreaks of these diseases and how they are controlled in other countries.

### **OIE LIST "A" DISEASES ERADICATED FROM CANADA AND LAST OCCURRENCE**

Disease	Year
Bluetongue	1988
Contagious bovine pleuropneumonia	1876
Foot and mouth disease	1952
Hog cholera	1963
Velogenic Newcastle disease	1973
Vesicular stomatitis	1949

### **OIE LIST "B" DISEASES ERADICTED FROM CANADA AND LAST OCCURRENCE**

Anaplasmosis	1986
Anthrax	1985
Brucellosis	1989
Dourine	1921
Equine Piroplasmosis	1987
Fowl typhoid	1983
Glanders	1938
Horse mange	1940
Porcine trichinellosis	1985
Pullorum disease	1989
Varroasis	1989



## LIST "A" OIE DISEASES NEVER REPORTED IN CANADA

African horse sickness  
African swine fever  
Lumpy skin disease  
Rift valley fever  
Rinderpest  
Peste des petits ruminants  
Sheep and goat pox  
Teschen disease  
Swine vesicular disease  
Fowl plague

## LIST "B" OIE DISEASES NEVER REPORTED IN CANADA

Aujesky's disease (pseudorabies)  
Heartwater  
Bovine babesiosis  
Theileriosis  
Trypanosomiasis  
*Brucella melitensis*  
Contagious agalactia  
Contagious caprine pleuropneumonia  
Nairobi sheep disease  
Contagious equine metritis  
Epizootic lymphangitis  
Horse pox  
Japanese encephalitis  
Leishmaniasis  
Surra (*T. evansi*)  
Venezuelan equine encephalomyelitis  
Porcine brucellosis (*B. suis* biovar 1)  
Porcine cysticercosis  
Screw worm (*C. hominivorax*)  
Viral hemorrhagic disease of rabbits  
Hemorrhagic septicemia

## LIST "A" OIE DISEASES OCCURRING IN CANADA

nil

A series of public education brochures is available on various foreign animal diseases. These publications emphasize the risks and effects that such diseases represent to Canada's agricultural economy.

Canada, the United States and Mexico are members of the North American Foot and Mouth Disease Vaccine Bank. The bank offers extra protection to the Canadian industry if a massive outbreak of the disease occurs that cannot be controlled by direct eradication procedures.

## Food Safety

### Salmonellosis

In 1979, a Canadian delegation was organized to examine the salmonella control programs of several Scandinavian and other European nations.

During the ensuing years, considerable domestic consultation led to the development and promotion of voluntary sanitation programs aimed at numerous points in the poultry production cycle. Staff preoccupied with bovine brucellosis eradication were hard pressed to contribute more to an area of activity that did not fit the traditional "production support" mold that shaped other field programs.

By 1989, the worldwide prevalence of reported human salmonellosis associated with poultry had risen dramatically. In an era characterized by overproduction, the orientation of international veterinary services had moved from production support to marketability. International concerns regarding dioxins, hormones, antibiotic residues, PCB's and food poisoning placed human health and safety considerations at the forefront of the marketability issue.

In the wake of *S. enteritidis* outbreaks in the United States and the United Kingdom, members of a second delegation witnessed the relative calm prevalent in the poultry industries of the Scandinavian nations. They returned home convinced that their methods were worth emulating.

The working group has initiated an integrated program which addresses fourteen key points in the poultry production chain. Individual efforts formerly aimed at feedmills, breeding flocks, hatcheries and abattoirs will now be coordinated by a management committee to progress at a pace considered cost-beneficial to industry and government.

Each key point, or 'element' will be addressed by a multi-disciplinary team made up of an epidemiologist, a bacteriologist, and one or more operational and program planning staff. A staff position has been established specifically for program planning and evaluation, building upon the success of a similar position created in bovine brucellosis eradication in 1978.

Another successful brucellosis program parallel, a consultative committee known as the Salmonella Review Board has been established with all poultry sectors and will include consumer advocates. It is anticipated the program will be regarded as a 10-year challenge before significant results are achieved.

## Trichinosis

Trichinosis has historically been a problem in one area of Nova Scotia and has been a reportable disease since 1971. The disease is diagnosed at slaughter using the trichinoscope. After investigation at the originating premises, animals are quarantined and ordered slaughtered under licence.

Compensation is paid for animals condemned at abattoirs following quarantine, but not for those found to be infected on regular kill.

As the result of a 1987 review of the trichinosis program, Trichomatic-35 digestion machines have replaced four trichinoscopes in swine abattoirs. Following a year's use of the Trichomatic-35, a decision will be made on whether to purchase machines for the other abattoirs.

A swine serum survey of 15 000 slaughter sows began in 1989. These sera will be tested using the enzyme-linked immunosorbent assay (ELISA) test. The results of the survey will be used for Canada's application for recognition of freedom from trichinosis in the national swine herd. No cases have been diagnosed in swine since 1985.

## Bovine Cysticercosis

Bovine cysticercosis occurs sporadically across Canada and was made a reportable disease in 1972. *Cysticercus bovis* is discovered during meat inspection and confirmed at the laboratory. The ELISA test, using an excretory/secretory antigen, has not been found to be a reliable field test.

Research is being conducted to determine the dose/antibody response of cattle infected with eggs of *Taenia saginata*. During 1989, 55 investigations were carried out on single-case positive submissions. While no source was determined, four of the cases were derived from imported animals.

## Eradication/Control Programs

### Bovine Brucellosis

Canada's cattle population was declared completely brucellosis-free on September 19, 1985, after eradication of the last substantive outbreak of brucellosis in Ontario during the previous year.

As far back as the early 1930s, a voluntary (BFLH) program was introduced, under which annual herd agglutination tests were performed and reacting cattle removed. This program was effective on herds but did little to lower the national infection rate. A national survey in the late 1940s revealed an animal infection rate of approximately 9.5 per cent.

In 1950, a joint federal-provincial Calfhood Vaccination Program began, using Strain 19 vaccine. This program reduced the infection rate to four per cent by 1956.

The next development in the eradication process was the introduction of the mandatory test and slaughter program in 1957. In a test of all cattle herds, (completed in 1966), more than 191 000 reactors were slaughtered with compensation paid to owners. This measure further reduced the herd infection rate to 0.2 per cent.



Two surveillance procedures, the Brucellosis Milk Ring Testing (BRT) and the Market Cattle Testing (MCT) programs were initiated in the early 1960s to monitor the tested herds. These screening programs virtually replaced "down the road" testing used in early programs.

Beginning in 1969, calfhood vaccination was emphasized less, revealing more hidden infection than was anticipated. With a more susceptible national herd, the test-and-slaughter approach was increasingly replaced by depopulating infected premises. Despite longer herd quarantines, shorter testing intervals and the use of traditional eradication techniques, the program continued to lose ground.

In 1977, the FPI Branch established a formal Brucellosis Consultative Committee with the livestock industry, provincial veterinary services and the Canadian Veterinary Medical Association. A revised program introduced on April 1, 1978 designated each province a region, and required certification and testing to move cattle between regions.

In some regions tests were required to move animals between farms. Presale testing was instituted at auction markets and cattle dealers were also regulated. As regions became infection-free, local controls were relaxed.

Since the eradication of brucellosis from the national cattle herd, owners can again move their cattle throughout Canada without restrictions.

In keeping with international practice, surveillance will continue at peak levels for several years. Completion of the transfer of the MCT surveillance program from auction markets to abattoirs took place in 1989. This improved the efficiency and maintained the effectiveness of this surveillance tool. The BRT surveillance of milking herds has been enhanced by more frequent use of automated testing techniques.

Surveillance programs have detected five foci since the declaration of freedom in 1985. One non-clinically infected animal in each of two herds was detected in 1986. Although the disease was not transmitted, both herds were destroyed.

The first clinically infected herd in three years was identified in Alberta in December

1986. The outbreak was transmitted by a latently-infected animal that had been exposed to infection in the 1970s. Eradication procedures were implemented and the outbreak was completely stamped out.

In 1989, one non-clinically infected animal was detected in Saskatchewan. Again, although there was no evidence of disease transmission, the herd and all other exposed cattle were destroyed. This involved more than 500 animals and almost \$500 000 in compensation was paid.

## **Bovine Tuberculosis**

Cattle were first tested for tuberculosis in Canada in 1897. The Municipal Tuberculosis Order passed in 1914 authorized municipalities to pass by-laws requiring milk herds to be tuberculosis-free. Compensation for tuberculosis reactors was paid by the federal Department of Agriculture. In 1923, area testing for tuberculosis began and by June 1961, the first general test of all areas in Canada was completed.

In the late 1970s, on-farm tuberculosis testing gave way to tracing the disease back to the herd of origin when lesions were found in animals at slaughter. This surveillance program established a target submission rate of one lesion per 2000 surveillance-eligible cattle (cows and bulls) slaughtered.

Federally inspected abattoirs have consistently achieved an average 200 per cent of the target testing over the past few years. To aid in eradication, Canada is divided into 10 accreditation areas corresponding to the 10 provinces. All areas, with the exception of Quebec, are accredited as tuberculosis-free.

In 1989, traceback investigations of slaughter lesions detected two tuberculosis infected herds, one in Quebec and the other in Saskatchewan. Each herd was destroyed, and movements extensively traced and tested. Approximately 800 animals were destroyed and almost \$500 000 in compensation was paid.

Canada anticipates achieving bovine tuberculosis freedom status by the early 1990s.



## Captive Wild Ungulates

The Captive Wild Ungulate (CWU) program was implemented in 1988 to eradicate brucellosis and tuberculosis from bison and other commercially ranched species of wildlife, primarily elk (wapiti) and deer.

The program calls for the identification and testing of all premises containing these species in a ranching operation. When brucellosis and/or tuberculosis infected herds are uncovered the appropriate disease eradication program is implemented. The initial inventory phase identified approximately 275 eligible ranching operations.

To date, the CWU program has uncovered two infected herds. In 1985, a bison herd in New Brunswick was found infected with tuberculosis (*M. bovis*) and was destroyed.

In 1988, the program detected a brucellosis infected bison herd in Ontario. This herd, which included elk and deer, was destroyed. Extensive investigation, tracing and testing revealed no transmission of the infection. The source, although not clearly established, is rumored have been the introduction of bison from a herd that had animals tracing back to the original infected bison herd in Buffalo Park, Wainwright, Alberta.



*Typical North American plains bison bull*

## Pullorum Disease and Fowl Typhoid

The pullorum and typhoid eradication program, in effect since 1982, tests primary breeding flocks and hatchery supply flocks, cultivates fluff samples from hatcheries, and regularly tests exhibition and game birds.

Surveillance of the national commercial poultry flock since 1970 has confirmed that it continues to be free from pullorum and typhoid. Isolated incidents occur in small exhibition flocks. Two backyard flocks were depopulated in 1989 as a result of positive cultures of a standard strain of *S. pullorum*. Both were thought to be 'tracebacks' to the original 1987/88 outbreak. Approximately 200 birds were destroyed and \$2880 paid in compensation.

## Rabies

The rabies control program aims to prevent human exposure to the disease. Rabies is a reportable disease and Operations staff investigate all suspected cases. The program provides diagnoses on specimens, imposes quarantines on domestic animals, pays compensation, recommends vaccination clinics and informs the public of rabies threats.

In 1989, 2366 cases of rabies were diagnosed in domestic and wild animals in Canada, a decrease of almost 7.7 per cent from 1988.

In a federal-provincial compensation program - to which the federal government contributes 40 per cent and the provinces (Manitoba, Ontario, Quebec and New Brunswick), contribute the remainder - the federal government paid \$133 009.50 for 462 livestock that died of rabies.

If rabies follows its normal three to five year cycle, then 1990 should see an increase in Canada. Monoclonal antibody tests on a red fox and a cow from Cape Breton Island, Nova Scotia, and a red fox in South Newfoundland, determined that the virus was the same strain found in a brown bat (*Eptesicus fuscus*) in Ontario.

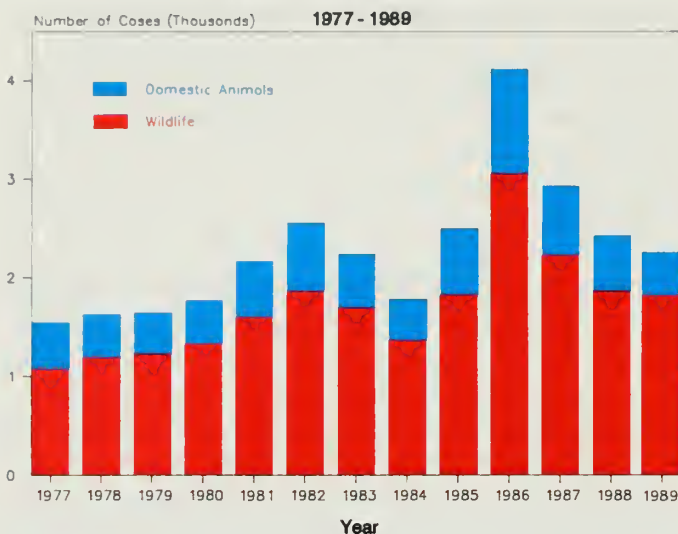
The main vectors of rabies continue to be the red fox in Ontario and Quebec, and the striped skunk in the Prairie Provinces. Insectivorous bats were the vector in the three cases of human rabies reported in the past 20 years.

The Federal Rabies Program was also assessed, as recommended by the 1987-88 NAHP review. This assessment recommended that Agriculture Canada study the feasibility of eradicating rabies in Canada.

This is to be accomplished through a cooperative field trial with the Ontario Ministry

of Natural Resources (OMNR), begun in October 1989. During that month 300 000 baits with vaccine and tetracycline (used as a marker) were dropped over Eastern Ontario.

#### Yearly Rabies Incidence



Over the next five years, the feasibility of this large scale vaccination program of wildlife will be determined. Should it prove successful, a Canada-wide rabies eradication program would be considered.

#### CASES OF REPORTABLE DISEASES, 1989

Anaplasmosis	-
Anthrax	-
Bovine cysticercosis	55
Brucellosis	1
Contagious equine metritis	-
Dourine	-
Equine infectious anemia	188
Equine piroplasmiasis	-
Fowl typhoid	-
Glanders	-
Mange in cattle	19
Pseudorabies	-
Pullorum disease	1
Rabies	2366
Scrapie	10
Sheep scab	-
Trichinosis	-
Tuberculosis	3
Vesicular exanthema	-

#### Scrapie

The first scrapie control program was instituted in 1945. It has been modified several times in response to industry needs and available scientific information.

The current program has been in place since 1983. It incorporates recent research indicating that scrapie is usually transmitted at the time of birth through close contact between dam and offspring. The program seeks to control the spread of scrapie when laboratory-confirmed cases are found.

All animals are designated as high, medium or low risk, depending on their bloodline relationship or physical contact with the affected animal.

Maternal relatives designated as "high risk" are traced, destroyed and compensation is paid. "Medium risk" contacts of the affected animals are identified and monitored for evidence of scrapie for 42 months after contact has ended. "Low risk" is a term applied to animals not known to have been in contact with the affected animal. There is no follow-up for low risk animals.

In 1989, 10 scrapie-infected sheep flocks were detected, six of them in Ontario, two in Quebec and two in Saskatchewan. In two of the cases, the extent of the infection required destruction of the entire flock.

#### Equine Infectious Anemia (EIA)

EIA was made a reportable disease under the Animal Disease and Protection Act, and a voluntary control program was initiated in 1972 when a reliable serological test (Coggins' test) became available.

All animals that react positively to the test are ultimately ordered slaughtered and compensation up to \$200.00 per animal is paid above any carcass value that may be salvaged. Some owners avail themselves of the option to permanently quarantine the reactor in suitable vector-proof facilities.

Approximately 80 000 to 100 000 tests are performed each year to meet routine domestic and international requirements for racing, exhibition, breeding, sale and stabling, as well as in response to suspected or confirmed disease situations. The reactor rate has



declined from 2.9 per cent in 1972, to 0.2 per cent in 1988.

Infection is known to be endemic in much of northern Canada, particularly in bush horses, native Indian horses, horses on pregnant mare urine production farms, and in wild horse herds.

EIA has virtually been eradicated from the remaining sectors of the horse population. They voluntarily request testing, which results in the periodic disclosure of reactors as a result of movements out of the endemic population. Approximately five per cent of all reactors demonstrate clinical signs of EIA.

In 1989, 82 128 horses were tested and 188 reactors were detected. To date, all EIA tests have been performed in federal laboratories. In 1988, Agriculture Canada developed a protocol outlining the process for the accreditation of non-federal laboratories to perform the Coggins' test for EIA. Following a period of consultation with all interested parties, the protocol was implemented in 1989. The current EIA program did not change in that all reactors continue to be reported to and dealt with by the Food Production and Inspection Branch, which also maintains statistics on negative tests.

### **Newcastle Disease in Pigeons**

To protect Canadian pigeons, import requirements were imposed in 1985 involving vaccination with killed Newcastle disease (ND) virus vaccine and post-importation quarantine. ND outbreaks in domestic pigeons are controlled through quarantine and vaccination. The outbreaks subside in the pigeons' lofts as soon as the younger birds are vaccinated. Annual vaccination of pigeons is a recommended procedure.

During 1989, 12 outbreaks were recorded, eight in Ontario, one in Manitoba and three in Alberta. It is not known whether these outbreaks are a continuation of the 1988 outbreak in Ontario.

All outbreaks in pigeons are examined in the laboratory to establish pathogenicity in poultry. There were no velogenic isolates found and velogenic ND has not been reported in Canada since 1973.

### **Mange**

Mange in livestock (cattle, horses, sheep and goats) is a reportable disease.

In the early 1900s, mange was major problem in western Canada and resulted in a blanket quarantine on moving cattle from southwestern Saskatchewan and southern Alberta.

A compulsory dipping program introduced in 1919 required two dippings, with the second to take place between 10-15 days after the first treatment. The program was a success and the quarantine was lifted.

A few cases of mange are diagnosed in cattle each year in Alberta and the Peace River area of British Columbia. Infected herds are quarantined and treated with an approved acaricide, supervised by an inspector of the Operations Directorate. All pens, corrals and barns must be cleaned and disinfected.

In 1989, Saskatchewan and Alberta experienced four serious outbreaks in community pastures that required the tracing and treatment of cattle on more than 150 premises.

### **Licensed Garbage Feeders**

The licensed garbage feeder program exists in order to ensure the practice of feeding garbage to domestic livestock does not pose a disease risk to the herd or flock or to other livestock operations. Anyone who collects garbage from commercial institutions such as hotels, restaurants and hospitals, and feeds the garbage to swine and poultry, must have a permit issued by Agriculture Canada. In 1989, approximately 40 permits were issued.

Licensed garbage feeders are visited regularly to determine the health of the swine, to ensure that garbage is completely cooked, and that the premises are clean and sanitary. Swine from these premises must be sold directly to an abattoir operating under full Government of Canada inspection.

