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CURRENT RESEARCH AND TRAINING ACTIVITIES OF THE PAN AMERICAN ZOONOSES CENTER

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CURRENT RESEARCH AND TRAINING ACTIVITIES OF THE PAN AMERICAN ZOONOSES CENTER*

Research accomplishments during the past year (June 1962 - May 1963) and future trends in the Pan American Zoonoses Center's research program are presented here in summary form. Mention is also made of training programs, as well as of technical services to the countries, insofar as those activities are carried out in support of research in other institutions.

Epizootiology and Epidemiology

The findings of the Echinococcus tapeworm in numerous specimens of the pampas gray fox from Buenos Aires province provided evidence that these wild carnivores are involved in the spread of hydatidosis(1). In order to determine whether eggs from the fox tapeworms are infective, and also as an aid in determining the species of the parasite, the material has been fed to a variety of laboratory rodents and domestic animals. Although this phase of the study is quite incomplete, it is now known that 2 of 5 cotton rats developed hepatic cysts from the fox material. Very recently, a weasel, trapped near Azul, was found to harbor Echinococcus tapeworms, thus making a new addition to the list of definitive hosts for that parasite.

The discovery of the natural occurrence of Leptospira pomona infection in the pampas cavy, Cavia pamparum(2), led to studies of the experimental disease in cage-bred animals of that species. One of the

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animals was found to be still shedding the organism in the urine at the 89th day after inoculation(3). Studies of the infection in the natural cavy population, and in the associated domestic livestock, have also been continued, and have been reported in part(4).

Serological evidence of bovine infection with leptospires of the L. abdomadis serogroup have been encountered frequently by investigators in many countries. A study, just completed by a graduate student at the Center, showed that more than half of serum samples from a large number of cattle from 38 ranches, widely scattered in the county of Azul, reacted positively for leptospires of that serogroup. The full significance of these findings is not clear, since the bovine infection appears to be almost invariably sublinical. It is hypothesized that a wildlife reservoir may serve as the source of infection for domestic animals, and perhaps man. Work now underway may shed light on this possibility.

Studies on Leptospira pomona infection in domestic cats have been completed(5), and a report on the isolation of Leptospira paidjan from opossums is being prepared(6).

Findings are in press on the investigation of a series of cases of infant diarrhea to determine the frequency of salmonellosis(7). With the collaboration of scientists in Argentina, Brazil, Chile, Colombia, Peru and Uruguay, studies on human and bovine Q fever are being carried out in those countries, using the capillary tube agglutination test. Although the work continues, 6,507 samples of bovine milk and bovine and human serum have been tested thus far.

Research on the natural history of leishmaniasis in Paraguay was begun recently, with the participation of scientists from that country.

A graduate student is carrying out serological tests for virus encephalitis, using paired serums collected from horses at selected ranches near Azul.

The pulmonary fungus Emmonsia was found in a variety of wild mammals, including armadillos, viscachas, skunks, patagonian hares, and wild cavies(8). The Zoological Collection continued to grow, with the addition of numerous specimens of wild vertebrates and arthropods, preserved, identified and catalogued for reference purposes. A range extension of a species of small cavy is being published(9).

Arrangements are being made for a short term consultant to spend two months at the Center, later this year, to assist in planning a program for typing mycobacteria isolated from man and animals and their environment. Plans for a project for investigating arbovirus reservoirs in southern South America, with special emphasis on migratory birds, have been completed and funds for the work are bing sought. It is hoped that the project may start operations late this year or early in 1964. Consideration is currently being given to plans for studying the influence of soil conditions on the survival of Bacillus anthracis spores, in cooperation with the Communicable Disease Center, USPHS, Atlanta.

Control Procedures

The anti-echinococcal treatment of dogs en masse is of fundamental importance for the control of hydatid disease. Arecoline hydrobromide

has been widely used for a number of years. However, it has certain definite disadvantages, the more important being that it fails to remove many of the E. granulosus from some dogs, the animals must be fasted prior to treatment, and the drug may cause vomiting so that its action against the parasite is lost. Several drugs and chemical compounds have been tested, using hundreds of dogs, in the search for an improved anthelmintic treatment for the elimination of the tapeworm - the causative agent of hydatidosis in man and animals. Two naphthalene derivatives have given very promising results and are being subjected to further series of tests. This work is partially supported by a grant from the NIH/USPHS.

