THE INSTITUTE REPORT

September, 1954 Vol. 4, No. 1

All persons from whom contributions have been received during the past year are listed under "acknowledgments". For this select group the Institute Report is prepared since their contributions help make possible our continuous long-range research.

With this Report to our sponsors, our fifth year of work begins. We hope that from the brief summary of studies thus far our long-range objectives can be seen more clearly, and the manner by which seemingly isolated findings fit eventually, just as do individual pieces of a puzzle, into a concise picture. In this picture of infectious disease, large patterns are formed by equally large problems in epizootics, epidemiology, microbiology and immunology, which must be combined by related analytical studies; individual portions are made by each separate finding, in each disease, in each species, which may be isolation of a specific organism, serological studies to identify it, laboratory techniques for growing it, or perhaps causing it to lose pathogenicity and serve as vaccine material.

Nature tends to repeat her patterns, including those of disease, spread of disease, and control of disease. The importance of correlated studies with many diseases and many species, not just in the laboratory, but as they occur first in nature, can be seen. We hope that our staff of workers, trained specifically in the veterinary sciences, may be able to observe and understand some of the little known reasons why each separate creature has its own infinite variations in susceptibility and resistance to each separate disease.

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Manuel Moro, Jr., D.V.M., M.S., Graduate Assistant

1Captain Mitchell was released from A.U.S. March, 1954, and has returned to his home.
2Dr. Sheffy, Assistant Professor of Animal Husbandry, is cooperating in certain nutritional phases pertaining to viruses.
3Dr. Celiker completed his assignment February, 1954, and has returned to Turkey where he is now Chief of the Bacteriology Laboratory, Pendik Institute, Istanbul.
4Dr. Gillespie, on sabbatic leave at Staats Veeartsenjkundig, Onderzoekingsinstituut, Amsterdam, The Netherlands, returned September, 1954.
5Dr. Moro completed work for M.S. degree February, 1954, and has returned to the Universidad Mayor Nacional de San Marcos, Facultad de Veterinaria, Lima, Peru, and is now Head of the Department of Bacteriology and Virology.
A Message from the Director

To Supporters of the Veterinary Virus Research Institute at Cornell:

Previous reports have dealt with developmental aspects, especially construction of buildings, the establishment of animal colonies, procurement of staff and coordination into an effective working unit. This Annual Report contains information from the laboratories, summarizing some of the more significant results of our work since we began operating as a research unit in 1950. I hope you are pleased with the progress thus far.

In reporting the results of our research, it is not possible to include all the detailed analytical work furnishing the basis for each finding. Indeed, in the brief space allotted to this report all findings are not included—these are found in the scientific publications whose titles are listed later. All findings thought to be of interest to you are discussed but, should you like further information, please let us know.

The Institute consists of two divisions: the Cornell Research Laboratory for Diseases of Dogs dedicated to research for dogs and the General Laboratory which studies diseases of farm animals. Support of research for farm animals is provided by the State of New York whereas research funds for the benefit of dogs must be donated each year in order to continue work the following year. This is being done by individuals, veterinarians, industrial companies, and dog clubs. In the beginning, the thought of undertaking the construction and operation of a permanent research center for dogs was a novel idea and a venture of doubtful outcome. The generosity, interest and cooperation shown by all of you have removed any doubt. With your continued interest, the future looks bright indeed.

I am most grateful for the support each of you has given and especially for the interest you have shown in our work.

Sincerely,

James A. Baker
When work at the Virus Institute began, many problems were considered. All could not be investigated immediately. Final choice was based on urgency of need for information, and availability of suitable space and equipment. Individual reports are given of these specific problems.

CORNELL RESEARCH LABORATORY FOR DISEASES OF DOGS

Distemper. Although the word distemper can mean any disease, to dog owners it means "the disease." For this reason, it was the first one selected for study and our primary aim became an analysis of distemper. We wanted to find whether more than one disease agent was involved or whether the many forms of illness attributed to distemper could be caused by a single virus.

Isolation and comparative studies of many strains of distemper virus have shown that only a single type is involved. We have found that a dog immune to one strain was immune to all others from many parts of the country, including egg-cultivated strains, those from generalized distemper, or from dogs with the nervous form. Distemper virus, therefore, appears to be the primary cause of this disease, but was found to act alone or in combination with either bacteria or another virus. On the basis of work thus far, the following clinical forms of distemper are tabulated and cause given.

