


Pan American Health Organization

PAHO/ACMR 16/12  
Original: English



SIXTEENTH MEETING OF THE  
PAHO ADVISORY COMMITTEE ON MEDICAL RESEARCH

Washington, D.C.  
11-15 July 1977

PAN AMERICAN ZOOSES CENTER  
SUMMARY OF RESEARCH ACTIVITIES IN PROGRESS

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PAN AMERICAN ZOOSES CENTER

SUMMARY OF RESEARCH ACTIVITIES IN PROGRESS\*

The Scientific Advisory Committee of the Director of the Organization for the Pan American Zoonoses Center met in Ramos Mejía, Buenos Aires, on November 1976.

The Group of Advisors reviewed the activities of the Center and the report of their recommendations was presented at the 10th Inter-American Meeting at the Ministerial Level for the Control of Foot-and-Mouth Disease and Zoonoses, held in Washington, D.C., during March 1977 (Document RICAZ-X/13).

The presentation given at this meeting will deal briefly with the main ongoing research activities carried out at the Center. An Appendix has been added at the end of this document to indicate who are the members of the Organization responsible for each of the various research aspects described in this document.

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\*Presented by Dr. Luis V. Meléndez, Director, Pan American Zoonoses Center, Buenos Aires, Argentina.

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## Rabies

Rabies continues to be a problem of significant public health and economic importance in the Americas.

In spite of the fact that good vaccines such as Suckling Mouse Brain (SMB) and Chick Embryo vaccines (CE) have been long available, the majority of countries in the Hemisphere have been unable to control this disease.

In fact, it has been repeatedly shown that the lack of vaccines has proved to be an obstacle in the effective development of these programs. The disease transmitted by vampire bats is the subject of continuing concern, particularly by agricultural and livestock authorities. If indeed, newer vampire bat control methods are being used, the vaccination of cattle remains, one of the most effective measures for prevention.

For a considerable number of years, CEPANZO has played a special role in research to improve and evaluate rabies vaccines for use in cattle. We have been able to show that SMB vaccine is capable of affording excellent protection against rabies in cattle (about a month after vaccination). The immunogenic capacity of SMB vaccine is even greater when the product is adsorbed with aluminum hydroxide.

It may be of interest to know of our recent work in the field of duration of immunity, using SMB vaccines with different adjuvants. A total of 2 groups each of 24 head of cattle were used in this determination. Each animal of the first group was vaccinated with 5 ml of SMB vaccine in aluminum hydroxide. Cattle in the second group were vaccinated subcutaneously with SMB and oil adjuvant. Serum samples were taken at routine periods thereafter. Two years post-vaccination, all animals were challenged using 85,000 LD<sub>50</sub>/0.03 ml mouse I.C. of rabies virus. A group of 8 non-vaccinated animals were used as controls. The results of the study are shown in the following figures.

In Figure I the specificity of the primary response is shown by the rapid elevation in titer occurring 30 days after challenge. It should be pointed out that the difference between the antibody levels produced by the two vaccines was statistically significant ( $p > 0.01$ ).

The challenge results are depicted in Table I. Both vaccines protected 96% of the vaccinated animals against a virus challenge which killed 63% of the unvaccinated controls. While there are no standard criteria for evaluating the efficacy of rabies vaccines in cattle, it is agreed, that a good vaccine should protect at least 80% of the animals exposed, and in the case of our studies, we were able to demonstrate a duration of protection for at least two years.

It is our conclusion, therefore, that a vaccine of high immunogenic power in cattle, such as SMB vaccine, can make a signal contribution to the control of this economically wasting disease.

Consistent with its responsibility of providing easier solutions to problems encountered in the field, CEPANZO is presently engaged in perfecting screening tests for serological studies used in epidemiological surveys for rabies. The Center, therefore, has been examining the usefulness of the counterimmunoelectrophoresis (CIF) test for rabies. This technique is simple, rapid and requires minimal amounts of antigen and serum. At this time, we present the results obtained when comparative serum neutralization and CIF studies were made on sera from previously vaccinated humans and animals. The sera of individuals who had not been previously vaccinated were included as controls.

We have been able to show that in 47 persons receiving pre or post-exposure treatment with SMB vaccine, the CIF test was as sensitive as the serum neutralization (SN) test (Table II).