The protection afforded by a product introduced for immunizing sheep against hydatidosis was studied under controlled conditions, using a flock of 118 lambs at the Center's Farm Annex. The results, supported by histopathologic examinations, provide no evidence that the vaccine conferred resistance to hydatidosis(10). Trials of an experimental method for immunizing dogs against echinococcosis are underway, in collaboration with the Hydatid Research Unit of the New Zealand Medical Research Council.

Results of the field study to determine the efficiency and duratio of protection provided by modified-live-virus vaccine in bat-transmitted bovine rabies are being evaluated, as are also results of a similar project to determine the value of L. pomona bacterin against bovine leptospirosis under South American conditions. Studies on the Sterne attenuated anthrax vaccine are continuing, with the assistance of a graduate student, the objective being to improve methods for its producti and potency testing.

A study was completed on the possible anamnestic effect of foot-and-mouth disease (FMD) vaccination on the brucellosis sero-agglutination titer of calves previously vaccinated with Strain-19 vaccine [11]. Three different FMD vaccines, each with a different coadjuvant combination, were used. Preliminary analysis of serological testing, carried out in many samples from each animal, indicates that vaccination for FMD had very little, if any, effect on the agglutination titer for brucellosis. These findings are important in countries where FMD vaccination and brucellosis testing programs are carried out simultaneously.

Substantial progress was made in the project for reducing wild fox population through an artificial interference in the reproduction rate. Field study areas were selected, and census calculations were made of the fox populations. Work was carried out on application of the lens technique for determining the age of pampas and patagonian gray foxes [12]. Various gametocides have been tested in small laboratory animals, and are being further tested in dogs and caged foxes. The project is now near the stage of distribution of a gametocide, in baits, in one of the study areas in order to measure the effect on the population structure and density. Unfortunately, this activity has been greatly reduced recently for lack of funds to continue the services of the principal investigator. The project will be continued, but only to the extent that prior commitments and available time of the Center's staff will permit.

Another interesting project that awaits funds is the evaluation of a vaccine that has been recommended for the control of swine brucellosis.

Diagnosis

Three different antigens were compared for sensitivity and specificity for the intradermal (Casoni) test for hydatid disease, and a standardized method for measuring the size of the reaction was described[13]. A modified slide latex screening test for the serological diagnosis of hydatidosis was developed in cooperation with the Communicable Disease Center, USPHS, Atlanta[14].

Samples of 43 plate and tube antigens (from 10 countries), for the sero-diagnosis of brucellosis in man and animals, were compared using tests for cell volume, pH, purity, sterility and sensitivity[15]. A graduate student is studying the reliability of a rapid whole-blood test that has been recommended for bovine brucellosis. The test would be advantageous as a screening process in eradication programs, providing it gives results consistent with those of the internationally-recognized sero-agglutination tests.

A large number of serum samples have been collected, from known infected and non-infected dogs, for use in studying serological methods for diagnosing canine echinococcosis. The project will be started as soon as available staff can give attention to it.

Human Zoonotic Infections

Studies were continued on rabies vaccine for human use. In one of them, the antibody response in persons who have received killed duck-embryo vaccine is measured, and the second involves comparative potency testing in animals of liquid and lyophilized phenolkilled vaccine. Good progress is being made in a project for testing as many as possible of the rabies vaccines prepared for human use in the Americas. All of the

15 vaccines, received to date from 7 countries, are being tested for potency and innocuity. Vaccines from several other countries are expected to be included in this study.

Socio-Economic Aspects

A review of the anthrax situation in animals and man in the Americas has been completed (16). A project designed to measure the economic loss of hydatidosis in sheep is pending availability of funds.

Research Training and Support Services

The needs and opportunities for research in connection with the zoonoses are vast, and the Center itself can be expected to cover only a very small part of them. Institutions, both public and private, in all countries, must continue and intensify their research work on these diseases, and it is considered to be an important responsibility of the Center to stimulate, support and aid such work. This is done, to the extent that available staff and funds will permit, by providing training, consultation, technical information, specific biological materials, and a testing service for certain antigens and vaccines.

With respect to training, graduate students are accepted for 12-month periods for special work on one or more phases of zoonoses problems; the program for each trainee is developed to suit his individual requirements. Twelve graduate students, each from a different country, have completed (or are completing) programs of this type. Numerous other trainees and visiting scientists have been received for shorter periods. Group training courses, of 2 or 3 weeks duration, have been held on specific aspects of such zoonoses as brucellosis and

leptospirosis. It is planned to repeat both of these courses during the coming months. Training activities at the Center can be expanded with an increase in staff and facilities.