<table>
<thead>
<tr>
<th>Form of Illness</th>
<th>Cause</th>
<th>Features of Illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accompanied by diarrhea</td>
<td>No additional organisms found</td>
<td>Watery fetid stools that may persist. Usually recover.</td>
</tr>
<tr>
<td>Nervous</td>
<td>Distemper virus alone</td>
<td>Epileptiform convulsions or myoclonia. Usually die.</td>
</tr>
<tr>
<td>Accompanied by pneumonia</td>
<td>Distemper virus plus <em>Brucella bronchiseptica</em>, PPL organisms or misc. bacteria such as streptococci, etc.</td>
<td>Cough, distress in breathing. Recovery doubtful.</td>
</tr>
<tr>
<td>With infectious canine hepatitis</td>
<td>Distemper virus plus infectious canine hepatitis virus</td>
<td>Same signs as generalized form but more severe. Usually die.</td>
</tr>
</tbody>
</table>
This table shows that distemper virus alone can cause disease; its typical pattern may be altered if other infectious organisms are present also; the nature of this additional complication depends upon the specific agent involved; we have some evidence that many types of bacteria are found at all times in normal bodies but that these bacteria alone cannot cause illness until after the body's defenses are first attacked and altered in some way by a more powerful agent, such as the virus of distemper. Complications, especially pneumonia, from these secondary invaders may be serious. If caused by *Bacillus bronchisepticus*, the pneumonia often can be treated successfully with antibiotics. An additional bacterial invader may prove of unexpected importance in the pneumonia picture, however, and, like the viruses, it does not respond readily to treatment with any drugs yet known. Isolated in our laboratories from the lungs of dogs with pneumonia, it proved to be a new member of the pleuropneumonia-like organisms (PPLO), a group of minute microbes which seem an intermediate form, between bacteria and viruses. We found that PPLO's isolated from normal lungs did not cause pneumonia in mice, but PPLO's from a dog with distemper did cause pneumonia in mice.

*Photograph of lung of a dog that died of distemper. Characteristic intracytoplasmic inclusion bodies in bronchial epithelium are indicated by arrow.*
Brain from a dog unaffected by distemper.

Brain from a dog with convulsions. The spaces represent nerve tissue destroyed by distemper virus.
Lung from a dog unaffected by distemper.

Lung from a dog that died from pneumonia. Contrast this with photograph of normal lung; note air spaces filled with cells. These cells are large monocytes of the type associated with PPL organisms.
For years virologists have studied "interference phenomenon", in which the presence of one virus disease automatically excludes another virus. Conversely, some laboratories have induced duplicate infection in mice. We have found dogs in which distemper virus was preceded, or accompanied, or followed by the entirely different virus of infectious canine hepatitis. Here again distemper seems the agent which can alter body defenses most seriously. Animals which had infectious canine hepatitis first were not as ill as those which had distemper first. In dual infection, with both viruses at once, incubation period was cut in half and disease was severe.

In distemper we have found that apparent recovery and convalescence can be complicated by ability of infectious organisms to remain alive. After apparent recovery, and with specific antibodies present in high titer in the blood, we have found that distemper virus can remain latent in the brain for over 6 weeks at least, before suddenly erupting into a condition called "the nervous form of distemper," characterized by epileptiform convulsions, usually followed by death.
In addition to a clinical picture which varies with the disease complications, many individual variations are found in reactions to distemper; some dogs seem to show no visible signs of disease at any time; some die. Similar degrees, ranging from mild to fatal, are found in nearly every infectious disease, and have been studied for years by specialists in epidemiology throughout the world.

**Infectious Canine Hepatitis.** This disease as seen in dogs was reported by Rubarth in Sweden in 1946. He called it "hepatitis" because a severely damaged liver was found on autopsy of animals that died from the acute form.

Feeling that this disease might prove important in this country, too, it was one of the first chosen for study. We hoped to find its exact effect on the host, how it spread from dog to dog, and what, if anything, could be done to prevent it.