Commercial institutions are visited to ensure that garbage is not being picked up by unlicensed garbage feeders.



## Paratuberculosis

Between 1959 and 1979, Agriculture Canada offered cattle owners a voluntary program to control paratuberculosis in which cattle were tested periodically, reactor animals were destroyed and compensation was paid. The program was modified in 1979 to end the need to destroy reactors and pay compensation.

The modified program increases the involvement of private practitioners and provincial veterinary services in helping livestock owners control paratuberculosis. This modified program has also been made available to sheep and goat owners. The Food Production and Inspection Branch co-ordinates these activities and offers diagnostic services and professional consultations.

The program emphasizes the elimination of clinical expression of paratuberculosis from the herd to increase productivity. Management, husbandry and hygiene considerations play an important part in the program.

In 1987, an epidemiological study, jointly funded by Agriculture Canada and the Province of Ontario was initiated to examine the prevalence and economic impact of paratuberculosis in dairy cattle. The results of the study are anticipated in 1990.

## Other

### Disease Surveillance and Epidemiology

The National Animal Health Information System (NAHIS) is the umbrella under which serological surveys and epidemiological studies are developed and analyzed. This facilitates the ongoing surveillance of Canadian livestock for economically important diseases.

One such survey, the swine serological survey involves the collection of 15 000 serum samples from sows at abattoirs across Canada. This five-month survey was initiated in late 1989. Serum samples were tested to portray freedom from pseudorabies (Aujeszky's disease) and *Brucella suis*, and trichinosis in our national swine population. The testing was essential to the continuation

and expansion of a strong swine export market.

As with two previous serological surveys conducted in 1979 and 1985, surplus serum was placed into a serum bank to allow for further testing and retrospective surveys.

A serological survey of maedi-visna infection and a study of health, production and management practices in sheep flocks was conducted on 14,000 sheep in 286 flocks across Canada and revealed a widespread distribution of the disease. An analysis of the serum test results and information on the flock portrayed the influence of flock size, breed, age, disease occurrence and management factors on the prevalence of this disease.

Another NAHIS study investigated the seroprevalence of bovine leukemia virus (BLV) infection in Ontario dairy cattle. An existing serum bank and production data, based on Dairy Herd Improvement Corporation records, were accessed for this study. The influence of BLV infection on milk production and calving interval, and the relationship between management practices and infection are being investigated.

A bovine serological survey of cows at abattoirs across Canada is being prepared for field implementation in 1990. The serum samples will be tested to portray Canada's freedom from bovine brucellosis, bluetongue and epizootic hemorrhagic disease (EHD).

### Voluntary Herd Certification

Agriculture Canada has offered Canada's livestock producers voluntary programs directed against one specific disease or another since 1919.

The most recent, the Brucellosis Free Listed Herd (BFLH) plan, had been adopted by more than 11,000 cattle herd owners in the late 1970s. The BFLH plan expired April 1, 1988, having served its purpose, the provision of several benefits to owners, such as animal identification, movement and inventory controls, export facilitation and disease prevention.

Cattle industry spokespersons expressed concern over the need for Agriculture Canada to certify herds free from disease. Decades of experience have also shown the voluntary

programs to have a value which transcended the specific disease entities against which they were directed. With this in mind, the NAHP established the Canada Health Accredited Herd (CHAH) program.

Although it incorporates an initial tuberculin test of the enrolled cattle herd, it is directed against the transmission of diseases in general through the application of fundamental principles. It emphasizes movement controls, isolation of additions, identification, herd registries and record retention. Initially offered to former BFLH owners, it is available to any qualifying cattle producer whose herd has existed as a unit for at least two years.

All enrolled herds will retain a basic CHAH status certified by Agriculture Canada. On a modular basis, however, herd owners may seek additional certification based on proven freedom from other diseases, which could include enzootic bovine leukosis (EBL), leptospirosis, IBR and paratuberculosis.

Diseases are selected for program inclusion based on their economic impact and the practicability of their control at the herd level. A CHAH-EBL module is being made available to interested herd owners in 1990.

Although the cattle industry is the initial recipient of the program, the swine and small ruminant sectors have expressed strong interest. A swine program, the National Swine Health Information Program (NSSHIP), aimed at standardized monitoring and reporting on six diseases, actinobacillosis, pleuropneumonia, enzootic pneumonia, atrophic rhinitis, swine dysentery, transmissible gastroenteritis and mange is being developed in collaboration with provincial veterinary services.

The goat industry is exploring caprine arthritis encephalitis herd certification, while the sheep industry is considering maedi-visna and scrapie certification.

These industries recognize this certification initiative as a means of building confidence among domestic and foreign buyers as to the high health status of their stock.

## **Animal Identification**

The 1987 National Animal Health Program (NAHP) review emphasized the need for an animal identification program. Improved animal identification is necessary to meet the NAHP

strategy in human health and safety and to protect the domestic animal population.

In 1988, development of a national animal identification program began. A broadly based NAHP working group identified key issues facing the industry and the program and several options for action.

Since affordable, practical, industry-wide electronic identification is still several years away, NAHP is moving to develop a system based on a conventional eartag. The program will concentrate first on identification of cattle.

A number of studies and projects have been undertaken to break ground for the development of a strategy for a national cattle identification program. These include: evaluation of a voluntary cattle identification project in Saskatchewan using a traditional eartag device; a study to evaluate the effectiveness of current identification systems in disease and residue investigations; a survey to quantify the levels and types of identification devices presently used; and a study to quantify, through a cost-benefit analysis, the implementation of an electronic identification system.

Funding was also provided for three pilot projects involving electronic devices in field trials through the Department's Livestock Identification Committee, where the NAHP is represented. This representation helps NAHP officials learn about industry trends and technological developments, especially in the area of electronic identification.

When electronic identification becomes practical, it will replace the existing tags. The NAHP is committed to implementing an affordable, practical, industry-wide identification system in support of a healthy domestic cattle population and safe animal products.

## **Exports**

The export component of the National Animal Health Program serves the Canadian livestock industry by providing access to international markets for quality animal genetics developed by producers and breeders in this country. Health certification conditions are established through bilateral negotiations with regulatory veterinary authorities of importing countries.



The export program is mandated under the Animal Disease and Protection Regulations to ensure compliance with the importation requirements of the country to which the animals or semen are being exported.



*Fallow Deer, imported from England, in quarantine in British Columbia*

The integrity and credibility of the health certification process is enhanced through the production and issuance of individual official export health certificates for specific commodities to each country.

In addition, offshore exports are tested, inspected and certified by full time staff of Agriculture Canada. Export tests and diagnostic services are conducted in federal laboratories according to internationally accepted norms.

More than five hundred export veterinary health certificates are currently on file. In 1989, livestock genetic resources in the form of live animals, semen and embryos were exported to more than eighty countries.

Canada's trading partners around the world continue to actively demand Canadian animals, semen, embryos and animal by-products, reflecting both the high health standards of Canadian livestock and confidence in the certification provided.

## Imports

The import program's objective is to permit the safe importation of animals, animal products and by-products without introducing animal diseases that would have a serious economic effect on Canada's livestock and related industries.

The Animal Health Division develops import policies, programs and procedures. This involves consulting with Canadian industry, inspecting foreign facilities, negotiating import conditions with foreign countries and monitoring exotic animal diseases in foreign countries.

In cooperation with Canada Customs, Operations Directorate staff implement the animal health import program in the field by inspecting animals, animal products and by-products, veterinary biologics and personal effects of travelers, at 85 airports, seaports and land ports of entry.

Program officials operate one maximum- and two medium-security quarantines, as well as border quarantines and approved private quarantines. Staff also control the disposal of garbage from international flights and ships at airports and seaports.

With few exceptions, animals (including embryos), animal products (dairy products, eggs, semen) and by-products (meat, hides, wool, bones, tankage, glands and organs, etc.) may only be imported from countries free from serious foreign animal diseases, such as foot and mouth disease, rinderpest, fowl plague, or Newcastle disease.

Fertilizers and feed-stuffs containing animal by-products are also restricted as are any feeds or seeds that could be used for animal food. Certain dairy products may be imported from other countries if processed satisfactorily.

Import conditions are based on an assessment of the degree of risk in the exporting country. Generally, an import permit, directive or certificate of origin is required.

In 1989, the Animal Health Import Program successfully kept foreign and other serious animal diseases out of Canada, and at the same time allowed an increasing number of importations from many countries.

Maintaining the embargo on the importation of honeybees from the United States throughout 1989 prevented varroasis from becoming established in Canada.

The new import certification requirements for bluetongue (BT) for the importation of cattle from the United States were refined and fully implemented. The number of BT tests depends on the season and the risk classification of the state of origin.



Large shipments of feeder cattle, breeding lambs and breeding deer from New Zealand were successfully negotiated and completed.

Bovine embryos were successfully imported from Austria. This represents the first importation of embryos from continental Europe.

The new import program that issues sample permits to allow clients to import samples of animal-based products from high-risk countries for evaluation, research and analysis was put in place.

The Import Manual of Procedures has been developed and distributed to field staff. All import documentation procedures and permits have been computerized for on-line access by field and headquarters staff.

## Artificial Insemination (AI)

A domestic AI health program ensures uniformity of the health and sanitary standards for semen donor animals. All centres producing and distributing semen must have governmental approval for their operations. Agriculture Canada veterinarians conduct health tests at AI centres, monitor sanitary procedures and maintain adequate records.

By consulting regularly with the Canadian Association of Animal Breeders, which represents the Canadian AI industry, problems that could disrupt semen export marketing can be promptly identified and addressed.

## Embryo Transfer (ET)

The domestic ET industry is a steadily growing service to the Canadian livestock industry. As of 1989, 45 ET centres were officially approved for exporting bovine embryos.

As more importing countries accept the guidelines for international movement of embryos recommended by the Office International des Epizooties (OIE), more foreign trade in embryos is expected.

## Veterinary Biologics and Biotechnology

The Veterinary Biologics & Biotechnology program was begun in the 1930s to ensure safe livestock, poultry and some human vaccines.

The program has since then taken on the regulatory responsibility for ensuring that livestock, poultry, pet, fur-bearing animal and fish vaccines manufactured, imported for use in Canada or exported, are pure, potent, safe and efficacious. This applies to those produced by conventional or biotechnological methods.

Product data submissions from both foreign and domestic manufacturers are reviewed with field data. This applies to all veterinary biologics, including vaccines, bacterins, toxoids, anti-toxins, sera, antisera, diagnostic reagents, materials of animal origin, as well as animal pathogens, specimens, etc., which may be approved for import, domestic manufacture or export. Permits are required for all veterinary biologics and related materials imported into Canada.

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### PERMITS ISSUED IN 1989

Single Entry	1366
Material of Animal Origin;	
from U.S. (Multi-year)	110
Biologics, Blood & Serum (Multi-year)	177
American Type Culture Collection	9
Animal Pathogen (Multi-year)	51
Veterinary Biologics (Annual)	35
Veterinary Biologics (Temporary)	45
<b>TOTAL</b>	<b>1 793</b>

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Facilities of 12 Canadian manufacturers are inspected annually for compliance with regulations and 82 foreign manufacturers' facilities are inspected triennially, as are those of 42 Canadian importers.

To prevent the introduction of serious foreign animal diseases into Canada and to ensure that products meet the required standards, Agriculture Canada has the authority to regulate the importation, preparation, manufacturing, preserving,

packing, labelling, storing, testing, transporting, disposing, sale and advertising for sale and conditions of sale of any veterinary biologic.

The Veterinary Biologics & Biotechnology Section coordinates the laboratory testing of existing and new products with the Biologics Evaluation Unit of the Animal Diseases Research Institute, Nepean, Ontario.

Currently, there are more than 1000 products available for commercial use in Canada.

Increased emphasis has been placed on the monitoring and regulation of biotechnology relating to animal health in order to ensure safety within the animal environment.

Recently, guidelines were published for the regulation of veterinary biologics produced by biotechnology. In addition, a "User's Guide to Biotechnology Regulation" was produced in cooperation with Health & Welfare Canada and Environment Canada.

## **Humane Transportation of Animals**

This program was designed to reduce mortality and stress in animals and poultry transported into, within and out of Canada by land, sea or air. Regulatory requirements and guidelines stipulate feed, water, rest periods, ventilation needs, segregation, handling and so on.

Codes of practice for rearing chickens, swine and veal calves, dairy cattle, mink and fox have been developed in consultation with agricultural and transport industries and the Canadian Federation of Humane Societies.

## **International Activities**

Health of Animals Directorate officers have frequent bilateral contact with their counterparts in foreign countries. Much of this contact establishes the health conditions for importing or exporting animals and their products.

Officers also have multilateral contacts through Canada's involvement with international agencies such as O.I.E., the Pan American Health Organization (PAHO), the Inter-American Institute for Co-operation on Agriculture (IICA), and the Food and Agriculture Organization (FAO) of the United Nations.

Program staff will often accompany livestock export shipments to ensure they comply with Canada's requirements for humane transportation. Officers frequently participate in departmental missions to other countries, or in hosting delegations from other countries.

In the past year, for example, officers were involved in missions to France, United Kingdom, The Netherlands, West Germany, Switzerland, Sweden and Finland; hosting delegations from Australia, Chile, India, Indonesia, Thailand, United Kingdom and the United States, as well as other visitors. These occasions provide opportunities to share information on animal disease incidence, diagnosis and research results.

Memoranda of understanding and official arrangements with many countries are the basis for regular exchanges and cooperation in animal health and disease research. Canada provides foreign assistance through the Canadian International Development Agency (CIDA) of the Department of External Affairs. Health of Animals program officers participate in development projects around the world by cooperating with CIDA and other international groups, such as the International Development Research Centre (IDRC) and the Consultation Group on International Agricultural Research (CGIAR) which is involved in technology transfer and technical assistance.



*Combined meeting of the Inter-American Commission on Animal Health and Office International des Épidémiologies Régionales pour l'Amérique, Buenos Aires, Argentina, June 26-30, 1989. (left to right) Dr. J. Crosnier, World Bank, Dr. H. Mussman, Director, Animal and Plant Health, IICA, Dr. R. Stevens, Health of Animals Directorate, Agriculture Canada, Dr. H. Campos, Deputy Director, Animal Health, IICA.*



Program officers are currently involved in developing animal quarantine facilities and associated laboratory training in the People's Republic of China, in developing veterinary diagnostic laboratories in South America, and in a CIDA-IIICA animal and plant disease reporting project in the Caribbean.

### **Headquarters Professional staff Animal Health Division**

W.S. Bulmer	DVM	Director
R.C.K. Stevens	B.Vet.Med., MRCV	Chief, Program Planning and Evaluation
B.L. Peart	DVM	Chief, Regulatory Affairs Animal Transportation and Welfare

### **Disease Control**

J.A. Kellar	DVM, DVPM	Associate Director
D.J. Gregory	DVM, MSc,	Chief, Poultry and PhD Zoonotic Diseases
Vacant	Chief,	Epidemiology
R.S. Morley	DVM, MSc, PhD	Chief, National Animal Disease Reporting
B.R. Jamieson	DVM,	A/Chief, Foreign Animal Diseases
M.A. Koller	DVM	Chief, Control Programs
Vacant	DVM	Staff Veterinarian, Foreign Animal Diseases
P. Shadbolt	DVM, MSc	Chief, Salmonella and Food

### **Import - Export**

C. Lavigne	DMV	Associate Director
J.B. Parliament	DVM	Chief, Export Semen and Embryos
W.R. Warrell	DVM	Chief, Export Negotiations - Livestock
B.R. Evans	BSc(AGR), DVM	Chief, Export Coordination - Livestock
W.J. McElheran	DVM	Chief Import Animals and Quarantine
W.C. Stadder	BA, DVM, DVPM	Chief, Import Animal Products and By-Products

### **Veterinary Biologics/Biotechnology**

H.T. Scott	DVM, DVPM	Associate Director
D.C. Alexander	DVM, MSc	Chief, Veterinary Biologics
B.S. Samagh	BVSc, DVM, MSc, PhD	Senior Staff Veterinarian
G. Gifford	DVM, MSc	Senior Staff Veterinarian

### **Special Projects Officers**

E. Broughton	DVM, MSc
R. Rogers	DVM, MSc
G. Roy	DVM
D.E. Jette	DVM, MSc

### **Professional Staff Health of Animals Laboratory Division/Headquarters**

M.F. Baker	DVM	Director, Diagnostic Services
J.F.C. Pantekoek	DVM, PhD	Director, Research
Vacant		Chief, Laboratory Safety
A. Wiegers	BSc, DVM	Quality Assurance Officer
C.E. Inch	BA, DVM	Special Projects Officer
S.B. Reid	BSc, DVM	Special Projects Officer

## **Health of Animals Laboratories**

### **Health of Animal Laboratory Sackville, New Brunswick**

This laboratory is located on the campus of Mount Allison University. It opened in 1948 as a general diagnostic laboratory to serve the Atlantic region.

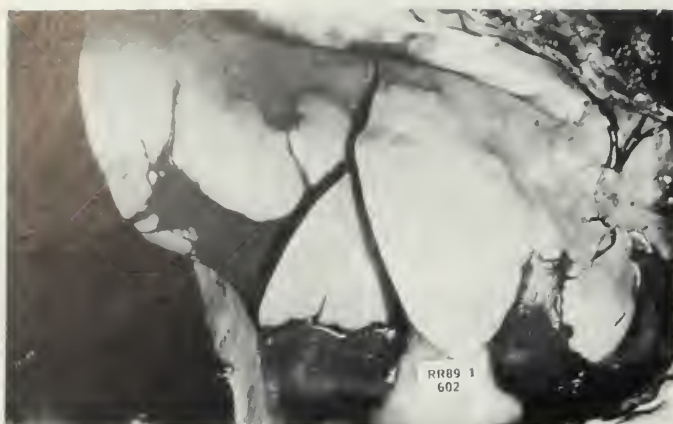
In its early years, its primary concern was the serological diagnosis of brucellosis. In subsequent years, bacteriology, parasitology, pathology, immunology and virology disciplines were added. The laboratory and nearby farm where experimental animals are maintained have a staff of 21.

The laboratory is divided into four main sections: parasitology, virology, diagnostic pathology/bacteriology and research bacteriology. Each section is responsible for diagnostic and research programs.

The virology section has diagnostic responsibilities for federal programs and testing samples for export or AI centers. Research focuses on sheep and goat diseases, emphasizing development of serological tests for retroviruses, such as maedi-visna of sheep and caprine arthritis-encephalitis of goats. The diagnostic pathology/bacteriology section provides microbiological services for meat safety programs. This includes testing of



environmental specimens and/or food products for microbial pathogens such as *Staphylococcus aureus*, *Salmonella spp.*, *E. coli* and *Listeria monocytogenes*.



Lung of an 18 week-old lamb inoculated intratracheally with diseased lung homogenate containing *M. ovipneumoniae*, followed later with *P. haemolytica*. Animals were euthanized on day 21. Note the consolidated, demarcated lesions.