Planning has reached an advanced stage on a proposed program of joint training between the Zoonoses Research Center at the University of Illinois and the Pan American Zoonoses Center in Azul. Under this program, candidates for M.S. or Ph.D. degrees would fulfill formal course requirements at Illinois, would then join the Pan American Zoonoses Center to carry out research on their chosen problems, following which they would be awarded their degree form the university. Financial support for this new joint training program has yet to be obtained.

A wide variety of biological materials is provided by the Center upon official request. The kind, volume and distribution of specific biologicals, during 1962, are shown in the accompanying table. It is noteworthy that the volume has increased in 1963, especially with regard to standard Brucella antigens. Several countries now depend upon the Center for their entire official requirements for these antigens. Recently upon request of its host country, the Center has begun the production and distribution of antigen for the intradermal (Casoni) test for hydatidosis, with a view to satisfying the need of that and other countries. There is wide interest in the Center undertaking the preparation and distribution of other diagnostic materials, e.g., standard tuberculin for bovine use.

The proposed Interamerican Serum Repository for Zoonotic Diseases to be established at the Center is considered to be a project with great potential for the support of future research. The application for a

grant to make this project possible is currently under consideration by NIH. Other important services that the Center might develop for the support of research in the Americas are projects for maintaining and distributing cell lines for tissue culture, as well as of different species, strains and bloodlines of laboratory animals.

References

(1) Blood, B.D.; Lelijveld, J.L. y Lord, R.D. Nota Preliminar sobre Equinococosis en el Zorro Gris Pampeano Dusicyon gymnocercus. Bol. Of. San. Panamer., 54: 127 (Feb.), 1963

(2) Blood, B.D.; Szyfres, B. and Moya, V. Natural Leptospira pomona Infection in the Pampas Cavy. Pub. Health Rep. (in press)

(3) Blood, B.D.; Moya, V. and Szyfres, B. Experimental Infection of the Pampas Cavy with Leptospira pomona (in preparation)

(4) Blood, B.D. Epizootiología y Profilaxis Regional de la Leptospirosis. Rev. Vet. Venezol., 13: 310-321 (Nov.),1962

(5) Blood, B.D. and Szyfres, B. Studies on Leptospira pomona infection in the domestic cat (in preparation)

(6) Szyfres, B. and Blood, B.D. Leptospira paidjan Isolated from Opossums in Argentina (to be published)

(7) Moya, V. y Cordeviola, J. Papel Etiológico de las Salmonellas en 207 casos de Diarreas Infantiles (to be published)

(8) Jellison, W.L. and Lord, R.D. The Pulmonary Fungus Emmonsia in Argentine Mammals (to be published)

(9) Lord, R.D. Range Extension of Galea musteloides littoralis (Thomas) in the Province of Buenos Aires. J. Mamm. (in press)

(10) Moya, V. and Blood, B.D. Ensayo de una Fracción de Líquido Hidatídico como Vacuna contra la Hidatidosis. (in press)

(11) Moya, V.; Blood, B.D. y Alvarez, N.O. El Efecto en Bovinos de Vacuna Anti-aftosa sobre el Título para Brucelosis.(to be published)

(12) Lord, R.D. Growth of the Lens of the Pampas Gray Fox (Dusicyon gymnocercus antiquus) and the Patagonian Gray Fox (Dusicyon griseus griseus). (to be published)

(13) Moya, V. y Santamarina, G. Estudio de la Reacción de Casoni en el Diagnóstico de la Hidatidosis. Bol. Chileno Parasit., 18: 2-5 (Enero-Marzo), 1963

(14) Szyfres, B and Kagan, I.G. A Modified Slide Latex Screening Test for Hydatid Disease. J. Parasit., 49: 69-72 (Feb.), 1963

(15) Moya, V.; Blood, B.D. and Szyfres, B. Standardization of the Diagnosis of Brucellosis in the Americas. II. Re-evaluation of the Sero-agglutination Test as Applied in Different Countries. (to be published)

(16) Moya, V. y Valdivieso, A. Estado Actual del Carbunclo en las Américas. Bol. Of. San. Panamer. (in press).