Signs of illness were studied, the pattern of infection established, and comparative studies with distemper virus started, as described under Distemper. Several vital facts about the nature of this disease were recognized. (1) The virus, unlike distemper, is not airborne. (2) This virus does not seem to alter the body in a way that allows the bacterial complications found with distemper. (3) This virus can remain alive, localized in the kidney, for nearly a year after apparent recovery from disease and spread new infection during all of this time through the urine. (4) This can occur also in dogs which had such mild original infection that no signs of disease were ever seen; their urine is just as infectious as that of a dog with severe disease originally. (5) Since the virus is so widespread nearly all dogs are exposed before they become very old; like distemper, it is, therefore, considered a disease of young dogs, and, like distemper, the mortality in young animals is high. (6) We found a mortality rate of 10% in all dogs, and, as did Rubarth, found severe liver damage in this 10% with acute initial disease. (7) In the other 90% of dogs which had apparently recovered we later found nephritis and kidney damage from persistent virus. (8) Considering the unexpected severity of this disease, we felt that a preventive vaccine was imperative.

Knowing that any killed-vaccine can give only temporary immunity, we developed and later perfected a live-virus vaccine to help control this disease. We combined it successfully with distemper virus so that one injection of this dual, live-virus vaccine could give permanent immunity to both of these diseases, if properly used. This product has been field tested extensively and some veterinarians are so enthusiastic that they state they would hate to have to stay in practice without it. It is not yet on the market. We do not, of course, manufacture or sell vaccines.
**Leptospirosis.** This disease has been recognized as a serious illness in dogs because of the effects on general health from kidney injury. Limited surveys thus far show an incidence of about 10%. Susceptible dogs taken from their home kennel and exposed to others, easily become infected. Dogs that travel to shows, field trials or on hunting trips are always in danger of being exposed to this disease. We have, therefore, developed a vaccine against *Leptospira canicola* that shows excellent promise of giving temporary but necessary protection to dogs that might become exposed. A successful live vaccine can be made in the laboratory but the leptospiras die if shaken violently in shipment; therefore, only a killed vaccine can be shipped; killed vaccines give only temporary immunity, which must be reinforced by booster injections.

Although the dog can be affected by several different species of leptospiras, the one known as *L. canicola* is diagnosed most often, now that methods for its study and identification are becoming more widely known. Many facts about all of the leptospiras remain to be learned. As a direct cause of death they may not seem important, but as a cause of systemic injury, such as nephritis, they are just beginning to be understood. Ultimate effects of such kidney damage may be as serious, although not as immediate, as those from more virulent organisms.

*Kidney from dog that died from infectious canine hepatitis. This dog developed a focal interstitial nephritis.*
Kidney from a dog with no abnormal pathology.

Kidney from a dog that died from *Leptospira canicola*. Arrow points to interstitial nephritis caused by this organism.
Hog Cholera. Losses from hog cholera amount to $50,000,000.00 some years. Many vaccines have been tried. None has been perfect.

We have developed a new type of modified hog cholera vaccine, passaged in rabbits. In the laboratory it worked perfectly and immunized successfully in every case. Used in the field it seemed to work most of the time, but not always. Studies were made to determine the reason for these failures. When 6 weeks old, the usual age for vaccination of swine, pigs from our disease free herd were given modified virus, followed by tests for the presence of virus in the blood. Virus was found circulating in the blood stream of these young pigs for weeks, and then for months, afterwards. Later we found that if the same amounts of the same virus were given to pigs after they reached 3 months of age, the virus immunized successfully and did not persist. Then we found that if the same amounts of the same virus were given to pigs 6 weeks old from sows immune to hog cholera, it also did not persist and immunized successfully. It became apparent that 2 factors, (1) age and (2) protection against hog cholera provided by the mother determined ability of virus to persist. Pigs in which virus persisted did not grow as well as those in which virus did not persist. Also pigs with persistent virus usually died sometime between 4 and 6 months of age in a manner similar to vaccination failures.

![Chart showing the comparative effects on pigs of virulent hog cholera virus and the same virus after transfer in rabbits. Virulent virus produced a prolonged fever and killed the pig whereas rabbit passed virus produced a slight elevation of temperature as the only sign of infection. Such modified virus makes a good vaccine.](image-url)
This work with hog cholera not only explains hog cholera vaccination failures but broadens our understanding of ability of virus to cause economic loss. Some of the stunting commonly seen in pigs is undoubtedly caused by persistence of hog cholera virus. Other viruses also cause stunting, as was found for transmissible gastroenteritis. Since virus does not persist if pigs get adequate protection from immunized mothers, it is of utmost importance to have highly immune breeding stock. The phenomenon of pigs dying between 4 and 6 months of age from virus acquired before 3 months of age may prove to have general application to other species of animals and other viruses, as for example, the convulsions seen in dogs after distemper.