Diagnostic tests on bovine semen are conducted so that specific requirements for export may be met. Laboratory testing for the bovine brucellosis eradication program is provided. The section also provides a pathology diagnostic service for abattoirs in the Atlantic region, and support for cooperative research projects with other sections. Support is also given to the fur-bearing animal industry through presentations and consultation on unusual problems.

The research bacteriology section is conducting studies to determine the penetration efficiency of *Salmonella enteritidis* in experimentally infected ungraded eggs. The effect of washing on the penetration of eggs by *S. enteritidis* will also be studied. Longer term studies have been designed to determine factors involved in colonization of the chicken intestine by *S. enteritidis*.

The primary focus of the parasitology section is research, but it also provides a reference service to Atlantic provincial veterinary laboratories and a diagnostic service for federal programs such as trichinosis, cysticercosis, mange and trichomoniasis. Current research involves epizootiology of trichinosis in herbivores in Canada, evaluating the ELISA in the diagnosis of cysticercosis, and investigating the etiology of eosinophilic myositis in cattle.

## Professional Staff

R.G. Stevenson	DVM, DVSM, PhD	Director
H.J. Smith	BSc(Agr), DVM, MVSc	Parasitology
J. Lopez	MVZ, MSc, PhD	Research Bacteriology
G.G. Finley	DVM, DDP	Bacteriology/ Histology
R. Heckert	DVM, PhD	Immunology/ Virology
C.L. Simard	DMV, MSc	Virology
F.A. Munroe	BA, BSc, DVM	Virology
Vacant		Microbiology

## Animal Diseases

### Research Institute (ADRI) Lethbridge, Alberta

The Animal Diseases Research Institute (ADRI) is located on the banks of the Oldman River, 16 km west of Lethbridge. It has been on its present site since 1905, the same year as the establishment of the Province of Alberta, and is the original veterinary research laboratory in Western Canada.

The institute is comprised of the main laboratory, a large animal experimental facility, service buildings, barns, corrals and houses. The associated farm consists of 700 hectares of native short grass prairie and 300 hectares of valley bottom land.

The commercial beef herd of 130 cows, mixed Hereford and Angus, is specific-pathogen-free (BVD and IBR). The herd is split into spring- and fall-calving units, the last calf crop being 100 per cent calves for the fall calving herd. The short grass prairie is pristine and under a rotational grazing scheme is slowly returning to its original productivity. Approximately 40 hectares of bottom land is irrigated and produces alfalfa and grass hay. The irrigation pastures and the short grass prairie are integrated to permit a sustainable grazing unit with minimal input of agricultural chemicals.

The institute has six sections, three scientific: immunology, microbiology, and pathology and clinical research; and three support: farm operations, maintenance, and administration. There are 62 employees, 11 of whom are professionals. This year we have added two post-doctoral fellows, Dr. Elizabeth Golsteyn Thomas and Dr. Sang-Geon Yeo.

ADRI continues to provide diagnostic services for the livestock industry, primarily in Western Canada.

Upon closure of the Health of Animals Laboratory (HAL) Division establishments in Winnipeg and Richmond, ADRI Lethbridge is the only Health of Animals Laboratory providing diagnostic services for the agricultural community in Western Canada. The only exception is the Brucellosis diagnostic services provided by HAL Saskatoon.

These above-mentioned responsibilities, a continually expanding export market, a reduction of Brucellosis testing at auction markets, changes in Artificial Insemination Centre health regulations and the import of cervid species has led to a 30 per cent increase in diagnostic sample submissions during the first six months of 1989 over the same period in 1988.

A reduction in EIA testing due to private laboratory accreditation has been considered in calculating the above figures. Flexibility of diagnostic and research technical staff has allowed the timely processing of these samples which arrive in large groups.

Staff continues to adopt new and improved test procedures and work towards improving test protocols. The ELISA program has been expanded to include testing of export and survey samples for pseudorabies. The virology technical staff now has the expertise to conduct the immunoperoxidase test for BVD. Training has been provided to Division personnel in IBR ELISA procedures to facilitate the transfer of this technology.

The diagnostic unit continues to provide support for research projects by providing diagnostic testing and reagent evaluation when requested.

ADRI Lethbridge's research mandate is to respond to the requirements of our two clients, the National Animal Food Safety and the National Animal Health Programs.

At present, research is in progress on the development of DNA probes for the identification of *Leptospira* serovars, the cloning of the genome of BVD virus and the leukotoxin gene of *P. haemolytica*, the development of anti-idiotypic antibodies mimicking the antigens of the BVD virus, the development of DNA amplification systems for the detection of *Listeria monocytogenes* in food and the development of monoclonal antibodies

against various epitopes of the BVD virus. Work on the evaluation of the efficacy of bovine respiratory vaccines is also in progress. Dr. L. Niilo, recently retired, has published a book called, "The River Bottom Station," that covers the history of the Animal Diseases Research Institute from 1905 - 1987.

### Professional Staff

P.H.G. Stockdale B Vet Med, MSc, Director  
PhD, MRCVS

### Immunology

R.C. Finlay	DVM	Head, Immunology and Associate Director
H.J. Cho	DVM, PhD	Immunologist (Viral Diseases)
S.A. Masri	BSc, MSc, PhD	Molecular Biologist

### Clinical Research

V.W. Lees	DVM, MSc	Head, Clinical Research and Animal Care Veterinarian
K.W.F. Jericho	DVM, PhD	Pathologist
W.O. Olson	DVM, MSc	Theriogenologist

### Microbiology

D. Deregt	BSc, DVM, PhD	A/Head, Microbiology and Virologist
S.P. Gale	DVM	Bacteriologist
V.P.J. Gannon	BSc, MSc, DVM, PhD	Bacteriologist
K.G. Loewen	BSc, DVM, MSc	Virologist

### Visiting Scientists

E. Golsteyn		
Thomas	BSc, MSc, PhD	NSERC Visiting Fellow
Sang-Geon Yeo	DVM, MSc, PhD	Post-doctoral trainee
S. Goubau	BSc, MSc	Post-graduate trainee



## Health of Animals Laboratory Saskatoon, Saskatchewan

This laboratory is located on the University of Saskatchewan campus next to the Western College of Veterinary Medicine. It was opened in 1976 as the new regional facility, but also became a national center for chemical residue analysis when staff and equipment were moved from other laboratories to Saskatoon in 1981. The 39 staff members comprise analytical chemists; veterinarians specializing in pathology, bacteriology, and immunology; technicians; and support personnel.

The laboratory is divided into two sections: Chemical Residue Analysis and Infectious Diseases. Chemical Residue Analysis provides national diagnostic services that confirm the presence in meat of various residues, including antibiotics, sulphonamides, growth promoters, and pesticides. Research programs in the chemistry section address the need for developing new and better methods of residue detection, both on-site and in the laboratory, to support regulatory programs in food safety and animal health.

The Infectious Diseases Section provides diagnostic pathology services for federal programs in Saskatchewan and Manitoba, and *Brucella* serology for Saskatchewan. It is also a national reference center for *Brucella* culture and biotyping.

Research includes studying the risk of disease transmission between domestic, wild, and game-ranched species of animals, improving microbiological techniques for *Brucella* diagnosis, and studying the immunological responses to herpesviruses.

The year's highlights included development of a quantitative (HPLC) method for the determination of penicillin G levels in meat. Although methods to detect this commonly used antibiotic have been available in the past, development of an exact quantitative method has been elusive, and is necessary to permit rational decisions on tolerance levels in light of currently used dosages.



*Analytical chemists working with the new mass spectrometer at Saskatoon. The new instrument will aid methods development for the detection of drug residues.*

Methods development of this type on other penicillins, tetracyclines, and other families of drugs used in livestock production will be greatly assisted by the purchase of a new mass spectrometer for the Saskatoon laboratory. The new instrument was installed in December of 1989 and will improve the Department's ability to protect the marketability of Canadian livestock and food products.

Staff from this laboratory provided training during the year to groups of inspectors, veterinarians, and veterinary students on such topics as the sulpha-on-site (SOS) test. This is a rapid test now used at packing plants to detect violative residues of sulphonamides in urine, in parallel with testing done in the United States.

The previously completed Ph.D. thesis, and continued surveys, on brucellosis and tuberculosis in the bison population of Wood Buffalo National Park provided detailed scientific information needed to solve this problem before it detrimentally affects other bison herds or the health status of Canada's national cattle herd. A panel for the Federal Environmental Assessment Review Office (FEARO) is conducting a definitive review involving public meetings and position statements from all interested parties.

In another PhD project nearing completion, some mechanisms have been elucidated whereby bovine herpesvirus-1 (IBR virus) causes immunosuppression that can result in pneumonia in cattle.

### Professional Staff

W.D.G. Yates	DVM, PhD	Director Food Animal Chemical Residues
J.D. MacNeil	BSc, MSc, PhD	Head
G.O. Korsrud	BSA, MSc, PhD	Methods Research & Development
J.O.K. Boison	BSc, MSc, PhD	Methods Research & Development
V. Martz	BSc	Quality Assurance/Safety Officer
J.R. Patterson	BSc, BEd, MSc	Chief, Analytical Services
A.C. Fesser	BSc, MSc	Supervising Chemist, Analytical Services
C.D.C. Salisbury	BSc	Supervising Chemist, Analytical Services

### Veterinary Research and Diagnostics

S.V. Tessaro	BSc, MSc, DVM, PhD	Head
L.B. Forbes	DVM, MSc	Diagnostic Services
D.L. Hutchings	DVM, Dipl VPM	Immunology

### Animal Disease Research Institute (ADRI) Nepean, Ontario

ADRI is the largest of the laboratories in the Division. This Institute grew from the research program of the Outremont farm of McGill University in the last part of the 19th century. After the federal election of 1902, the work in progress at Outremont moved to Ottawa.

After several weeks on Queen St., a new Biological Laboratory was opened on the Central Experimental Farm. In 1922, the Institute moved to Cliffe St., west of Parliament Hill with a farm in Hull. A new Institute was built on this farm at 100 Gamelin Blvd. in 1927. A virology unit was established in 1946.

Gradually, diagnostic support of federal programs, research into animal diseases and production of biologicals increased. In 1974 the Institute moved to the new building on Fallowfield Road in Nepean while the virology unit remained in Hull.

ADRI, Nepean has a staff of 198 and is comprised of the following sections: Laboratory Operations, Immunology, Microbiology Research, Microbiology Services, Pathology, Reproduction, Serology, Biologics Evaluation, Virology and Administration & Resources. The work done at ADRI, Nepean covers all four Divisional programs: Foreign Animal Diseases (FAD), Food Safety, Program Indigenous Diseases and Non-Program Indigenous Diseases. ADRI conducts both research and diagnostic testing. Research is discussed under specific projects.

Laboratory Operations coordinates diagnostic information, the receiving of samples and issuing of results. The design and implementation of the automated Laboratory Information Management System (LIMS) will be coordinated within the Section. The Section is also responsible for large and small animal care and farm, fleet and equipment maintenance.

In the Immunology Section purification and production of *Mycobacterium paratuberculosis* antigens is done for serodiagnostic testing. Monoclonal antibodies for FAD, food safety and indigenous diseases are being developed for diagnosis.

The Microbiology Services Section has four major responsibilities: general microbiology, leptospirosis, mycobacteriology and bioproduction.

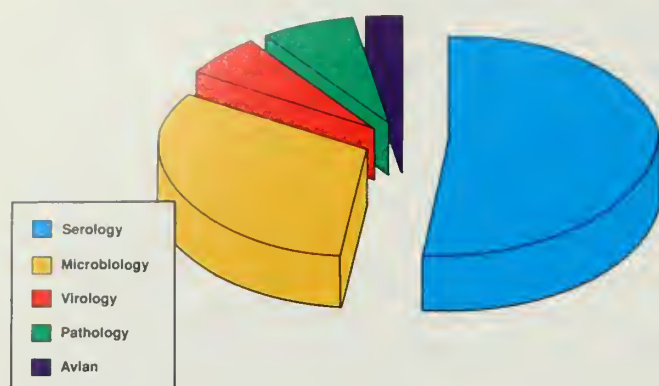
Culture of semen for export is performed by the general microbiology unit, which also does routine diagnostic culture for various diseases including campylobacteriosis, anthrax, listeriosis and salmonellosis.

More than 100 000 microscopic agglutination tests are performed annually for leptospirosis export certification and AI Centre monitoring programs. The mycobacteriology unit is responsible for all diagnostic culture under Canada's tuberculosis eradication program and Johne's disease program.

In 1988-89 the unit did 358 cultures for tuberculosis and 2702 cultures for Johne's disease.



TEST PERFORMED AT ADRI In FISCAL YEAR  
88 / 89



The bioproduction unit produces between four and five million diagnostic test reagent doses per year including mycoplasma, campylobacter and brucellosis test antigens, johnin, mallein, bovine and avian tuberculin, and reagents such as complement for the complement fixation test.

In 1989 the unit also undertook responsibility for preparation and maintenance of the national serum banks for cattle and swine. Every three years, 15 000 new bovine or porcine sera collected by systematic sampling at sale barns and abattoirs across Canada are added.

The Pathology Section consists of five main groups: the rabies unit (including rabies diagnosis and research), avian diseases, diagnostic pathology, pathology research and electron microscopy.

In 1988-89 the Section conducted 28 446 tests (rabies-10 039; avian diseases-8500; histopathology-9000; hematology-907). The rabies unit utilizes fluorescent antibody testing and the rabies tissue culture test, adopted in 1986.

Diagnostic pathology is involved in both gross and histopathology, serum testing from poultry flocks, testing chicks and hatching eggs for export, examining imported birds that die in quarantine, and testing suspect samples from meat and poultry processing establishments in support of the food safety program. The Section also examines tissues submitted by meat production establishments to support the ongoing tuberculosis eradication program

emphasizing tracing back to herds of origin. A robotic slide stainer is now functional for peroxidase-anti-peroxidase staining of tissue sections for rabies and tumor antigens.

The Serology Section consists of six units: complement fixation (CF), agar gel immunodiffusion (AGID), agglutination (AGG), enzyme immuno-assay (ELISA), quality assurance (QA) and methods development and transfer (MDT).

In 1989-90, the four diagnostic units performed approximately 175 000 tests for approximately 20 different diseases. Approximately 80 per cent of the tests performed were related to the import/export market and the bulk of the remaining 20 per cent of tests represented national surveys and QA testing.

The Serology Section, acting as a technology transfer and reference centre, has undertaken a major role in the decentralization and privatization of testing services. The section is responsible for the accreditation of private laboratories for the performance of AGID tests for equine infectious anemia and enzootic bovine leukosis.

As well, the Serology Section is involved in the proficiency testing of other laboratories within the HALD for the performance of CF, AGID, AGG and ELISA tests for various diseases. As such, the section has a heavy commitment in the area of QA and a special QA unit has recently been created for this purpose.

The ELISA unit is expanding at a steady rate and now tests for pseudorabies, *Brucella* and bluetongue. The MDT unit is responsible for the standardization and automation of new ELISAs developed in the research sections of ADRI. The MDT initiates the initial transfer of these assays to the diagnostic ELISA unit of the Section and assists in the transfer to other laboratories of the HALD.

A Biologics Evaluation Laboratory (BEL) became functional in 1987. Its mandate is to develop and conduct a quality assurance monitoring program for veterinary biologics (vaccines and test kits) for purity, potency, safety and in some cases, efficacy.

The BEL comprises a microbiology laboratory (including a HEPA-filtered air supply clean room for sterility testing), a cell culture and virology laboratory, a serology laboratory and other special laboratory support within the

Institute. Products are selected and tested as described in the Quality Assurance Monitoring Protocols (QAMP) which have been carefully prepared for this purpose.

Current priorities are the bovine respiratory agents. BEL is doing post release testing of these and other products at the request of its client, the Biologics Section of the Animal Health Division.

The Virology Section functions as both a diagnostic and research centre for FAD and selected indigenous diseases. Its primary objective is to maintain an up-to-date readiness for diagnosis of FAD. This is achieved through constant research on improving diagnostic techniques and producing and maintaining reagents for diagnostic tests performed at ADRI, Nepean and other divisional laboratories.

The routine diagnostic techniques include: virus isolation, electromicroscopy, hemadsorption, immunofluorescence (FA), immuno-peroxidase (IP), serum neutralization, hemagglutination, hemagglutination inhibition, AGID and ELISA.

A DNA probe for pseudorabies virus has been developed and is currently used for detection of the viral genome in tissues of experimentally infected animals.

In 1989 the Section conducted its annual exotic disease course with emphasis on the management of disease outbreaks and eradication programs.

Serological monitoring of the sentinel cattle stationed in the Okanagan Valley, British Columbia for bluetongue and epizootic hemorrhagic disease (EHD) of deer did not reveal any activity in 1989. Lentogenic (mild) Newcastle disease (ND) virus was also isolated from samples received from imported birds held in quarantine. Pigeon paramyxoviruses were isolated from several submissions of pigeons with no significant pathogenicity for chickens. Following the closure of the Richmond Laboratory, B.C., the number of avian samples received for diagnosis has increased by 20 per cent in 1989.

The competitive ELISA for bluetongue was applied as a confirmatory test and will replace the AGID for national surveys of bovine sera. It is more specific, more sensitive and less labor intensive than the AGID test. The IP test

which replaced the FA test for bovine viral diarrhea (BVD) virus isolation was used extensively for monitoring bulls resident at or awaiting entry to the artificial insemination centres in Canada.

This technology was adapted at ADRI, Nepean from a technique in place at the Central Veterinary Laboratory, Weybridge, U.K. and was transferred to ADRI, Lethbridge and HAL, Sackville. The increased sample capacity and decreased costs associated with the IP has prompted the section to compare the performance of the test with that of the FA for BVD virus isolation from bull semen samples.

Furthermore, a similar test is being adapted for isolation of border disease virus in serum and semen samples collected from rams. The section performed 24 000 diagnostic tests in 1988-89; about 75 per cent of these tests were related to the import and export market.