Biological Materials Provided by the
Pan American Zoonoses Center (CEPANZO), 1962

Description	Quantity	Country and No. of shipments
ANTHRAX		
B. anthracis, Sterne $34F_2$	1 ampule	Argentina (1)
BRUCELLOSIS		
Br. abortus, 1119-3	4 cultures	Argentina (3)
	1 culture	Ecuador (1)
	2 cultures	Brazil (1)
	1 culture	Uruguay (1)
	1 culture	Colombia (1)
Br. abortus, 19	4 cultures	Argentina (2)
	2 cultures	Brazil (1)
	1 culture	Uruguay (1)
Br. abortus, 544 (Weybridge)	1 culture	Argentina (1)
Br. ovis	8 cultures	Argentina (1)
Br. abortus, CEPANZO isolates	17 cultures	Poland (1)
Br. suis, CEPANZO isolates	1 culture	Poland (1)
Br. melitensis, CEPANZO isolates	16 cultures	Poland (1)
Br. ovis, CEPANZO isolates	8 cultures	Argentina (2)
Antigen, plate	9,714 ml.*	Argentina (19)
	30 ml.	Spain (1)
	30 ml.	Ecuador (1)
	30 ml.	Uruguay (1)
	240 ml.	Haiti (1)
	120 ml.	Mexico (1)
	30 ml.	Colombia (1)
	180 ml.	Panama (1)
	30 ml.	Venezuela (1)
Antigen, tube	60 ml.	Ecuador (1)
	120 ml.	Haiti (1)
	90 ml.	Mexico (1)
	30 ml.	Colombia (1)
	30 ml.	Venezuela (1)

*Includes 7,509 ml. for the Pilot Program of Brucellosis Control (BRU-4) and 510 ml. for the Dirección General de Sanidad Animal, Argentina.

Biological Materials Provided by the
Pan American Zoonoses Center (CEPANZO), 1962 (cont'd)

Description	Quantity	Country and No. of shipments
Antigen, ring test	120 ml.	El Salvador (1)
	840 ml.	Guatemala (3)
	190 ml.	Haiti (2)
	60 ml.	Mexico (1)
	30 ml.	Argentina (1)
	60 ml.	Brazil (1)
	30 ml.	Colombia (1)
	30 ml.	Venezuela (1)
Serum, bovine, positive	69 ml.	Argentina (2)
Serum, bovine, known titers	2 sets (20 tubes each)	Argentina (2)
	40 ml.	Venezuela (1)
Serum, bovine, negative	10 ml.	Venezuela (1)
Serum, ovine, positive (Br. ovis)	35 ml.	Argentina (1)
Monospecific serum, Br. abortus	2 ml.	Colombia (1)
Monospecific serum, Br. melitensis	2 ml.	Colombia (1)
HYDATIDOSIS		
Antigen, Casoni	32 ml.	Argentina (2)
	100 ml.	Ecuador (1)
	600 ml.	Colombia (1)
Liquid, cyst, human	10 ampules	U.S.A. (1)
Serum, human, positive	4 ampules	U.S.A. (1)
Cyst, ovine (preserved)	1 bottle	U.S.A. (1)
Adult teniae (preserved)	1 ampule	U.S.A. (1)
LEPTOSPIROSIS		
Collection of 12 serotypes	12 cultures	Brazil (1)
	12 cultures	Haiti (1)
	24 cultures	Mexico (2)
Strain S91 (L. pomona)	1 culture	Spain (1)
Serum Anti-L. pomona	2.5 ml.	Spain (1)

Biological Materials Provided by the
Pan American Zoonoses Center (CEPANZO), 1962 (cont'd)

Description	Quantity	Country and No. of shipments
Q FEVER		
Serum, human, positive	2 ml.	Argentina (1)
Milk, positive	6 ml.	Chile (1)
Antigen, CAT	10 ml.	Argentina (2)
	10 ml.	Brazil (1)
	2 ml.	Chile (1)
RABIES		
Virus, CVS-24	3 ampules	Brazil (3)
	3 ampules	Argentina (2)
	1 ampule	Venezuela (1)
Virus, PV-10	2 ampules	Argentina (2)
	1 ampule	Brazil (1)
	1 ampule	Ecuador (1)
Virus, Flury LEP	1 ampule	Argentina (1)
	1 ampule	Brazil (1)
Virus, Flury HEP	1 ampule	Venezuela (1)
	1 ampule	Argentina (1)
	3 ampules	Brazil (3)
TRICHINELLOSIS		
Larvae T. spiralis	- -	Argentina (1)