These two pigs are littermates. The larger pig was given colostrum; the smaller pig was not given colostrum. When 6 weeks of age both pigs were still the same size. At this time both were exposed to hog cholera virus. The pig that had been given colostrum continued to eat normally and showed no signs of illness. No virus was found in its blood; it is immune to hog cholera. The smaller pig, which had no colostrum after it was born, had live hog cholera virus in its blood stream but did not die from the disease. It did not grow normally and remains stunted in size.

Transmissible Gastroenteritis of Swine. We were asked to investigate a disease characterized by diarrhea and high mortality. Our studies soon showed that the disease was transmissible gastroenteritis. Strains of virus
were isolated and studies made of their effects on pigs of various ages, concentration of virus in various organs, and maintenance and spread of virus. We found that the natural route of infection was by way of the mouth. Virus was recovered from feces of infected pigs for intervals of time extending from 2 to 8 weeks after inoculation. Since virus was recovered from the lungs and kidneys of infected pigs for only a short time after inoculation, from the blood during the period of clinical illness and in no instance from the urine, it was concluded that persistence of virus in the intestines accounted for spread. Infected pigs that showed persistence of virus in the intestines were stunted and did not gain weight as well as pigs that did not harbor virus.

When considered with stunting produced by hog cholera virus and, perhaps others, the importance of controlling such viruses to increase swine production can be seen.

Chart shows stunting of pigs caused by persistence of transmissible gastroenteritis virus. Uninfected pigs or pigs in which virus did not persist grew almost twice as fast.
Leptospirosis in Swine. To gain information about the incidence of this disease, a serological survey was made of 285 serums collected in large packing houses; 22% were found positive. *L. pomona* infection is characterized by interstitial nephritis. Since many apparently normal swine coming to market have similar lesions, an attempt was made to correlate positive serological findings for *L. pomona* with the presence of such lesions in swine slaughtered at abattoirs. The results showed that a significant number of swine with lesions also had other evidence of leptospirosis.

*L. pomona* exists in both cattle and swine; this suggested the possibility that these animals might serve as reservoirs of infection for each other. Leptospirosis was found to spread readily from infected pigs to susceptible ones, and from infected pigs to calves. This ease of transmission is understandable when consideration is given to the tremendous number of organisms eliminated in the urine of carrier pigs. In areas where swine and cattle exist on the same premises, therefore, it seems logical, in outbreaks of bovine leptospirosis, to suspect swine as a possible source of infection, although, of course, the disease is entirely capable of spreading through a herd without the mediation of swine.

This brings up the important question of the natural host for *L. pomona*. Although rodents are the hosts for most known species of leptospiiras, evidence to date has not indicated that this is so for *L. pomona*.

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**EFFECTS OF L. pomona INFECTION ON CATTLE AND SWINE**

<table>
<thead>
<tr>
<th>DAYS</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>120</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEPTOSPIRURIA</td>
<td>SWINE</td>
<td>CATTLE</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>LEPTOSPIREMIA</td>
<td>CATTLE</td>
<td>SWINE</td>
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<table>
<thead>
<tr>
<th>INAPPROPRIATE INFECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELEVATED TEMPERATURE - FREQUENT</td>
</tr>
<tr>
<td>DECREASED MILK PRODUCTION - USUAL</td>
</tr>
<tr>
<td>THICKENED MILK - USUAL</td>
</tr>
<tr>
<td>BLOOD IN MILK - RARE</td>
</tr>
<tr>
<td>HEMOGLOBINURIA - RARE</td>
</tr>
<tr>
<td>ICTERUS - RARE</td>
</tr>
<tr>
<td>ABORTIONS - VARIABLE</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>DEATH</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN YOUNG ANIMALS - RARE</td>
</tr>
</tbody>
</table>

* Only symptom shown

Chart showing clinical features of leptospirosis in cattle and in swine.
Infection by this organism has been reported in other animals, including the dog, rat, horse, and man; either swine or cattle appear to be the probable host for this organism. Unlike swine, the infection in cattle, although important in the United States and Australia, does not seem to be spread throughout the world—yet. If growth and multiplication of leptospira in the host are criteria, the organism is better adapted to pigs, as evidenced by the excessively large numbers excreted in their urine as well as by the duration of the carrier state. Cattle have not been shown to harbor leptospira for longer than 3 months; in swine they may remain alive for over a year; it appears that swine may be the natural hosts for *L. pomona*.