**Animal Diseases Research Institute  
(ADRI)/Nepean, Ontario**

**Professional Staff**

B.W. Stemshorn	BSc, DMV, PhD	Director (leave of absence)
P.R. Ide	DVM, MSc, PhD	Acting Director
J.A.J. Carrière	BA, DMV	Special Projects, Accomodation, Safety
A. Bouffard	DMV, MSc, PhD	Manager, Scientific Projects
P.J. Cairns	DVM	Special Assignment, Virus Lab

**Laboratory Operations**

M. Collins	DVM	Head
F. Lord	DMV	Diagnostic Coordinator
J.J. Morgan	BSc(Agr), DVM	

**Immunology**

E.A. Sugden	BSc, PhD	Head
D. Henning	BSc	
K.H. Nielsen	BSc, MSc, PhD	
O. Surujballi	BSc, PhD	



### Microbiology Research

J.L. Spencer	DVM, MS, PhD	Head
B.W. Brooks	BSc(Agr), MSc, PhD	
A.D.E. Fraser	BSc(Hon), MSc, PhD	
M.M. Garcia	BSA(Hon), MSc, PhD	

### Microbiology Services

J.B. Stevens	BSc, DVM, MSc, PhD	Head
G. Falk	BSc	
K.L. Malkin	BVSc, Dipl Vet PM	
R.B. Stewart	BSc(Hon)	
C. Turcotte	DMV, MSc	(study leave)

### Pathology

K.M. Charlton	DVM, PhD, ACVP	Head
A. Bundza	DVM, Dipl Clin Path	
G.A. Casey	BSc	
S. Chen	DVM, MS	
T.W. Dukes	DVM, MS	
F. Gilka	DVM, MS Med Vet, MVD, CSc, PhD, ACVP	
N. Smart	DVM, MS	MRC Fellowship
A.I. Wandeler	Bacc, MSc, PhD	
W.A. Webster	BSc	

### Reproduction

E.L. Singh	BSc, MSc	Head
A. Bielanski	PhD, DVM, Dr Habilit VM	
A.M.P. Bouillant	DMV, MS, PhD	
M.D. Eaglesome	BVMS, MRCVS	
W.C.D. Hare	MA(hc) BSc, PhD, DVM&S, FRCVS	
C. Lutze-Wallace	BSc, PhD	NSERC Fellowship
S. Nadin-Davis	BSc, MSc, PhD	
G.C.B. Randall	BVSc, PhD, MRCVS	

### Serology

P.F. Wright	BSc, MSc, PhD	Head
J. Bossé	DMV, MSc	
W. Kelly	BSc(Hon)	
E.A. Taylor	BSc, DVM	

### Biologics Evaluation

F.C. Thomas	DVM, PhD	Head
J.R. Duncan	BSA, DVM, MSc, PhD, ACVP	
R.P. Lacroix	BSc, MSc	
G.M. Ruckerbauer	BA, MA, DVM	
S. Wilson	DVM, MSc	

### Virology

A. Afshar	DVM, PhD	Acting Head
N.H.A. Al-Shakarchi	BSc, MSc, PhD	
D. Denicourt	BSc, MSc, PhD	
C. Dubuc	DMV, MSc	
G.C. Dulac	DMV, MSc, PhD	
M.L. Harper	DVM, MSc	HAD Secondment
D.J. Myers	DVM, MSc	
T.U. Obi	DVM, MVM, PhD	Rockefeller Fellowship
C.E. Rigby	BSc, MSc, PhD	(leave of absence)
M.F. Traykova-Adonova	BSc, PhD	
NSERC Fellowship		

## Health of Animals Laboratory Guelph, Ontario

This laboratory was established in 1958. Formerly situated on the University of Guelph Campus, it was relocated in 1986 to 110 Stone Road, West, close to the University of Guelph.

A staff of 30 people provide diagnostic services and conduct research to support the Canadian livestock and poultry industry.

Specialized facilities are available for research in microbiology and biotechnology, and include containment areas for work on virulent pathogens.

Diagnostic services support Agriculture Canada's national animal disease program by testing for brucellosis, equine infectious anemia (EIA), campylobacteriosis, trichomoniasis, pseudorabies and infectious bovine rhinotracheitis (IBR).

Testing is also conducted to ensure that Canadian livestock and livestock products meet the health requirements of importing countries before leaving Canada. This is accomplished through a series of tests for brucellosis, EIA, IBR, enzootic bovine leucosis (EBL), bluetongue, epizootic hemorrhagic disease (EHD), trichomoniasis, and pseudorabies. In addition, this laboratory now provides serotyping service for non-human *Salmonella* isolates for the FPI branch.

The Meat Safety Unit conducts bacteriological examination primarily of fresh and processed meats for *Staphylococcus aureus* counts, *Salmonella* species and verotoxigenic *Escherichia coli*.

The unit also have the capability of testing for *Listeria monocytogenes* both by the USDA method (meats and poultry products), and the FDA method (egg products). Also available are tests for thermophilic *Campylobacter* species, *Clostridium perfringens*, and *Staphylococcus aureus* enterotoxin which was expanded this year to include canned mushrooms as well as meats.

For dry cure products, pH and water activity can be determined. The unit also provides in-plant investigation services to support federal meat hygiene programs in abattoirs and processing plants, in particular, analysis of environmental swabs for *Listeria monocytogenes*.

Species verification testing is also part of Guelph HAL's mandate. An immunodiffusion test is used for raw meats, whereas a sensitive ELISA test is used for cooked meat species verification. The "Health of Animals Meat Safety Procedures Manual" is updated and revised by this unit as required to reflect changes in current methodologies and in our clients' sampling plans. The meat safety unit conducts bacteriological examination primarily of fresh and processed meats for *Staphylococcus aureus* counts.

Research activities at HAL/Guelph are focused on detecting and controlling pathogenic microorganisms and other contaminants in animal and poultry meat products.

Development of rapid methods for detection of *Salmonella* sp. and verotoxin-producing *Escherichia coli* (VTEC) has continued, using oligonucleotide (DNA-RNA) probes and amplification systems. Experiments on VTEC are being conducted to examine the role of adhesions and the specificity of verocytotoxins for host cells in order to develop improved diagnostic kits.

Also, in response to increasing concern over egg-borne *S. enteritidis* infections, a nation-wide survey was conducted to determine the prevalence of this organism in Canadian layer flocks. A similar survey of broiler flocks is planned for 1990.

Follow-up material from the survey has contributed to related investigations into possible sources of food-borne *Salmonella* infections, and to development of improved microbiological and serological tests for detection of *S. enteritidis* and other strains of *Salmonella* sp. in poultry.

Identification of *Salmonella* strains isolated in these studies has been facilitated by the recent transfer of the *Salmonella* serotyping service to the Guelph laboratory. In addition, these and other *Salmonella* strains are being examined for potential virulence markers by plasmid profile analysis and hybridization studies. This aspect will soon be expanded to include characterization of *S. enteritidis* strains by phage typing.

### Professional Staff

T.R.S. Bhatia	BVSc and AH, MSc, MRCVS	Director
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### Diagnostic Services

P.T. Shettigara	BVSc, MSc, PhD, Head, DVM, DTVM, DECH, MRCVS	Animal Health Serology (leave of absence)
E.D. Mann	DVM, MSc	Acting Head Serology
L. Boag	BSc, DVM, MSc	Serology
P.J. Cairns	DVM	Seconded to ADRI/Nepean
S.C. Read	DVM	Head, Meat Safety
A.M. Lammerding	BSc(Agr), MSc	Meat Safety (Education Leave)

### Meat Safety Research

R.P. Johnson	DVM, MSc, PhD	Acting Head Bacteriology
R.C. Clarke	BSc, DVM, PhD	Bacteriology
C. Poppe	DVM, MSc, DVP, PhD	Bacteriology
C.M. Forsberg	PhD	Bacteriology
R.J. Irwin	DVM, MSc	Epidemiology
W.B. McNabb	DVM	Epidemiology
A. Valdivieso- Garcia	DVM, MSc, PhD	Bacteriology (Post Doctorate fellow)





Cytotoxic effects of verocytotoxin-producing *Escherichia coli* are studied as part of a project to develop better test for these pathogens.



*Salmonella* organisms are isolated as part of the national surveys conducted by the branch.



Serological tests in support of branch animal health programs are conducted by HSL/Guelph.



A mechanical "Chicken Shaker" based on USDA design was constructed to develop tests to enumerate bacterial load on chicken carcasses.



An epidemiologist, Dr. Revecca Irwin, was added to the research team. Epidemiologists are playing an increasingly important role in research and diagnostic programs.



An ELISA test for species verification of cooked meats is used at HAL/Guelph.

## Health of Animals Laboratory Saint-Hyacinthe, Quebec

The Saint-Hyacinthe Health of Animals Laboratory is housed in an ultramodern building on the Saint-Hyacinthe Agri-Food High Technology Campus. Located nearby are the Food Research Centre (part of the Research Branch of Agriculture Canada) and the Faculty of Veterinary Medicine of the University of Montreal.

The laboratory offers a range of services.

As part of the bovine brucellosis eradication program nearly 200 000 blood samples are analysed every year.

The laboratory also provides support for Canadian artificial insemination centres. Cattle are screened for bovine leukemia, campylobacteriosis and trichomoniasis, and swine for Aujeszky's disease.

Microbiological tests on raw and processed meats as part of the agri-food safety program are conducted to detect microbial agents possibly responsible for food poisoning, such as *Staphylococcus aureus*, *Salmonella spp.*, *Clostridium perfringens*, *Compylobacter jejuni*, *E. Coli* 0157:H7, and *Listeria monocytogenes*.

A pathology advisory service is provided in support of the meat inspection program.

Research at the Saint-Hyacinthe Health of Animals Laboratory is concentrated on infectious diseases in animals, especially swine. Research subjects include swine enteritis caused by atypical rotaviruses and transmissible gastroenteritis rotaviruses, and virulence factors associated with certain strains of *Treponema hyodysenteriae*. Other research focuses on bovine virus diarrhea and food poisoning caused by *Listeria monocytogenes*.

### Professional staff

A.N. Gagnon	D.M.V., M.Sc.	Director
S.P. Carrier	D.M.V., M.Sc.	Head, Serology
Y. Robinson	D.M.V., M.Sc., Dipl. L.A., I.P.S.A.V.	Head, Pathology
J.B. Phaneuf	D.M.V., M.Sc.	Pathology
S. Messier	D.M.V., M.Sc., Ph.D.	Head, Bacteriology
R. Magar	B.Sc., M.Sc., D.M.V., Ph.D.	Head, Virology

## Experimental Station Grosse Île, Quebec

Grosse Île Experimental Station, located on the St. Lawrence River, has been administered by the Health of Animals Laboratory Division for many years.

Animal quarantine facilities on the island are kept ready in case live imported animals need to be quarantined under conditions of maximum security.

Until 1988 a course for federal veterinarians on exotic diseases of animals was held annually in this maximum-security laboratory. Foreign livestock diseases that could be introduced into Canada were demonstrated in vivo.

Unfortunately, a fire in June 1988 destroyed the animal cubicles, forcing the cancellation of the 1989 course. Arrangements for future courses are being considered.

Grosse Île has been a quarantine and disease treatment center since the early 1830s. At the height of its prominence during the 19th century, it was the first and last tragic stop for many potential immigrants infected by small pox or diphtheria.

In recognition of its national historic significance, the departments of agriculture and environment have agreed to transfer most of the island to Environment Canada for a national historic park managed by the Canadian Parks Service.

Agriculture Canada will continue to use the island for the animal disease and quarantine program. Public use of Grosse Île will be prohibited while courses on animal diseases or maximum-security quarantines are conducted. Otherwise, Grosse Île will be open to the public from late June to early September.

**Officer in charge:** François Duchaine

## Diagnostic National Reference Centre For Exotic Virus Diseases Winnipeg, Manitoba

Agriculture Canada has embarked upon a project which will see a new diagnostic centre for exotic viral diseases of livestock constructed in Winnipeg, Manitoba.



Scheduled for completion by 1996, the centre will fulfill three objectives: diagnosis of animal diseases caused by viruses normally foreign to Canada, the development of new or improved diagnostic techniques, and the training of veterinarians from Canada and abroad in the recognition of foreign animal diseases.

The successful fulfillment of this mandate will require a physical plant and operating characteristics unique to Canada and rarely found in the rest of the world. The facility will be built to exacting standards of maximum containment to prevent the escape of exotic disease agents. Operations at the centre will similarly follow strictly enforced safety and containment procedures.

This facility will be incorporated into a complex being constructed jointly with Health and Welfare Canada, which is designing accommodation for its Bureau of Microbiology.

The complex will be situated in downtown Winnipeg close to the Health Sciences Centre.

## **Diagnostic Services**

### **Background to the 1987 Review**

Tests performed by the Health of Animals Laboratory Division assure Canadian livestock owners, consumers and trading partners of the health and safety of Canadian livestock and meat.

In six laboratories across Canada, more than 200 people are busy providing that assurance. The work they do is becoming more complex at the same time as it is increasing in volume.

These and other challenges prompted a (task force) review of diagnostic services in 1987. The ensuing recommendations were designed to carry the Division into the 1990s. An assessment of the present status of diagnostic services will serve to assist in future divisional policy making.

### **Business and Strategy**

The following business statement, or mission, for the program was affirmed by the task force:

to ensure (through in-house analysis, contracting out or accreditation of private laboratories) timely, analytical testing service in support of the National Animal Health and Food Inspection Programs.

To perform this task for clients, a strategy was developed which formed the basis for the recommendations. A two-pronged approach was developed, namely:  
to increase speed of response to client needs;  
and to increase efficiency and effectiveness.

## **Progress on the Recommendations**

### **Client Needs**

Demand for laboratory tests is increasing as the need to reassure the public of food safety increases. As well, the demand for export tests is paralleling the remarkable growth of international markets for Canadian livestock, semen and embryos. During 1989, for example, the number of export tests performed in the Division's laboratories increased by 30 per cent over the previous year.

The task force recommended the speedy accreditation of non-federal laboratories as a means of increasing national testing capacity. In 1989, testing for offshore export remained the responsibility of the federal laboratories but the transfer of testing responsibilities for other purposes was begun.

An immediate obstacle to accreditation was unclear legal authority. As a result, clarification of the diagnostic mandate was the first recommendation to be addressed.

Obstacles were removed and in 1989 a prototype accreditation scheme was successfully launched with the agar gel immunodiffusion test for equine infectious anemia (EIA). Accreditation for enzootic bovine leukosis (EBL) testing followed shortly after.

To date, 11 laboratories have been accredited for EIA and 10 for EBL, with eight more pending for both diseases. Inspection of facilities, provision of training and performance of proficiency tests form the basis of the accreditation program to ensure a continuing high standard and uniformity of service to the livestock industry. Accreditation will continue

to expand in the coming years to keep up with the need for more tests and more laboratories to serve the livestock and food industries.

Client service recommendations included provisions for ready access to disease and diagnostic expertise. This objective will be met in a way unforeseen by the task force.

Within the division's quality assurance program, all laboratories will be responsible for standardization and the quality of certain tests. It follows that identifying such "reference laboratories" to clients, along with a list of the appropriate scientist for each test or disease in that reference laboratory, will provide the access which was sought.

An aspect of service delivery that has received higher priority as a result of the review is technology development and transfer. Increased dialogue with clients about their needs and better planning within the division have spawned several new methods.

In 1989, an immunoperoxidase test for bovine viral diarrhea virus and a variety of ELISA procedures have joined the ranks of routine tests. Among the improvements offered by these techniques is increased efficiency - another means of enlarging testing capacity.

**TABLE 1 NEW TESTS AVAILABLE**

	DISEASE	TEST
Virus Isolation	Bovine	
	viral diarrhea	Immunoperoxidase
Serology	Pseudorabies	Indirect ELISA
	Bluetongue	Competitive ELISA
	Maedi-visna	Indirect ELISA
	Caprine arthritis-encephalitis	AGID
	Caprine arthritis-encephalitis	Indirect ELISA
Bacteriology	<i>Actinobacillus pleuropneumoniae</i>	Indirect ELISA
	<i>Salmonella</i>	Modified semisolid Rappaport-Vassiliadis culture

During the review, clients asked that testing be made available closer to where samples are being drawn. As a result, an effort has been made to increase the numbers and types of tests being offered in the Guelph, Ontario, Lethbridge, Alberta, St-Hyacinthe, Quebec and Sackville, New Brunswick laboratories in order to improve services to clients.

**TABLE 2 DECENTRALIZATION OF EXPORT TESTING**

LABORATORY	DISEASE	TEST
Guelph	Enzootic bovine leukosis	AGID
	Bluetongue	AGID & CF
	Epizootic hemorrhagic disease of deer	AGID & CF
	Brucellosis	CF
	Paratuberculosis	CF
	Pseudorabies	ELISA
	Infectious bovine rhinotracheitis	ELISA
Lethbridge	Bluetongue	AGID
	Enzootic bovine leukosis	AGID
	Bovine viral diarrhea	Immunoperoxidase
	Pseudorabies	ELISA
St-Hyacinthe	Enzootic bovine leukosis	AGID
	Pseudorabies	ELISA
Sackville	Bovine viral diarrhea	Immunoperoxidase

As in every large enterprise, good communication was identified as a prominent client need and some recommendations on possible improvements were put forward. They have been implemented at a local level in various laboratories where lists of tests and turnaround times have been provided to clients.



## Efficiency and Effectiveness

The search for internal means of increasing efficiency led to the closure of two of the smaller laboratories located in Winnipeg, Manitoba and Richmond, British Columbia. A study had revealed that the services provided from these laboratories could be delivered from other laboratories in the branch without significant adverse effects on clients.

The review also identified the need to reduce, or eliminate, if possible, services that did not directly support the two client programs, Animal Health and Food Safety. Various steps have been taken which have gradually reduced the number of tests performed for non-client groups.

For example, in fiscal year 1988-89, five per cent of tests were in that category. The following year, 1989-90, it was reduced to 3.5 per cent. A few questions of obligation remain outstanding and are in the process of being resolved. Quality assurance recommendations are on track largely due to the hiring of a divisional quality assurance officer. Quality control is central to laboratory testing but the need for impeccable procedures and documentation was heightened with the laboratory accreditation initiative. The good reputation of the Division was verified during the review when the provincial and private sectors suggested that HALD should set standards for veterinary diagnostic testing in Canada.

Accomplishments during the year included the standardization of test protocols and the development of new principles and procedures for design and analysis of inter-laboratory proficiency tests.

Another recommended initiative was the negotiation of a guaranteed 24-hour courier service to deliver specimens to our laboratories. The availability of test results is often delayed because samples have taken a long time to reach the laboratory. In the past, the division has considered this to be a factor beyond its control and initial attempts seemed to confirm that. It may prove to be difficult to improve upon present courier service but further investigation is warranted due to the importance of this issue.

Another concern about turnaround is the time taken for results to be mailed to

submitters. The task force recommended that electronic terminals be installed in district offices as quickly as possible to allow test results to be reported electronically.

The division is developing a computerized sample tracking system which will make results and other information readily available on-line. The component which supports export testing is scheduled for implementation in May 1990.

## The Future

The trend is towards increased use of accredited laboratories for those tests which are amenable to it. The net effect for the Division will be a substantial change in the type of work performed by diagnostic staff. Rather than doing large volume testing, they will increasingly be asked to provide training, expertise and quality assurance for other laboratories.

A divisional priority that has occupied a great deal of time, and that was not dealt with during the review, is laboratory safety. Pressures from new legislation and policies have stimulated a great deal more activity in this area. All divisional laboratories have made exceptional efforts to comply with new safety requirements and have met their objectives. Safety will remain a priority, and a safety officer to coordinate these activities centrally will be hired in the near future.

Another efficiency which has been implemented, and which was outside the scope of the review, is the consolidation and collocation of branch laboratories.

For example, food laboratories previously located in Toronto, Montreal and Moncton are being consolidated in space provided in the Laboratoire d'hygiène vétérinaire in St-Hyacinthe. In addition, the Central Plant Health Laboratory is being accommodated in the Animal Diseases Research Institute in Nepean.

As mentioned, 1990 will see the first stage of implementation of the Laboratory Information Management System. The potential for increasing client satisfaction and internal efficiency with a computerized system of this size is great. Export testing, which is expected to provide the largest payback, is

being designed first and will be in place in May 1990 at the Animal Disease Research Institute in Nepean. The system's migration to other laboratories across the country will occur simultaneously with the design of other parts of the system, such as import and disease control testing.

Periodic reassessment of progress with respect to the recommendations of the 1987 review allows divisional managers to see how the environment has evolved since that time. This will assist in more accurately predicting future directions for the diagnostic component of the laboratory division.

The following table illustrates services provided during 1989:

Services	Number of tests
Foreign animal diseases (including surveys)	4 220
Brucellosis eradication	445 169
Tuberculosis eradication	3 650
Reportable and other diseases of national concern	86 284
Artificial insemination	119 710
Import	30 327
Export	346 534
Food safety	41 114
Support Services	99 970
Biologics production (test doses)	2 948 378

## Research

More than 100 staff tackled about 60 projects in 1989 to meet the research needs of the National Animal Health and the Agri-Food Safety Programs. A strong research component is present in each of the six laboratories of the Health of Animals Laboratory Division. The research and diagnostic interests often complement each other.

The following section highlights research achievements. The numbers in parentheses in the text refer to the project numbers in the "List of Research Projects" which follows the research report. For direct inquiries, the location of the researchers is provided. Further information on projects may be gained through the list of publications.

## I Foreign Animal Diseases

The most important responsibility of the National Animal Health Program is diagnosis and control of diseases foreign to Canada that would threaten our susceptible livestock population if allowed to gain entry. Estimates on the cost should such a disease gain entry to Canada run to several million dollars a day in lost export markets alone.

The research conducted requires highly specialized facilities and expertise which are only available within the federal government. Researchers are looking for ways to prevent entry of exotic diseases and to ensure rapid diagnosis in the event of their intrusion.

Embryo transfer promises to be a preventive tool that will allow Canadians to purchase genetic stock from countries which are known to have serious animal diseases. Most foreign animal disease research concentrates on improving the diagnostic methods available to recognize these diseases.

This year, accolades were received from the United States Department of Agriculture for Dr. A. Afshar and his team at the Animal Diseases Research Institute in Nepean for the competitive enzyme-linked immunosorbant assay (ELISA) for detection of antibody to bluetongue virus. This test outperforms conventional agar gel methods and also has an advantage over the indirect ELISA as it does not require several anti-species conjugates.

In general, ELISA test technology is being rapidly developed within the division for detection of both foreign and indigenous pathogens. This test offers many advantages, among which is its potential for automation.

### Embryo transfer

Research on diseases which can be transmitted by embryos has two primary thrusts. First, researchers are trying to discern which pathogens are transferred by embryos harvested from diseased animals. Secondly, they are developing embryo handling techniques which eliminate some pathogens and minimize the risk of transmitting others.



Embryo handling techniques developed by researchers in the Nepean Institute have become the international sanitary standard and this work has served to ease restrictions on the international movement of embryos (1).

Researchers continued to investigate the threat of specific pathogen transmission through embryos. There was collaboration with Dr. C. Mebus of the United States Department of Agriculture in which embryos from sheep infected with foot-and-mouth disease virus were collected, assayed and found to be foot-and-mouth disease free. This finding is very significant for ovine embryo trade.

Work was also carried out on bluetongue in sheep. Recent findings are conclusive that the virus is not present in embryos following prescribed embryo handling techniques. Earlier studies had established freedom from these diseases in bovine embryos.

Collaborative embryo transfer research with the United States is very appropriate as researchers on both sides of the border work to keep North America free from the more serious foreign animal diseases and yet open the door to the genetic potential available in other parts of the world.

Embryo transfer research and technology is also being applied to indigenous diseases in order to expedite our export market. Treatments are being explored which render embryos free of all infectious agents.\*

### **Diagnostic Development for Foreign Animal Diseases**

The research effort aimed at improved diagnosis of foreign animal diseases has concentrated this year on bluetongue, epizootic hemorrhagic disease of deer (EHDV), vesicular stomatitis, pseudorabies and turkey rhinotracheitis viruses.

The same team that successfully developed the competitive ELISA for antibody to bluetongue virus is nearing completion of an indirect ELISA for detection of antibody to EHDV in bovine sera. Their test uses conjugated monoclonal antibody against bovine immunoglobulins (M23, ADRI/Nepean)

and its successful performance was determined by testing several thousands of negative and positive field samples (2).

Efforts to increase speed and efficiency in foreign animal disease serology has led to the development of an indirect ELISA using combined bluetongue and EHD viral antigens. Preliminary work indicates the usefulness of this method for screening bovine sera for the presence of antibodies to bluetongue and EHD virus. Reacting sera are tested by the bluetongue competitive ELISA for group specific antibody. This tandem approach may replace the conventional agar gel tests for national surveys which are required to maintain our international trade status with respect to these diseases (2).

Another approach to bluetongue antibody detection is being explored with the development of "a dipstick" using blocking dot ELISA technology. This year two appropriate bluetongue antibodies have been developed and are in the process of being evaluated on several hundred field samples.

Although it is not as sensitive and quantitative as the competitive ELISA, the "dipstick" test may have the potential as a rugged test for field application (8).

Vesicular stomatitis virus is exotic to Canada and is a differential to be determined in cases of suspected vesicular disease. Three technologies are being explored to improve diagnosis of this disease.

One is an indirect ELISA for the detection of antibody to vesicular stomatitis virus in bovine sera using combined New Jersey and Indiana type antigen and conjugated monoclonal antibodies (M23). Evaluation of this test is continuing on several thousands of field sera including 1500 samples from a national survey panel.

ELISA tests are also being developed for detection of antibodies to vesicular stomatitis in sera of species other than bovine (2). As well, a capture ELISA is being developed and preliminary work on the application of immuno-electronmicroscopy using gold-labelled reagents proves the diagnostic potential of this rapid diagnostic test (3).

The third technology being brought to bear on vesicular stomatitis disease diagnosis is the development of a nucleic acid probe. This project is in its infancy for vesicular stomatitis

\* See Research Projects.

but has successfully produced a pseudorabies virus probe which is now being evaluated (7).

Pseudorabies virus detection is also being developed in a project being conducted in collaboration with a Rockefeller Foundation visiting scientist.

Researchers have defined the optimal parameters of an immunoperoxidase staining technique for detection of pseudorabies virus in infected tissue culture and tissues from pigs using monoclonal and polyclonal antibodies(4).

Work on the application of DNA recombinant technology for expression of pseudorabies virus in prokaryotic and eukaryotic systems is progressing. This project uses the tools of biotechnology to bypass the use of animals in viral antigen production (9).

Development and assessment of an ELISA for detection of antibodies to avian viruses is being directed towards turkey rhinotracheitis, an emerging disease in several countries (6).

Binary ethyleneimine (BEI) was found to be a reliable inactivant for viruses such as pseudorabies, swine vesicular disease and hog cholera, without compromising their antigenicity. This information has been invaluable in assuring freedom from exotic diseases on biological products leaving the high security laboratory. It is also being applied in work with indigenous viruses.

It has been determined that BEI also inactivates bovine virus diarrhea virus present in bovine serum used as supplement in tissue culture. No detrimental effect on the growth and other characteristics of several cell lines were observed when BEI-treated calf serum was used (5).

## II FOOD SAFETY

Consumers are increasingly aware of the potential for foodborne disease and have high expectations of the regulatory system designed to provide them with safe food in their supermarkets. Visual inspection alone is no longer considered adequate and the system now requires the sophisticated tools provided by research to effectively assure Canadians of safe, wholesome food.

In recent years, research on salmonella diagnosis and control has received a great

deal of emphasis but this year was the first time that a Canadian national survey of the commercial egg-laying and broiler flocks was conducted to determine the prevalence of *Salmonella spp.* at the farm level.

The survey generated data on the *Salmonella* situation in Canada that will serve as the basis for future program decisions. As well, recent outbreaks of *Salmonella enteritidis* in Great Britain and the United States required that Canada assess the status of our national flocks for the presence of this organism.

The Saskatoon chemical analysis section achieved an international breakthrough by development of a test to detect penicillin G. Previous methods could not differentiate penicillins from the rest of the beta lactans group. The test has many practical regulatory applications as well as achieving international status for that section among standard-setting organizations.

This year the development of rapid, effective tests for the detection of bacterial, parasitic and chemical contamination of food products has resulted in technology transfer to diagnostic programs.

## Bacteriology

As stated above, foodborne salmonellosis continues to be a major international public health problem. Investigations of the outbreaks of *S. enteritidis* in other countries have implicated eggs as potential reservoirs of disease. In our Canadian commercial egg-laying flock survey, 300 randomly selected flocks were tested for *Salmonella* by sampling feces, dust/fluff and feed. The results indicated that approximately 55 per cent of premises surveyed, and six per cent of the feed samples were contaminated with one or more serotypes of *Salmonella spp.* *S. enteritidis* was isolated from 2.7 per cent of the premises surveyed (10).

Phase II of this survey was initiated to determine the utility of the pullorum test for detecting *S. enteritidis* contaminated laying hens. *Salmonella* culture is resource intensive and requires that hens be sacrificed. It was hoped that existing serological techniques could be used.



Seven contaminated flocks identified from phase I and several *Salmonella*-negative flocks were included in the study. 300 birds from each flock were blood sampled.

A micro-agglutination test was used to test the sera against three antigens; *S. pullorum* standard, *S. pullorum* variant and *S. enteritidis*. Forty sero-positive (positive with both pullorum standard and *S. enteritidis*) and 20 seronegative birds from each flock were culled for post-mortem examination and culture. Testing of these samples is underway.

Material obtained from the above survey was used, with experimental materials, in the evaluation and improvement of serological tests for *S. enteritidis* infection (11). Agglutination assays lacked sensitivity and specificity, although homologous *S. enteritidis* whole cell antigens provided some increase in sensitivity over heterologous antigens, and standard or variant *S. pullorum* antigens.

ELISA tests using partially purified LPS and flagellin of *S. enteritidis* proved more sensitive than agglutination tests, but specificity was inadequate. Plans include identification of *S. enteritidis* specific antigens and use of monoclonal antibodies to these antigens in a competitive ELISA.

Investigations have also been undertaken to characterize *S. enteritidis* isolates for the presence of potential virulence markers which could be used to identify virulent strains causing egg borne infections and disease in humans (12). The characteristics examined were biochemical properties, drug resistance, plasmid profiles, hybridization with a DNA probe derived from the virulence sequence of the 90Kb plasmid of *S. typhimurium* and phage type.

So far, 70 *S. enteritidis* strains have been examined. All except three carried the 36 Mdal plasmid, the only plasmid presently associated with virulence. Plasmid with other molecular sizes were found in 11 strains and two strains lacked plasmids. *S. enteritidis* strains containing the 36 Mdal plasmid were more virulent for day-old chicks and Balb/c mice than strains that did not harbour this plasmid. Only the 36 Mdal plasmid hybridized with a probe consisting of virulence genes taken from a plasmid of *S. typhimurium* that has been reported to encode virulence

characteristics such as resistance to the bactericidal effect of serum, and invasiveness to internal organs (liver and spleen).

About half of the Canadian (primarily poultry) *S. enteritidis* isolates have been phage-typed. Phagetype 8 is the most common, which is similar to the phagetype identified in poultry isolates of *S. enteritidis*, in the U.S. Other phagetypes that were found are phagetypes 8a, 13, 13a and 23.

Development of rapid detection systems for *Salmonella* spp., including *S. enteritidis*, remains a major area of research in the Guelph Health of Animals Laboratory (13). In collaboration with Cangen Corporation and the University of Guelph, experiments are continuing to develop and evaluate *Salmonella* genetic probes and amplification systems. This approach will allow small numbers of *Salmonella* to be detected without the time-consuming pre-enrichment steps required in standard methods.

To improve sampling of chicken carcasses for bacteriological examination, a mechanical chicken-shaker based on a USDA design was constructed. It will be used in conjunction with the automated hydrophobic grid membrane filter interpreter system to enumerate the bacterial load on chicken carcasses.

At the Animal Diseases Research Institute in Nepean, efforts continue toward the development of a rapid antemortem diagnostic test for *Salmonella* in broiler chickens. Approaches include the development of sample processing technology and rugged, simple-to-use detection systems. In the case of sample processing, a filtration system for rapidly separating *Salmonella* cells from fecal material was further developed to be completely disposable for ease of use in the field. The intention is to patent this system.

In the same project, two detection systems were developed - a dot ELISA on nitrocellulose membrane and a fluorescent antibody slide test (FAST). The dot ELISA is a rugged, simplified, ELISA test format which should see use as a field test. The FAST will be a very rapid laboratory test for *Salmonella* detection which requires minimal sample preparation. A preliminary small-scale field evaluation of all systems is in progress (14).

In another project, efforts are concentrated on two other detection systems; a dot-blot

assay which is economical and practical, and a competitive ELISA involving the reading of a plate with a photometer.

Both assays currently depend on culture of *Salmonella* for a few hours for reasons of sensitivity. Currently, the reproducibility of the assays is evaluated using field samples. Efforts to improve the sensitivity through amplification are also carried out (15).

In recent years the verocytotoxin-producing *Escherichia coli* (VTEC) have been shown to be important pathogens transmitted from animals to man through the food chain. The lack of adequate detection systems for these pathogens presents a major research challenge.

Workers at the Guelph laboratory are collaborating with Cangene Corporation and the University of Guelph to evaluate oligonucleotide probes for three types of verotoxin (SLT-I, SLT-II, SLT-IV). The probes were found to correctly differentiate these toxins and will be used to detect VTEC in food samples, in combination with amplification systems.

Research is also being conducted to determine which animal VTEC isolates have the potential to cause disease in humans. These studies involve the examination of the effects of verotoxins on cells from various species.

It was discerned from these studies that human colonic adenocarcinoma cells were the least susceptible to the toxic effect of SLT-IV. Porcine endothelial cells were 1400-fold more susceptible than bovine endothelial cells. This finding is consistent with SLT-IV being specific for the porcine species (16).

The gene encoding a fourth variant of *E. coli* shiga-like toxin has been cloned and sequenced. The *E. coli* strain from which the gene was obtained was isolated from a case of infant diarrhea. The toxin gene appears to belong to the SLT-II family and is most closely related to SLT-IV which, as mentioned above, is a variant produced by *E. coli* strains associated with disease in pigs.

Current collaborative studies with workers at the Hospital for Sick Children in Toronto are attempting to determine the cell receptor specificity of this variant of the toxin. Knowledge about different variants of this toxin is necessary if effective diagnostic tests are to be established (17).

In the past decade, *Campylobacter spp.* have been recognized as important foodborne pathogens. Epidemiological studies will be facilitated by improved characterization and classification of strains belonging to these species. Multilocus enzyme electrophoresis was used to study cephalothin-susceptible and resistant strains of *Campylobacter coli* serogroup 20, biotype 1.

All southern Ontario isolates were found to be monomorphic in all enzyme loci, suggesting that they are clonal. Sodium dodecylsulfate polyacrylamide gel electrophoresis of whole cells indicated that, except for one environmental strain from the U.K., all showed almost identical protein profiles.

These results suggest that Multilocus enzyme electrophoresis also can be used effectively to further discriminate *Campylobacter* strains.

Monoclonal antibodies are being produced for evaluation in rapid detection of thermophilic *Campylobacter* in foods. Collaboration has begun with a Canadian biotechnology company in improving and evaluating potential commercial kits for rapid identification of campylobacters by restriction endonuclease analysis (18).

Work on *Listeria monocytogenes* has focused on rapid and sensitive methods of detection. Synthetic oligonucleotide probes based on the organism's hemolysin gene have been constructed and are being tested for species specificity. Work has begun on amplification of target DNA sequences using the latter probes as primers in the polymerase chain reaction (19). Studies of the virulence and genetic characteristics of *L. monocytogenes* are also being conducted (20).

## Residue detection

As mentioned in the introduction, a new analytical method for penicillin G residues in tissue, with a sensitivity in the low parts per billion range, was developed and is now undergoing evaluation prior to routine implementation. This method will allow quantitation and more specific chemical identification of penicillin G residues in meat.

Research will continue on related penicillin and cephalosporin compounds. Research is also in progress to develop improved methods



for streptomycin and related aminoglycoside antibiotics (21).

A new analytical method for monensin residues in poultry tissues was developed and published. This research, using the technique of thin-layer chromatography-bioautography, was then extended to enable the separation and identification of the related ionophore compounds, lascaiocid and salinomycin. A study of the stability of monensin residues in frozen tissues was also published (21).

Research was continued to develop detection methods for antibiotic residues in eggs, both in the laboratory and by inspectors and producers. Dosing studies are now in progress to produce eggs containing known residues for method evaluation (24).

A collaborative research project is underway with the Animal Research Center, Research Branch, Agriculture Canada, to develop specific methodology for organoarsenical compounds used as feed additives and their metabolites, then to study the metabolism of these compounds. The efficacy of organoarsenicals is also of interest in this study (22).

Research has been conducted on tests to replace or supplement those now used by inspection staff for residue detection in meat. The Brilliant Black Reduction Test (24) and the Charm II Receptor Assay (25) have both performed well in laboratory evaluations to date and will be studied further under conditions of routine application. Other test technologies are also being monitored.

## Parasitology

Workers in the Sackville laboratory are studying the infectivity parameters of various parasites at different ages in food animals.

*Trichinella spiralis spiralis* is the species most commonly found in cattle but *Trichinella spiralis nativa* is also of concern due to its presence in wild species and possible transmission to cattle.

In ongoing work on trichinosis, clinical signs were observed between 10 and 30 days post-infection and all animals seroconverted between 7 and 14 days post-infection. Antibody levels in cattle exposed to *T. spiralis nativa* were lower and for both species of

*Trichinella*, the antibodies dropped off between 182 and 369 days post-infection.

Focal lesions of eosinophilic myositis were observed up to about 90 days post-infection but after that little cellular reaction was observed except for cysts undergoing disintegration. These findings will provide program planners with parameters to design antemortem examination and serological testing protocols for *Trichinella* (27, 28).

Antibody levels to *Cysticercus bovis*, after experimental inoculation of varying dosages, were studied using a newly-developed ELISA test. Maximal levels of antibody occurred between 40 and 60 days post-infection and a second rise between 160 and 200 days was attributed to death of cysticerci with release of antigen. The results on the ELISA were equivocal with light infections, indicating that further fine tuning of the tests is required (30).