**Leptospirosis in Cattle.** Following our isolation of a leptospira from cattle in the United States, and recognition that this organism was responsible for serious economic losses in dairy cattle, efforts were made to reduce these losses. In general, information needed for a control program was: (1) an accurate means of diagnosis, (2) a protective vaccine for exposed animals and (3) means to eradicate reservoirs of infection.

For diagnosis, a practical serological test was devised. In order to protect exposed animals, an effective vaccine was made. This vaccine, made from a strain of *L. pomona* propagated only in eggs, is now available from commercial sources, and is proving effective under field conditions. It cannot give permanent immunity, but provides temporary protection, which must be maintained by booster injections.

While vaccination reduces loss, it holds no promise of eradication. Any consideration of eradication must include elimination of all reservoirs of infection. Original studies on cattle indicated that other animals maintained this organism, which later proved true, as described for swine. Antibiotics have been tested and seem promising as eliminators of carrier conditions in swine.

**Miyagawanella bovis.** From portions of the intestine and from feces of apparently normal calves, a virus that produces elementary bodies was isolated. Comparison of serological, pathogenic, and other properties indicated that this virus from calves is a new member of the psittacosis-lymphogranuloma group and in keeping with classification practices it is provisionally named *Miyagawanella bovis*.

*M. bovis* produced an infection of the intestinal tract without clinical manifestations in calves 4 to 6 months of age. This virus remained alive for months, and was found to be eliminated in the feces. Studies of *M. bovis* have been extended recently to younger calves. If young calves are not allowed to have colostrum, *M. bovis* produces serious infection
Miyagawanella bovis virus particles (1) as they appear under the ordinary microscope, (2) with electron microscope and (3) with electron microscope but after particles have been shadowed with uranium.

characterized by diarrhea and death, while calves that receive colostrum remain essentially free from signs of illness. These preliminary studies suggest that *M. bovis* may be important in fatal diarrheas of newborn calves; and re-emphasize the importance of colostrum, which many farmers throw away.

**Virus Diarrhea.** From a cow in New York State and from another in Maine, 2 strains of virus diarrhea (VD) virus were obtained that proved antigenically related in cross immunity tests.

Transfer of virus to rabbits was accomplished. After serial passages in rabbits, the virus became modified and in the 75th transfer produced a slight decrease in the leukocytes and a temperature elevation that lasted for 1 day only. This modified virus immunized against fully virulent virus and should prove useful for vaccine purposes.
Pneumonitis in Cats. Feline pneumonitis is a highly infectious, debilitating disease of unusually long duration, characterized by sneezing and coughing, accompanied by a mucopurulent discharge from the eyes and nose. The organism was isolated and found to produce elementary bodies. It has now been classified with the psittacosis-lymphogranuloma group because of its mode of reproduction and antigenic structure, and is called Miyagawanella felis. Cats, while acutely affected and for long periods afterwards harbor virus in the eyes and respiratory tract. In sneezing, droplets are sprayed into the air, and susceptible cats become infected easily through inhalation.

Recovery from natural disease does not lead to complete immunity, since relapses occur. The virus multiplies in superficial tissues, and whenever the antibody level falls, any disturbance of equilibrium between host cell and parasite results in active disease, although milder in character than the initial attack. Inoculation parenterally with active virus, while not completely immunizing against intranasal instillation of virus, reduces severity of signs of illness; this may offer a means of vaccination.
Cat showing typical feline pneumonitis, featured by mucopurulent discharge from eyes and nose. In addition, this cat sneezed and coughed. The inset shows an electron microscope photograph of the causative organism, a virus called Miyagawanella felis.

*Q Fever In Cats.* Infection with *Coxiella burnetii* has been studied in cats. No signs of serious illness were seen in infected cats, except lack of appetite, lethargy and elevated temperature. The organism was shown to be present in the blood of some infected cats for at least 1 month and in the urine for at least 2 months. Complement-fixing antibodies and agglutinins were demonstrated in sera from infected cats for 1 to 2 months after infection.
PUBLICATIONS FROM THE CORNELL RESEARCH LABORATORY FOR DISEASES OF DOGS

RECENT


(3) Encephalitis in Dogs Produced by Distemper Virus. Gillespie, J. H., Submitted for publication.


PREVIOUS


PUBLICATIONS FROM THE GENERAL LABORATORY RECENT


PREVIOUS


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