In an experiment to determine the infectivity of *Toxocara canis* with a calf host, about 21 000 embryonated eggs were given to each of two calves. Eosinophil counts started to rise at six days post-infection and peaked at 29 days. Post-mortem at 29 and 48 days respectively did not reveal lesions, suggesting that toxocariosis is not associated with bovine eosinophilic myositis (29).

## III Eradication/Control Program Diseases

Over the years, research in support of Animal Health Program diseases formed the basis upon which policy makers have designed programs. The declaration of bovine brucellosis freedom in Canadian domestic livestock put Canada in the forefront of disease control internationally and this achievement owes much to research on brucellosis.

Brucellosis is still present in Northern bison and the nature of the bacterium is such that continued surveillance will be required to maintain the "free" status in livestock. Researchers will continue to refine diagnosis because it is more difficult to detect the disease at very low incidence.

All cases of suspected rabies in animals in Canada are submitted to one of the two Animal Disease Research Institutes. This wealth of diagnostic information has been analyzed over

the years and provided the background for research which won a departmental merit award this year for three Nepean scientists.

Messrs. G.A. Casey, W.A. Webster and A.R. Bourgon will share a monetary award for "outstanding contribution to the diagnosis and control of rabies". Their work has clarified much about inter-species transmission and epidemic cycles of rabies; information which has assisted in vaccination and control programs.

## Rabies

In 1989, work continued on the correlation between the distribution of rabies antigens in tissues and the clinical signs in skunks exposed to street rabies virus (31). Research on oral rabies vaccines (partly funded by the Ontario Ministry of Natural Resources) indicates that a human adenovirus recombinant vaccine (McMaster University) is effective orally in skunks and foxes.

## Brucellosis

An atypical isolate of *Brucella* from a cow was found to be stable and to closely resemble both *B. abortus* biovar 5 and *B. melitensis* biovar 1, making it the first *B. melitensis*-like isolate recovered from an animal in Canada (32).

Research is ongoing on the purification of the O-chain antigen of *B. abortus*, necessary for the performance of the ELISA test. The methods of purification are being evaluated (33).

*B. abortus* biovar 4, not previously reported in bison, was identified in a domestic bison herd. This finding provides good evidence of cattle-to-bison transfer of infection, a much-debated issue in recent months as the fate of northern diseased bison is being decided. Serological studies in this herd indicated that the tests used to detect brucellosis in cattle were also sensitive in bison (34).

## Tuberculosis

Aerial surveys in conjunction with Alberta Fish and Wildlife, located bison up to 75 km outside the western boundary of Wood Buffalo National Park. Of five of these bison that were

necropsied, one had lesions compatible with a diagnosis of tuberculosis.

This evidence adds weight to the concern that the diseased bison of the park will transmit brucellosis and tuberculosis to cattle and healthy bison in nearby areas (34).

## Scrapie

This project is approaching a significant breakthrough in the identification of this elusive agent. This year, researchers continued to screen recombinant plasmids prepared from nucleic acids extracted from scrapie infectivity enriched preparation to identify a scrapie-specific sequence.

Utilizing digoxigenin labelled probe (non-radioactive probe) and S35 labelled probe, in-situ hybridization has been applied to animals (35).

## Paratuberculosis

*Mycobacterium paratuberculosis* presents a continuing challenge to researchers. Diagnosis is difficult and many questions remain outstanding regarding its pathogenesis.

This year, the Nepean project continued to evaluate the performance of ELISA tests using purified liparabinomannan and purified protein D antigens. Results from a large Ontario survey are being analyzed and the first phase of a study being done with Cornell shows good repeatability.

This test is being developed to replace the CF test and once it is available, there will be international interest in this much more sensitive technique.

An evaluation of pyruvate as a supplement in medium used for the primary isolation of *M. paratuberculosis* from bovine feces was undertaken as part of continuing studies to improve cultural procedures for this organism. Results suggest very little enhancement of isolation generally.

Histological and cultural evaluation of tissues from selected cattle revealed a focal intestinal infection in areas from which samples for diagnostic testing are usually not taken.

Much remains to be learned about inter-species infectivity with *Mycobacterium sp.* For example, paratuberculosis developed in sheep



following oral inoculation with tissues from an infected antelope and infection with *M. paratuberculosis* was established in a sheep inoculated with fecal material from infected cattle.

As well, *M. avium* serotype 2 pathogenicity studies in sheep and goats determined that while this infection induced a serological response indicative of paratuberculosis, it caused a self-limiting intestinal infection. In sheep, a dose response with age relationship was demonstrated.

Long-term studies continued in an industry sheep flock to evaluate current test performance in paratuberculosis and Maedi-visna control (36).

#### **IV Indigenous Diseases**

Research on indigenous diseases is often initiated in response to problems that the Animal Health Program is having with certification of animals for export, import or artificial insemination centre residency.

Questions arising from the veterinary biologics control program are another source of these investigations. The research may aim at clarifying disease mechanisms, defining parameters or developing a test. Frequently, it has broader implications outside the program's immediate needs and results in an increased body of knowledge for use by industry in their own disease control efforts.

#### **Leptospirosis**

The present serological method used for leptospirosis diagnosis leaves some doubt as to the significance of low and transient titres. This problem has been amplified in artificial insemination centres where many apparently healthy studs have been excluded from residency or international semen trade based on these tests.

ELISA tests appear to hold some promise but the appropriate antigen has been evasive. Research at Nepean will focus on the use of polysaccharide and protein antigens which have been purified from outer sheath and whole cell preparations, and the use of anti-*Leptospira* monoclonal antibodies for detection of antibodies and antigens through competitive ELISA protocols (37).

In order to further characterize antigen isolated from tissue, blood and urine, work has begun at Lethbridge to develop serovar-specific DNA probes.

A gene library was constructed for each of five serovars which commonly infect livestock in North America: *L. pomona*, *L. hardjo*, *L. grippotyphosa*, *L. icterohaemorrhagiae* and *L. bratislava*. DNA from these serovars were extracted, digested and recombinant plasmids were harvested from selected clones. They are labelled with 32 P-labelled nucleotides and used to hybridize blots of genomic DNA extracted from 32 homologous and heterologous serovars.

All probes examined to date hybridize with at least one heterologous serovar, as well as homologous DNA; for example, a probe derived from a field isolate of *L. pomona kennewicki* is specific to *L. pomona pomona* as expected, but also to *L. canicola* UV IV. A *L. hardjo bovis* probe will hybridize to *L. hardjo h'prajitno*, and to *L. tarassovi prepelisin* as well.

As testing continues, it is possible that a completely non-specific probe, common to all serovars, will be found; this would be useful as a preliminary treatment of tissues (38).

#### **Bovine Viral Diarrhea**

This organism is a major cause of economic loss to the producer as well as a barrier to international livestock trade. Lethbridge ADRI has concentrated its efforts at unravelling this complex virus and five projects are currently underway at this laboratory.

A new BVD viral ELISA antigen to detect antibody against BVD virus was 100 per cent specific and 97.8 per cent sensitive when tested against 403 sera. This technology holds promise for replacement of the resource intensive serum neutralization tests for routine diagnosis (39).

A second body of BVD research continues to use biotechnological techniques to produce BVDV anti-idiotypic monoclonal antibody which appears to mimic BVDV antigen, but further testing is required to confirm its identity (40).

Two of the antibodies produced from these projects have been selected for use in collaborative research which aims to develop a test to detect BVD antigen in formalin-fixed

tissues (41). Workers at the St-Hyacinthe laboratory are also collaborating with the Lethbridge projects with the objective of producing non-radioactive gene probes to BVDV. The ultimate objective of this work is to develop a simple serum test to detect BVD virus carrier animals (44).

The Lethbridge BVD work is augmented by the development and maintenance of a BVD-free beef herd at the Institute (42). The status of the herd is difficult to maintain with a virus of this nature but repeated testing confirms negativity. It is planned that this work be extended into control techniques for commercial herds to reduce the incidence of the virus (43).

## Respiratory Disease

### Bovine

Both the Lethbridge and Nepean Institutes are establishing tools and protocols to assist in the resolution of respiratory vaccine efficacy and potency questions that perplex livestock owners.

In Lethbridge, 25 calves were used in three experiments to evaluate the suitability of standard BHV-1 (frozen - glycerol stabilized) cultures (produced by the Biologics Evaluation Laboratory) for standard challenge of bacterin vaccinated calves.

Both cultures were found suitable with the proviso that exposure to the bacterial aerosol occurred while a nasal-tracheal tube was in place.

Thirty-six calves were used in two experiments to evaluate the efficacy of a commercial *Pasteurella haemolytica* bacterin when compared to a positive control bacterin (ADRI Bacterin). The protective effect of the commercial product was 37 per cent whilst that of the control bacterin was 63 per cent. This provided valuable data for licensing decisions by the Veterinary Biologics Section.

This evaluation was the first step in the Canadian effort to recognize for the consumer the best of many *P. haemolytica* vaccines on the market.

The leukotoxin gene cluster of *P. haemolytica* A1 has been cloned into a multicopy plasmid vector at Lethbridge. The recombinant leukotoxin and antiserum to this cloned toxin may be of value in the Branch's vaccine evaluation studies.

In Nepean, the development of potency assays for *P. haemolytica* vaccines, with the collaboration of scientists from Cornell University, H&W, NRC and VIDO has involved the following: 1) the evaluation of PAGE to quantitatively and qualitatively evaluate protein and polysaccharide antigens in conjunction with the development of processing procedures for adjuvanted products; 2) the development and standardization of immunoassays for LPS (lipopolysaccharide), CPS (capsular polysaccharide) and LKT (leukotoxin) from *P. haemolytica* serotype 1.

These assays are being evaluated for the quantitation of serum antibody in cattle, sheep and mice and the quantitation of specific antigen in pre-and-post-adjuvanted vaccines; 3) the evaluation of a murine animal model for potency assay (45).

Studies in Saskatoon on how bovine herpesvirus-1 infection of cattle causes immunosuppression found that live or inactivated virus causes white blood cells to produce a soluble suppressive factor. This factor inhibits cellular immunity to a variety of other agents by blocking lymphocytic proliferative responses to interleukin. Current experiments are aimed at characterizing specific antibody responses that will protect cattle from immunosuppression (46).

### Ovine

Investigations in Sackville have focused on determining the pathogenic mechanisms and etiology of atypical pneumonia in sheep. Hundreds of clinical isolations from diseased lungs invariably revealed the presence of *Mycoplasma ovipneumoniae* and frequently *P. haemolytica* but the significance of these organisms requires definition.

This year it was learned that microscopic lesions of proliferative interstitial pneumonia developed after the ovine lung was exposed repeatedly to whole cell antigens of *M. ovipneumoniae* but lung involvement was not extensive. Vaccinated animals also developed these microscopic lesions. In an attempt to reproduce the extensive lesions characteristic of atypical pneumonia, a pooled homogenate of diseased lamb lungs from a local abattoir were inoculated intratracheally into lambs. Seven days later, they were exposed to *P. haemolytica* by the same route, as described in work done in Scotland. One hundred per cent of the animals developed extensive lesions.



The same trial determined that live organisms were needed for extensive involvement, as well as demonstrating the augmentation of disease which the presence of lung cells appears to play.

Another trial substantiated speculation that *M. ovipneumoniae* loses its virulence upon culture in vivo. It is unlikely that this represents a genetic change but rather is a phenotypic phenomenon related to expression of virulence determinants in vivo.

While this project has clarified much on ovine atypical pneumonia, questions are outstanding on the role of cell mediated immunity and vaccine production. As well, *M. ovipneumoniae* receptors in the respiratory tract remain to be determined (47).

## Reproductive Diseases

### Bovine

A study is continuing on the potential of using DNA probes to monitor embryonic health. Since BVD and IBR viruses are not always embryocidal, the morphology of embryos may not indicate infection.

Diagnostic probes for these viruses are being developed. These probes will be used on embryo biopsies to determine whether an embryo is infected. Development of this methodology may, for the first time, allow for the direct certification of embryos rather than testing the sperm and embryo donors for these diseases (48).

A research project on micro-organisms in bull semen has made several significant findings this year. A sperm penetration assay has been developed which evaluates bovine sperm function in vitro and is being used in three studies: the differentiation between pathogenic and non-pathogenic strains of organisms, the efficacy of antibiotics in semen, and the transmission of micro-organisms by sperm at fertilization.

An enzyme immunoassay has been developed to detect *Mycoplasma bovis* and *M. canadense* in semen and preputial secretions. This project also studies methods of disinfecting bull semen and evaluates the effectiveness of antibiotic treatment of bull semen used by the AI industry to control microbial contaminants. This information should help the industry promote its product in both domestic and foreign markets (49).

### Porcine

A project investigating perinatal mortality has investigated methods for reducing losses in swine production caused by mechanical problems during delivery and to identify the variables in survival in the first hours after birth.

Marked differences have been shown in umbilical cord strength, both between and within litters. Cord weakness is strongly associated with stillbirth.

In a model using chronically catheterized fetuses, studies were made of the role of the fetal pituitary-adrenal axis in glycogen deposition, fetal lung maturation and the triggering of parturition, as well on the influence of the number of fetuses on the length of gestation (50).

### Ovine

A two-year study was completed to document the prevalence of *Haemophilus somnus* carriers in young rams and identify risk factors for the flock of origin. Of 473 rams entering Alberta and Ontario Record of Performance stations at 50 days of age, 43 (9.1 per cent) were infected with *H. somnus* in the preputial cavity. Based on these rams, 22 of the 80 eligible flocks of origin (27.5 per cent) were classified as infected with *H. somnus*.

In the model developed, 12 per cent of the bred ewes in infected flocks failed to lamb, compared to a rate of 6 per cent in non-infected flocks, suggesting *H. somnus* is associated with reduced fertility in sheep.

It was identified that purchasing replacement animals and having cattle on the farm were risk factors for *Haemophilus* infection in the flock. When replacements had been purchased within the previous year, the risk of flock infection rose 8.5 times.

On farms where cattle as well as sheep were present, the risk rose 13.2 times, suggesting interspecies transmission may play a role in the epidemiology of *Haemophilus* infections (51).

## Gastrointestinal Disease

### Porcine

The Health of Animals Laboratory located on the campus of the Faculty of Veterinary Medicine in St-Hyacinthe optimizes its location by maintaining a close relationship with the college. The laboratory has a growing interest and expertise in diseases of swine, a species of relative prominence in Québec.

This year three projects related to porcine gastrointestinal disease were spawned from collaboration with the veterinary faculty. A project on transmissible gastroenteritis virus is attempting to characterize specific monoclonal antibodies for diagnostic purposes.

Techniques for local monoclonal production are being developed at the same time that prototypic strains obtained elsewhere are being used for comparison to clinical cases. Dr. P. Talbot, a human coronavirus expert from the Institut Armand Frappier, is collaborating on the project.

An interesting offshoot of the work may be the ability to differentiate coronavirus causing gastrointestinal disease from an emerging strain which colonizes the respiratory tract. That distinction may become important for export certification, as well as disease diagnosis (52).

In 1989, an atypical porcine rotavirus was isolated from 12 per cent of 114 outbreaks of gastrointestinal disease submitted to the college. Since the presence of this virus in porcine gastrointestinal disease outbreaks was first documented in the United States in 1982, it has appeared in the United Kingdom and South America.

Only one strain of the virus grows in tissue culture and is used in vaccine production. This means that the porcine population is virtually unprotected from most strains of atypical rotavirus.

Work in St-Hyacinthe has succeeded in identifying these problems in Québec and is concentrating on determining nucleic acid diversity of the virus by using polyacrimide gel

electrophoresis and comparing field strains to prototypic strains propagated in specific pathogen free pigs. An indirect fluorescent antibody test has been developed to test sera for antibody to the virus. Eventually an ELISA test is to be developed (53).

The determination of plasmids associated with antimicrobial resistance to *Treponoma hyodysenteriae* is the purpose of a third project in the St-Hyacinthe laboratory. As with the other two projects described, this effort uses sophisticated techniques just being introduced in the new laboratory so that preparation has preoccupied the initial portion of the research (54).

### Poultry Diseases

Marek's disease virus is responsible for outbreaks of the disease in vaccinated chickens. The Nepean workers have isolated what appears to be chick anemia agent from an Ontario outbreak and are conducting studies to determine the role of the immunosuppressive agent on the development of Marek's disease (55).

Antibody to avian leukosis virus was detected by ELISA and virus neutralization in feather pulp of naturally and experimentally infected chickens. As feather pulp is convenient to obtain and could be useful in disease monitoring programs, pulp and serum from vaccinated chickens for antibody to Newcastle disease and avian encephalomyelitis was also tested. For both diseases there was a highly significant correlation between tests for antibody in the two types of specimens.

In cooperation with the Animal Research Centre, Ottawa, progress continues to be made in experiments aimed at developing strategies for eradicating exogenous avian leukosis virus from chickens and in determining the significance of endogenous leukosis viruses in meat and egg laying stocks (56).



## V Diagnostic Development in Program/Indigenous Diseases

Research which will be applied directly to the diagnosis of specific diseases or to a diagnostic technology is described in this section. This work is designed to meet the immediate needs of clients, so it is generally short term and considered to be 'low risk' in terms of the return on investment. It requires that a diagnostic unit be situated close to the research to allow for on-going validation of the results.

Research is progressing on the development of monoclonal antibodies to porcine immunoglobulins for use in the serodiagnosis of porcine diseases. Monoclonal antibodies to various subclasses of porcine immunoglobulins are being evaluated (57).

Work is also under way on antigen production and test development in the area of retrovirology. Retroviruses can cause disease in large animals and the exogenous virus is transmissible. The work is emphasizing the development of methods of antigen production and the development of assay systems that can be used for detecting bovine immunodeficiency virus (BIV) (58).

One approach being investigated is the use of BIV-specific DNA probes. Towards this end, the genome of BIV is being cloned and characterized. Portions of such a clone will then be assessed for use as a diagnostic probe (61). A reliable diagnostic test for this virus would allow both animal products and vaccines to be certified.

Development, modification and implementation of automated systems continues for use with diagnostic enzyme immuno-assay techniques. So far, the primary emphasis has been on automating ELISA reading, and data expression, handling and storage, data analysis and reporting and quality control.

Systems have been developed for both indirect and competitive ELISAs. These currently include diagnostic systems for brucellosis, pseudorabies and bluetongue. Others will be added as they come on line (59).

Research is also being conducted on automating liquid handling, sample tracking and identification (i.e. bar coding).

Results of studies on a persistent infection of a cell line with street rabies have been published. Comparison of growth characteristics of field rabies virus strains in cell culture is continuing (60).

Potency tests for non-live adjuvanted products such as *Pasteurella*, field trial designs for efficacy of respiratory vaccine and potency tests for colostrum-whey products are being developed at Nepean.

For the detection of cattle which have antibody to infectious bovine rhinotracheitis (IBR), Lethbridge developed a qualitative ELISA which performed very well after modifications were made to the initial antigen.

For quantitation of antibody concentration, utilization of either simple end-point titration methods or comparison of final absorbance of the substrate can be immunochemically misleading and, in some cases, invalid.

Kinetic-based ELISA (KELA) assays are truly quantitative assays. KELA is based upon the fact that the initial velocity of substrate conversion is directly proportional to the antibody concentration. A computer program for the KELA test for IBR antibody which is being used at Cornell University, has been installed at Lethbridge by Dr. Richard Jacobson of Cornell University.

To test the KELA, 10 calves were aerosol-inoculated with IBR virus to obtain serial serum samples to determine whether the kinetic ELISA assay can replace the SN test in export tests which require testing for IBR twice with no significant increase in antibody titre (62).

## **VI List of Research Projects**

### **1. Foreign Animal Diseases**

#### **Embryo Transfer**

1. Embryo transfer as a means of controlling disease.  
E.L. Singh, G.C. Dulac, C. Dubuc (ADRI, Nepean), C.A. Mebus (PIADC-USDA).

#### **Diagnostic Development in Foreign Animal Diseases**

2. Application of enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against selected animal viruses, exotic to Canada.  
A. Afshar, N.H.A. Al-Shakarchi, G.C. Dulac, P.F. Wright, C. Dubuc, D.J. Myers (ADRI, Nepean).
3. Application of enzyme immunoassay (ELISA) to detect vesicular disease viral antigens in cell culture and tissues of animals infected experimentally and application of related techniques to other foreign animal disease viruses.  
C. Dubuc, P.F. Wright, A. Afshar, G.C. Dulac, W. Kelly (ADRI, Nepean).
4. Immuno-enzymatic techniques for rapid detection of selected animal viruses, exotic to Canada.  
A. Afshar, T.W. Dukes, G.C. Dulac, N.H.A. Al-Shakarchi, K. Nielsen, D. Henning (ADRI, Nepean), T.U. Obi (Univ. of Ibadan, Nigeria).
5. Inactivation of selected exotic animal viruses with binary ethyleneimide.  
G.C. Dulac, C. Dubuc, D.J. Myers, A. Afshar (ADRI, Nepean), R. Morley AHD).
6. Application of the enzyme-linked immunosorbent assay for the detection of antibody to selected avian viruses.  
D.J. Myers, G.C. Dulac, A. Afshar, P.F. Wright (ADRI, Nepean).

7. Nucleic acid probes for detection of foreign animal diseases viral nucleic acid.  
O. Surujballi, D. Denicourt, M.F. Traykova-Andonova, G.C. Dulac, C. Dubuc (ADRI, Nepean).
8. Evaluation of the blocking "Dot ELISA" for detecting antibodies to bluetongue virus.  
A. Afshar, G.C. Dulac, P.F. Wright (ADRI, Nepean).
9. Application of recombinant DNA technology for production of exotic virus antigens.  
O. Surujballi, D. Denicourt, C. Dubuc, G.C. Dulac, A. Afshar, P.F. Wright (ADRI, Nepean)

### **2. Food Safety**

#### **Bacteriology**

10. National Survey of *Salmonella enteritidis* in laying flocks. Phase I, II.  
R. Irwin, C. Poppe, R. Clarke, R. Johnson (HAL Guelph).
11. Development and evaluation of improved serological tests for *Salmonella enteritidis* infection.  
R. Johnson, C. Poppe, (HAL Guelph).
12. Characterization of *Salmonella enteritidis* isolates of animal and poultry origin.  
C. Poppe (HAL Guelph).
13. Development and evaluation of rapid *Salmonella* detection systems.  
R. Clarke, K. Rahn (HAL Guelph) and C.L. Gyles (University of Guelph).
14. Development of sample processing technology for a rapid antemortem *Salmonella* detection test in broiler chickens.  
A.D.E. Fraser, M.M. Gracia, B.W. Brooks (ADRI, Nepean).



15. Rapid detection of *Salmonella* sp. antigens in chicken material.  
K. Nielsen, E.A. Sugden, D. Henning (ADRI, Nepean).
16. Detection of verocytotoxigenic *Escherichia coli*.  
R. Clarke, A. Valdivieso-Garcia, S. Read (HAL Guelph), C.L. Gyles (University of Guelph) H. Lior (National Enteric Reference Centre, LCDC, Ottawa).
17. Characterization of *E. coli* shiga-like toxin (SLT-IIv).  
V.P.J. Gannon (ADRI, Lethbridge).
18. Methods for improved detection of campylobacters in animals and foods for the diagnosis of *Campylobacter* infections.  
M.M. Garcia, A.D.E. Fraser, B.W. Brooks (ADRI, Nepean).
19. Development of DNA amplification system for the detection of *Listeria monocytogenes* in meat and cheese using DNA probes.  
V.P.J. Gannon, (ADRI, Lethbridge).
20. Characterization of *Listeria monocytogenes* from processed meats.  
S.Messier, (HAL, St-Hyacinthe).
21. Development of new methodology for analysis of antibiotic residues in meat products.  
J. D. MacNeil, J. R. Patterson, C. D. C. Salisbury, A. C. E. Fesser, V. Martz (HAL, Saskatoon).
22. Development of new methodology for analysis of non-antibiotic residues in meat products.  
J. R. Patterson, J. D. MacNeil, A. C. E. Fesser, C. D. C. Salisbury, V. Martz, (HAL, Saskatoon).
23. The development of mass spectrometric methods for the screening and confirmation of antibiotic residues in edible tissues and biological fluids of food producing animals.  
J. O. K. Boison, J. D. MacNeil, C. D. C. Salisbury, J. R. Patterson (HAL, Saskatoon).
24. The development of alternative and/or improved methods of analysis for the screening of antibiotic residues in the edible tissues and biological fluids of food-producing animals.  
J. O. K. Boison, J. D. MacNeil, A. C. E. Fesser, C. D. C. Salisbury, J. R. Patterson (HAL, Saskatoon).
25. Laboratory evaluation and testing of the Charm Test II receptor assay for antibiotic residue analysis.  
G. O. Korsrud (HAL, Saskatoon).
26. Adaptation of chromatographic reference methods for penicillin G and tylosin to be used in evaluating screening tests (swab test on premises and thin layer bioautography), effects of extra-label dosages, and loss during storage.  
G. O. Korsrud, J. D. MacNeil (HAL, Saskatoon) and M. G. Papich (University of Saskatchewan).

#### Residue detection

#### Parasitology

27. Investigation of trichinosis in cattle.  
H.J. Smith, K.E. Snowdon, G.G. Finley (HAL, Sackville)
28. Presence of anti-*Trichinella* antibodies in cattle.  
H.J. Smith, K.E. Snowdon (HAL, Sackville)

29. Ascarid infections in cattle.  
H.J. Smith, K.E. Snowdon (HAL, Sackville)
30. Evaluation of the ELISA for the serologic diagnosis of cysticercosis in cattle.  
H.J. Smith (HAL, Sackville), P.H.G. Stockdale (ADRI, Lethbridge)

### 3. Eradication/Control program diseases

#### Rabies

31. Studies of the pathogenesis of rabies in skunks.  
K.M. Charlton, G.A. Casey, W.A. Webster, A. Bundza (ADRI, Nepean).

#### Brucellosis

32. Improved bacteriological and serological methods for the diagnosis and characterization of *Brucella* species infecting wildlife and domestic animals.  
L.B. Forbes, S.V. Tessaro (HAL, Saskatoon).
33. Development of monoclonal antibodies for primary binding assays. I. Application to *Brucella* and *Mycobacterium paratuberculosis* enzyme immunoassay and detection of antigens.  
K.H. Nielsen, J.R. Duncan, P.F. Wright, A.M.P. Bouillant, D. Henning (ADRI, Nepean).

#### Tuberculosis

34. Survey of diseases and parasites in commercially-utilized wildlife.  
S.V. Tessaro (HAL, Saskatoon).

#### Scrapie

35. Characterization of the etiological agent of scrapie.  
H.J. Cho (ADRI, Lethbridge).

### Paratuberculosis

36. Diagnosis and immunopathogenesis mechanisms of bovine, ovine, and caprine paratuberculosis.  
B.W. Brooks, T.W. Dukes, E.A. Sugden, J.R. Duncan, A.D.E. Fraser, M.M. Garcia (ADRI, Nepean) in collaboration with K.H. Nielsen (ADRI, Nepean), A. Muckle (Ontario Veterinary College).

### 4. Indigenous Diseases

#### Leptospirosis

37. Leptospira antigen characterization: application to serological diagnosis.  
E.A. Sugden, K. Malkin, O. Surujballi, M.D. Eaglesome, D. Henning, K. Nielsen, J.B. Stevens, P.F. Wright, B.W. Brooks (ADRI, Nepean).
38. DNA probes for the identification of leptospires.  
S.P. Gale (ADRI, Lethbridge).

#### Bovine Viral Diarrhea

39. Studies on the BVD virus on the molecular level.  
H.J. Cho, S.A. Marsi, D. Deregt (ADRI, Lethbridge).
40. BVD virus anti-idiotypic monoclonal antibodies.  
D. Deregt (ADRI, Lethbridge).
41. Identification of BVD virus antigen in formalin-fixed tissues.  
D. Deregt (ADRI, Lethbridge).
42. Production of a BVD-free beef herd.  
V.W. Lees (ADRI, Lethbridge).
43. Controlling BVDV in purebred and commercial cattle.  
V.W. Lees (ADRI, Lethbridge).
44. Utilization of non-radioactive gene probes to detect BVDV.  
R. Magar (HAL, St-Hyacinthe).



## Respiratory Disease

### Bovine

45. Development of potency tests for *Pasteurella haemolytica* vaccines. J.R. Duncan, R. Lacroix, T.W. Dukes (ADRI, Nepean); in collaboration with M. Perry, J. Richards, D. Bundle (National Research Council, Ottawa), L. Babiuk, A. Potter, S. Acres (VIDO), A.J. Winter (Cornell University, U.S.A.)
46. Cell-mediated immunity to bovine herpesvirus-1. D.L. Hutchings (HAL, Saskatoon) and L.A. Babiuk (University of Saskatchewan and VIDO).

### Ovine

47. A study of *Mycoplasma/Ureaplasma* from sheep and goats in the Maritime provinces and their interrelationship with bacteria and viruses in specific disease conditions. J. Lopez, G.G. Finley, C. Simard (HAL, Sackville).

## Reproductive Disease

### Bovine

48. The application of microtechniques to the study of infectious disease in early embryos. W.C.D. Hare, A. Bielanski, A.M.P. Bouillant, C. Lutze-Wallace (ADRI, Nepean).
49. Microorganisms in bull semen. M.D. Eaglesome, W.C.D. Hare, K.H. Nielsen, M.M. Garcia, F. Gilka (ADRI, Nepean).

### Porcine

50. Perinatal mortality: the contribution of perinatal maturation and adaptation to losses of newborn animals. G.C.B. Randall (ADRI, Nepean) in collaboration with University of Texas Medical School, University of Western Ontario, University of Ottawa, McGill University.

### Ovine

51. Hemophilosis in Alberta rams - epidemiology. V.W. Lees (ADRI, Lethbridge)

## Gastrointestinal Disease

### Porcine

52. Development and characterization of monoclonal antibodies to TGEV. R. Magar (HAL, St-Hyacinthe).
53. Identification and prevalence of atypical porcine rotavirus. R. Magar (HAL, St-Hyacinthe).
54. Plasmid associated antimicrobial resistance in *Treponema hyodysenteriae*. S. Messier (HAL, St-Hyacinthe).

## Poultry Diseases

55. Studies on the etiology, pathogenesis, epizootiology and control of Marek's disease. J.L. Spencer, F. Gilka (ADRI, Nepean) in collaboration with J.S. Gavora, M. Lessard (Animal Research Centre, Ottawa).
56. Studies of lymphoid leukosis in chickens. J.L. Spencer, F. Gilka (ADRI, Nepean) in collaboration with J.S. Gavora, R.W. Fairfull, J.R. Chambers (Animal Research Centre, Ottawa).

## 5. Diagnostic development in Indigenous Control Program diseases

57. Development of monoclonal antibodies for primary binding assays. II. Production of monoclonal antibodies for detection of antibody in porcine sera. K.H. Nielsen, D. Henning (ADRI, Nepean).

58. Production of viral antigens by cell culture techniques.  
A.M.P. Bouillant, K.H. Nielsen (ADRI, Nepean).
59. Assay automation - application to disease diagnosis.  
W.A. Kelly, P.F. Wright, K.H. Nielsen, E.A. Sugden, A. Afshar (ADRI, Nepean).
60. Methods development - rabies diagnosis.  
W.A. Webster, G.A. Casey, K.M. Charlton (ADRI, Nepean).
61. Development of DNA probes for animal lentiviruses.  
S.A. Nadin-Davis (ADRI, Nepean).
62. ELISA development for IBR.  
H.J. Cho (ADRI, Lethbridge).



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## Patents

MASRI, S.A.

1. Sonication method for water treatment  
(Patent Pending)
2. Sonication method for sewage treatment  
(Patent Pending)
3. Flow-through horn for cell description  
(Patent Pending)

## External appointments

AFSHAR A - Member, Graduate  
Advisory Committee, Dept.  
of Biology, Carleton  
University, Ottawa

ALEXANDER DC - Member, Biologics  
Committee - U.S. Animal  
Health Association

BOISON JOK - Adjunct Professor  
(Chemistry), University of  
Saskatchewan

BOUFFARD A - Member, Animal Research  
Centre Advisory  
Committee, Ottawa

BULMER WS - Chairperson, Editorial  
Board Canadian  
Veterinary Journal and  
Canadian Journal of  
Veterinary Research

CARRIER SP - Board Member, Quebec  
Professional Association of  
Veterinary Medicine

CHARLTON KM - Adjunct Professor,  
Queen's University,

CLARKE RC - Adjunct Professor, Ontario  
Veterinary College  
University of Guelph

DULAC GC - Canadian Technical  
Advisor, North American  
Foot and Mouth Disease  
Vaccine Bank

EVANS BR

- Board of Directors,  
Canadian Embryo Transfer  
Society
- Member, Import/Export  
Committee, International  
Embryo Transfer Society
- Member, Regulatory and  
Government Liaison  
Subcommittee,  
International Embryo  
Transfer Society

FINLAY RC

- Member, Regional Training  
and Development  
Committee

FRASER ADE

- Coordinator BIONET
- Chairperson, Algonquin  
College Joint Advisory  
Committee for Chemical  
(Biochemical) Tech-  
nology, Chemical  
(Bioengineering)  
Technology  
and Chemical Engineering  
Technology Programs,  
Ottawa

GANNON VPJ

- Member, Regional  
Information Systems  
Committee
- Member, Computer Use  
and Development  
Committee, Lethbridge  
Research Station

GARCIA MM

- Expert, United Nations  
Brucellosis Network

GREGORY D

- Member, Rabies Advisory  
Committee of Ontario

HARE WCD

- Chairperson, Research  
Group, International  
Embryo Transfer Society  
(IETS)
- Member, Import/Export  
Committee, IETS



	<ul style="list-style-type: none"> <li>- Member, Import/Export Committee, U.S. Animal Health Association</li> </ul>		<ul style="list-style-type: none"> <li>- Departmental representative, Cornwall Island Fluoride Committee</li> </ul>
IDE PR	<ul style="list-style-type: none"> <li>- Member, Grosse-Ile Joint Management Committee (Agriculture-Parks Canada)</li> </ul>	MARTZ V	<ul style="list-style-type: none"> <li>- Program Coordinator (Meat and Meat Products), Canadian Association of Pesticide Control Officials</li> </ul>
	<ul style="list-style-type: none"> <li>- Member, Ontario Animal Research and Services Committee</li> </ul>		
JOHNSON RP	<ul style="list-style-type: none"> <li>- Associate Member, Faculty of Graduate Studies University of Guelph</li> </ul>	MESSIER S.	<ul style="list-style-type: none"> <li>- Associate Faculty Member, Faculty of Veterinary Medicine, University of Montreal</li> </ul>
KELLAR JA	<ul style="list-style-type: none"> <li>- Chairman of Health Committee, Canadian Swine Improvement Advisory Board</li> </ul>	MORIN G.	<ul style="list-style-type: none"> <li>- Secretary-Treasurer, High Technology Committee for agricultural food products of St-Hyacinthe</li> </ul>
	<ul style="list-style-type: none"> <li>- Organizing Committee, VI Triennial Symposium on Veterinary Epidemiology and Economics, Ottawa 1991</li> </ul>	MORLEY RS	<ul style="list-style-type: none"> <li>- Adjunct Professor, Department of Health Management, Atlantic Veterinary College, University of P.E.I.</li> </ul>
LEES VW	<ul style="list-style-type: none"> <li>- Member, Animal Health Committee, Lacombe Research Station</li> </ul>	NIELSEN KH	<ul style="list-style-type: none"> <li>- Member, Rabies Research Committee, Ontario Ministry of Natural Resources</li> </ul>
	<ul style="list-style-type: none"> <li>- Member, Large Animal Health Committee, Lethbridge Research Station</li> </ul>		<ul style="list-style-type: none"> <li>- Member, Natural Sciences and Engineering Research Council of Canada (NSERC), Strategic Grants Committee</li> </ul>
LOEWEN KG	<ul style="list-style-type: none"> <li>- Member, Rabies Control Committee (Alberta)</li> </ul>		
	<ul style="list-style-type: none"> <li>- Member, Electron Microscope Committee, Lethbridge Research Station</li> </ul>		<ul style="list-style-type: none"> <li>- Member, NSERC University/Industry Committee</li> </ul>
MACNEIL JD	<ul style="list-style-type: none"> <li>- Adjunct Professor (Toxicology), University of Saskatchewan</li> </ul>		<ul style="list-style-type: none"> <li>- Vice-Chairperson, World Health Organization (WHO) informal meeting on enzyme immunoassay (ELISA) for Brucellosis diagnosis</li> </ul>
	<ul style="list-style-type: none"> <li>- Member, ad hoc Working Group on Methods of Sampling and Analysis, Committee on Veterinary Drug Residues in Foods, Codex Alimentarius Commission</li> </ul>	OLSON WO	<ul style="list-style-type: none"> <li>- Member, Library Committee, Lethbridge Research Station</li> </ul>

PEART B	<ul style="list-style-type: none"> <li>- Representative from O.I.E. to the Live Animals Board, International Air Transportation Association</li> </ul>		<ul style="list-style-type: none"> <li>- Member, Embryo Movement Subcommittee, U.S.A.H.A.</li> </ul>
		SINGH EL	<ul style="list-style-type: none"> <li>- Member, Research Group, IETS</li> </ul>
RANDALL GCB	<ul style="list-style-type: none"> <li>- Adjunct Professor, MacDonald College, McGill University</li> </ul>		<ul style="list-style-type: none"> <li>- Member, Import/Export Committee, IETS</li> </ul>
REID IR	<ul style="list-style-type: none"> <li>- Member, North American Foot and Mouth Disease Vaccine Bank</li> <li>- Member, Executive Committee, United States Animal Health Association</li> <li>- Member, Foreign Animal Diseases Committee, United States Animal Health Association</li> </ul>	SMITH HJ	<ul style="list-style-type: none"> <li>- Member, Board of Governors, IETS</li> <li>- Chairman, Atlantic Veterinary College Advisory Council</li> <li>- Member, Strategic Planning Group for Veterinary Medical Education at Atlantic Veterinary College</li> </ul>
	<ul style="list-style-type: none"> <li>- Departmental Representative on International Air Passenger Traffic Committee (I.A.P.T.C.)</li> <li>- Member of I.A.P.T.C. Task Force on Transfer Lounge Initiative</li> <li>- Project Manager CIDA/China Tanggu Animal Quarantine Upgrading Project Phase II</li> </ul>	SPENCER JL	<ul style="list-style-type: none"> <li>- Member, Alternatives Committee, Canadian Council of Animal Care</li> <li>- Member, Avian Tumour Virus Committee, American Association of Avian Pathologists</li> <li>- Editor, Newsletters of the World Veterinary Poultry Association</li> <li>- Member, Editorial Board, Journal of Avian Diseases</li> </ul>
ROBINSON Y.	<ul style="list-style-type: none"> <li>- Associate Faculty Member, Faculty of Veterinary Medicine, University of Montreal</li> <li>- Assistant Editor, Canadian Veterinary Journal</li> <li>- Adjunct Professor, and Member of School of Graduate Studies, University of Ottawa</li> <li>- Assistant Editor, Canadian Journal of Veterinary Research</li> </ul>	STEMSHORN B	<ul style="list-style-type: none"> <li>- Member, Scientific Advisory Committee on Brucellosis, U.S.A.H.A.</li> <li>- Coordinator, Animal and Plant Diseases Information System, Inter-American Institute for Co-operation on Agriculture, Tacarigua, Trinidad</li> </ul>



- |               |   |  |
|---------------|---|--|
| STEVENS JB    | <ul style="list-style-type: none"> <li>- Member, Committee on Tuberculosis in Animals, International Union Against Tuberculosis and Lung Disease</li> </ul>   | <ul style="list-style-type: none"> <li>- Chair, Technical Committee, Waterton Biosphere Reserve</li> </ul>   |
| STEVENS RCK   | <ul style="list-style-type: none"> <li>- Process Leader, Organization, International Office of Epizootics (IOE) 1989 Regional Conferences</li> </ul>  | <ul style="list-style-type: none"> <li>- Chair, NSERC Site Visit Committee, Industrial Research Chair in Poultry Biotechnology, McGill University</li> <li>- Member, Editorial Subcommittee, Joint Working Group on the Medical Research Council Committee on Biosafety Guidelines</li> </ul>  |
| STEVENSON RG  | <ul style="list-style-type: none"> <li>- Member, Canadian Agricultural Research Council</li> <li>- Member, Atlantic Provinces Agricultural Services Coordinating Committee</li> <li>- Chairman, Atlantic Provinces Veterinary Conference</li> <li>- Council Member, Canada, Commonwealth Veterinary Association</li> </ul>  | <p>TESSARO SV</p> <ul style="list-style-type: none"> <li>- Adjunct Professor (Veterinary Pathology), University of Saskatchewan</li> <li>- Bison Specialist Group, International Union for the Conservation of Nature and Natural Resources, Geneva</li> <li>- Education Committee, Wildlife Disease Association</li> <li>- Public Awareness Committee, Wildlife Disease Association</li> <li>- Expert Panel, Saskatchewan Game Farming Technical Advisory Committee, Government of Saskatchewan</li> <li>- Chairman of the Organizing Committee, Western Canada Wildlife Diseases Conference, 1989</li> </ul> |
| STOCKDALE PHG | <ul style="list-style-type: none"> <li>- Assistant Editor, Canadian Journal of Veterinary Research</li> <li>- Member, Editorial Board, Canadian Veterinary Medical Association</li> <li>- Associate Editor, Canadian Journal of Zoology</li> <li>- Adjunct Professor, University of Calgary, Department of Biological Sciences</li> <li>- Member, Biological Safety Committee, University of Lethbridge</li> <li>- Member, Agriculture Development Committee (Alberta)</li> </ul> | <p>WANDELER AI</p> <ul style="list-style-type: none"> <li>- Member, WHO Expert Committee on Rabies</li> </ul>  |

**WILLIS NG**

- Vice President, International Animal Health Code Specialist Commission, OIE
- Commissioner, North American Foot and Mouth Disease Vaccine Bank
- Member, Advisory Council, Atlantic Veterinary College
- Member, Advisory Council, Ontario Veterinary College
- Member, Advisory Council, Western College of Veterinary Medicine
- Member, Canadian Agricultural Research Council
- Chairperson, Expert Committee on Animal Health, Canada Committee on Animal Production Services, Canadian Agricultural Services Coordinating Committee
- Co-chairperson, Agriculture Canada Animal Welfare Coordinating Committee
- North American Ministerial Representative to Hemispheric Committee for the Eradication of Foot and Mouth Disease
- Member, Animal Welfare Committee, United States Animal Health Association

**WRIGHT PF**

- Brucellosis Expert, International Atomic Energy Agency, Joint IAEA/FAO Division
- Advisor, United Nations University Brucellosis Network Project
- Co-chairperson, WHO informal meeting on ELISA for Brucellosis diagnosis
- Western Regional Director at Large, Spectroscopy Society of Canada
- Scientific Editor, Canadian Veterinary Journal
- Adjunct Professor (Veterinary Pathology), University of Saskatchewan

**YATES WDG**

- Scientific Editor, Canadian Veterinary Journal
- Adjunct Professor (Veterinary Pathology), University of Saskatchewan

## Awards

Ms. Lammerding from the Health of Animals Laboratory, Guelph received the John C. Ayres Award for best Graduate Student Poster Presentation in food microbiology at the Inst. Food Technology 1989 Annual Meeting.

Messrs. G.A. Casey, W.A. Webster and A.R. Bourgon of the Rabies Unit, Animal Diseases Research Institute, Nepean, received a group merit award by the Departmental Merit Award Committee in recognition of their outstanding contribution in the diagnosis and control of rabies.



## Tests Available

Disease/ Microorganism	Test	SACKVILLE	ST. HYACINTHE	NEPEAN	GUELPH	SASKATOON	LETHBRIDGE
Actinobacillus pleuropneumoniae	ELISA		x				
	CF			x			
Anaplasmosis	CF			x			
Anthrax	Culture			x			x
African Swine Fever	Isolation			x			
	FA			x			
	IFA			x			
Avian Influenza	AGID			x			
	HI			x			
	Isolation			x			
	Pathogenicity			x			
<i>Bacillus cereus</i>	Culture		x		x		
Bluetongue	AGID			x	x		x
	CF			x	x		x
	EM			x			
	Isolation			x			
	ELISA			x			
	SN			x			
Bovine Leukosis	AGID		x	x	x		x
Brucellosis	Biotyping			x		x	
	BPAT	x	x	x	x	x	x
	BRT	x	x	x	x	x	x
	CF			x	x		x
	Culture			x		x	
	ELISA			x			
	SPAT			x	x	x	x
	STAT	x	x	x	x	x	x
Brucella ovis	CF			x			x
	ELISA						x
Bovine Virus Diarrhea	Isolation	x		x			x
	SN	x		x			x
<i>Campylobacter</i>	Culture	x	x	x	x		x
	FA	x	x	x	x		x
	VMA			x			x
Caprine Arthritis-Encephalitis	AGID	x					
	ELISA	x					
Chlamydiosis	CF			x			
	Isolation	x		x			
	Special stain	x		x			
<i>Clostridium perfringens</i>	Culture	x	x	x	x		

Disease/ Microorganism	Test	SACKVILLE	ST. HYACINTHE	NEPEAN	GUELPH	SASKATOON	LETHBRIDGE
Contagious Equine Metritis	CF			x			
	Culture			x			
Cysticercosis	Histology	x	x	x			x
	Parasitology	x					
	ELISA	x					
Dourine	CF			x			
Enzootic Abortion of Ewes	CF			x			
<i>Eperythrozoon suis</i>	IHA			x			
Epizootic Hemorrhagic Disease	AGID			x	x		x
	CF			x	x		
	EM			x			
	Isolation			x			
	SN			x			
Equine Arteritis	Isolation			x			
	SN			x			
Equine Encephalomyelitis	CF			x			
	Isolation			x			
Equine Infectious Anemia	AGID			x	x		x
Equine Paratyphoid	Tube Agg			x			
Equine Rhinopneumonitis	SN			x			
<i>Escherichia coli</i> 0157:H7	Culture	x	x		x		
Glanders	CF			x			
	Culture			x			
Hemagglutinating Encephalomyelitis Virus	SN			x			
Hog Cholera	FA			x			
	IFA			x			
	Isolation			x			
	SN			x			
Infectious Bovine Rhinotracheitis	ELISA				x		x
	FA	x					
	Isolation	x		x			x
	SN	x		x			x
Leptospirosis	Culture						x
	FA						x
	MA			x			x
<i>Listeria monocytogenes</i>	Culture	x	x	x	x		
Maedi-Visna	AGID	x					
	ELISA	x					
Mange	Identification	x					x



Disease/ Microorganism	Test	SACKVILLE	ST. HYACINTHE	NEPEAN	GUELPH	SASKATOON	LETHBRIDGE
Mastitis	Culture			x			
<i>Mycoplasma/Ureaplasma</i>	Agg			x			
	CF			x			
	Culture	x		x			x
Newcastle Disease	HI	x		x			
	Isolation	x		x			
	Pathogenicity			x			
Parainfluenza-3 virus	HI			x			x
Paratuberculosis	AGID			x			x
	CF			x	x		x
	Culture			x			
Porcine Influenza	HI			x			
	Isolation			x			
Porcine Parvovirus	HI			x			
Piroplasmosis	CF			x			
Pseudorabies	ELISA	x	x	x	x		x
	FA			x			
	Isolation			x			
	SN			x			
Pullorum/typhoid	Tube Agg			x			
	Culture			x			
Q Fever	CF			x			
Rabies	FA			x			x
	Histology			x			x
	MIT			x			x
	TCT			x			
Rinderpest	Isolation			x			
	SN			x			
<i>Salmonella</i>	Culture	x	x	x	x		
	Serotyping				x		
Scrapie	Histology			x			
<i>Staphylococcus aureus</i>	Enterotoxin				x		
<i>Streptococcus</i>	Lancefields grouping			x	x		
Teschen Disease	Isolation			x			
	SN			x			
Transmissible Gastroenteritis	Isolation	x		x			
	SN	x		x			
Toxoplasmosis	CF			x			
Trichinosis	Digestion	x					
	ELISA	x					
	Histology	x	x	x			x

Disease/ Microorganism		Test	SACKVILLE	ST. HYACINTHE	NEPEAN	GUELPH	SASKATOON	LETHBRIDGE
<i>Trichomonas</i>		Culture	x	x	x	x		x
		Micro Exam						x
Tuberculosis		Culture			x			
		Histology			x			x
Vesicular Diseases		CF (antigen detection)			x			
		EM			x			
		Isolation			x			
		SN			x			
Various		Culture	x		x			x
		EM		x	x			x
		Histology	x	x	x		x	x
		Virus Isolation	x		x			x
		Necropsy	x	x	x		x	x
		Parasitology	x					x
		Bacterial Profiles on Meat Products	x	x		x		
		Water Activity Determination				x		
		Species Verification of Raw Meat			x	x		
		Species Verification of Cooked Meats				x		
		Serum Banking and Inventory	x ovine		x			
		Semen microbiology	x		x			x



The following testing for residues in food products is conducted at the Saskatoon laboratory:

Residue	Test	Residue	Test
Antibiotics	TLC/BA	Ivermectin	HPLC
Carbadox	GC	Melengestrol acetate	GC
Chloramphenicol	GC	Nitrofurans	HPLC
Clopidol	GC	PCB, PCP	GC
Decoquate	HPLC	Pesticides	GC
Diethylstilbestrol	GC-MS	Sulfa	TLC, GC-MS
Dimetridazole	GC	Tetracyclines	HPLC
Heavy Metals	AA	Zeranol	GC-MS

## Test abbreviations key

AA	atomic absorption	IHA	indirect hemagglutination
AGID	agar gel immunodiffusion	MA	microagglutination
Agg	agglutination	Micro Exam	microscopic examination
BAPT	buffered antigen plate agglutination test	MIT	mouse inoculation test
BRT	brucellosis milk ring test	MS	mass spectrometry
CF	complement fixation	RIA	radio immunoassay
ELISA	enzyme-linked immunosorbent assay	SN	serum neutralization
EM	electron microscopy	SPAT	serum plate agglutination test
FA	fluorescent antibody	TCT	tissue culture test
GC	gas chromatography	TLC	thin layer chromatography
HA	hemagglutination	TLC/BA	thin layer chromatography/ bioautography
HI	hemagglutination inhibition	STAT	serum tube agglutination test
HPLC	high-performance liquid chromatography	VMA	vaginal mucus agglutination
IFA	indirect fluorescent antibody		

## **Address list**

### **Food Production and Inspection Branch**

#### **HEADQUARTERS**

Sir John Carling Building  
930 Carling Avenue  
**Ottawa, Ontario**  
K1A 0C5

and

Halldon House  
2255 Carling Avenue  
**Ottawa, Ontario**  
K1A 0Y9

#### **Assistant Deputy Minister**

Dr. J.B. Morrissey  
Sir John Carling Building  
Telephone: (613) 992-2114  
Telex: CANAGRIC OTT 053 3282  
FAX: (613) 992-5040

#### **Senior Advisor to the Assistant Deputy Minister**

Dr. I.R. Reid  
Halldon House  
Telephone: (613) 995-5433  
Telex: CANAGRIC OTT 053 3846  
FAX: (613) 993-4334

#### **Chief Veterinary Officer and Veterinary Director General Health of Animals Directorate**

Dr. N.G. Willis  
Sir John Carling Building  
Telephone: (613) 992-2114  
Telex: CANAGRIC OTT 053-3978  
FAX: (613) 952-0677

#### **Animal Health Division**

Halldon House  
Telephone: (613) 995-5433  
Telex: CANAGRIC OTT 053-3846  
FAX: (613) 993-4336

#### **Health of Animals Laboratory Division**

Halldon House  
Telephone: (613) 995-5433  
Telex: CANAGRIC OTT 053 3846  
FAX: (613) 993-4334

### **Inspector General Operations Directorate**

Dr. G.E. Dittberner  
Sir John Carling Building  
Telephone: (613) 992-2114  
Telex: CANAGRIC OTT 053-3282  
FAX: (613) 995-8464

### **Health of Animals Laboratories**

#### **Atlantic Region**

P.O. Box 1410  
4 College Street  
**Sackville, New Brunswick**  
E0A 3C0  
Telephone: (506) 536-0135

#### **Ontario Region**

110 Stone Road West  
**Guelph, Ontario**  
N1G 3W4  
Telephone: (519) 822-3300  
FAX: (519) 822-2280  
FAX: (506) 536-1801

#### **National Capital Region**

##### **Animal Diseases Research Institute**

P.O. Box 11300, Station H  
3851 Fallowfield Road  
**Nepean, Ontario**  
K2H 8P9  
Telephone: (613) 998-9320  
Telex: CANAGRIC OTT 053-4339  
FAX: (613) 952-8072

and

#### **Virology Section**

100 Gamelin Boulevard  
**Hull, Quebec**  
J8Y 1V9  
Telephone: (819) 997-3303

and

#### **Grosse-Île Experimental Station Montmagny County, Quebec**

G5V 3S2  
Telephone: (418) 248-2770



**Quebec Region**

3400 Casavant Blvd. West  
**St-Hyacinthe, Quebec**  
J2S 8E3

Telephone: (514) 773-7730  
FAX: (514) 773-7786

**Manitoba and Saskatchewan Regions**

116 Veterinary Road  
**Saskatoon, Saskatchewan**  
S7N 2R3

Telephone: (306) 975-4071  
Telex: CANAGRIC 074-2471  
FAX: (306) 975-5711

**Alberta and British Columbia Regions****Animal Diseases Research Institute**

P.O. Box 640  
FAX: (819) 953-6399

**Lethbridge, Alberta**

T1J 3Z4  
Telephone: (403) 381-8182  
FAX: (403) 381-1202

**Operations Directorate  
Regional Offices****Atlantic Region**

4th Floor, 30 Highfield Street  
P.O. Box 6088

**Moncton, New Brunswick**

E1C 8R2  
Telephone: (506) 857-7652  
FAX: (506) 857-6557

Mr. R. Greene, Director General  
Dr. W.R. Anderson, Regional  
Director, Animal Health

**Quebec Region**

Guy Favreau Complex  
Suite 1002(V), E. Tower  
200 Rene Levesque Blvd. West  
**Montreal, Quebec**

Telephone: (514) 285-8888  
Telex: 05-828660

FAX: (514) 283-3143

Dr. G. Meilleur, Director General,  
Dr. P. Brisson, Regional Director Animal  
Health

**Ontario Region**

Suite 1210, 4900 Yonge Street  
**Willowdale, Ontario**  
M2N 6G7

Telephone: (416) 226-9262  
Telex: 06-986116 • FAX: (416) 224-4388  
Mr. Larry Hillier, Director General  
Dr. T. Wilson, Regional Director,  
Animal Health

**Manitoba Region**

269 Main Street  
613 Federal Building

**Winnipeg, Manitoba**

R3C 1B2  
Telephone: (204) 949-2200  
Telex: 075-57451 • FAX: (204) 983-5926  
Dr. A. van der Meulen,  
Director General (949-2204)  
Dr. V.J. Kjernisted, Regional Director  
Animal Health (942-2203)

**Saskatchewan Region**

330, 2100 Broad Street  
P.O. Box 8070

**Regina, Saskatchewan**

S4P 4E5  
Telephone: (306) 780-5210  
FAX: (306) 780-5177

Mr. P. Amundson, Director General  
Dr. A. Choquer, Regional Director,  
Animal Health

**Alberta Region**

Harry Hays Building  
220-4th Avenue S.E.,  
Room 750 Station M, Bag 2998

**Calgary, Alberta**

T2P 3C3  
Telephone: (403) 293-4301  
FAX: (403) 292-6132  
Dr. L.K. Anderson, Regional  
Veterinary Director (292-5818)  
Dr. Z. Petran, Regional  
Veterinarian, Animal Health (292-5823)

**British Columbia Region**

Suite 203, 620 Royal Avenue  
**New Westminster, British Columbia**  
V3L 5A8

Telephone: (604) 666-8900  
FAX: (604) 666-6130  
Mr. A. Oliver, Director General  
Dr. M. Martin, Regional Director,  
Animal Health







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