Similar studies carried out using animal sera confirmed these findings (Table III).

Though SMB and CE vaccines are both useful and practical in the establishment of control programs, CEPANZO is aware that there are other methods which might offer a more useful and simpler means to produce rabies vaccine. It is for this reason that we have been involved in the possible application of tissue culture techniques already being used successfully for controlling Foot-and-Mouth Disease (FMD). It is well known that baby hamster kidney cells (BHK<sub>21</sub>) provides a suitable substrate for the multiplication of this viral agent. CEPANZO is now engaged in the utilization of these same cells to develop a rabies vaccine. The product is inactivated using binary ethylenimine, and the vaccine produced maintained its stability for at least one month at 37°C. This is an important characteristic to preserve the vaccine under field conditions.

The vaccine evokes an excellent serological response in vaccinated dogs. As can be seen in Table IV, our results are compared with those of Sikes et al. (J. Amer. Vet. Med. Ass. 159: 1491-1499, 1971).

The results obtained in challenge tests at varying intervals of time after vaccination are depicted in Table V. Other laboratory tests indicate that the vaccine is an excellent antigen for use in cattle.

We are hopeful that these encouraging laboratory results can be successfully applied to industrial production. This latter point can be sought particularly when experience is already present in several countries, using this methodology for the production of FMD vaccine in BHK cells.

### Brucellosis

Brucellosis is a common zoonosis in many countries of the Region. The Center has developed continuing monitoring and review of existing diagnostic tests for both the acute and chronic phases of the disease in

man and animals. Such tests as mercaptoethanol and card tests are simple to carry out and of value in identifying human chronic cases. However, they have limitations and we are studying how to overcome these.

In the field of animal disease the complement fixation test is the one of choice because of better sensitivity and specificity. We know however that countries encounter serious difficulties in carrying out this test.

The Center has studied the susceptibility of goats to infection by B. ovis and concluded that while these animals are indeed susceptible, the animal does not appear to be very important from the epidemiological standpoint. In addition, the disease has not yet been diagnosed in man.

The Center offers a reference typing service to the countries. Through this, we now know what species and subspecies of brucella are prevalent in 13 countries.

Studies proceed on the improvement of existing vaccines. We are looking at factors which help to maintain the antigenic capacity of the vaccines, and which aid in the survival of live vaccines.

We undertake to examine vaccine samples taken periodically from stock and from production in the countries of the Americas. Following studies carried out at the Center in the setting up of a laboratory animal model for testing vaccine potency, we have determined the conditions under which such tests may be carried out. We have studied challenge strains, and determined the limitations and advantages of each standard strain. New vaccines are submitted to potency tests in laboratory animals. Those which give satisfactory results are considered for evaluation in the species in which the vaccine will be used.

Of increasing interest to all agencies responsible for multifaceted animal control programs, is the prospect of simultaneous vaccination for a variety of zoonoses, including brucellosis. This is a complex immunological field and at this time we are looking into interference or synergistic effects encountered when simultaneous vaccination with strain 19 and FMD vaccine is carried out.

With regard to porcine brucellosis, a large variety of vaccines have been tested; we have not found one with sufficient antigenic capacity which could be recommended for extensive use. In spite of this, various trials have been carried out in guinea pigs, and those vaccines which failed have been removed from the list of possible antigens for use in pigs. We have found it necessary to undertake trials of pathogenicity of challenge strains, and to standardize the doses and potency tests in pigs.

The Center is also carrying out collaborative work with several official agencies to ascertain the most appropriate methods for control of brucellosis in the field.

### Hydatidosis - Field Studies

The burden of human suffering in hydatidosis is well known. Uruguay alone has 500 surgical human cases per year. Case fatality rates following such surgical intervention are high. In contrast with this, little is known of the impact of the disease in sheep, the principal host, and the Center has, therefore, undertaken in collaboration with the "Comisión Honoraria de Lucha contra la Hidatidosis" in Uruguay, a study to determine economic aspects of this disease. Four hundred lambs (200 infected and 200 non-infected) are maintained in an isolated peninsula of Río Grande in Uruguay to study live weight gain, wool production and reproductive capacity in the two groups.

It is recognized that the cycle of infection must be broken at the dog/human point of contact. Various drugs of different ovicidal and vermifugal effects have been used in the past with limited success, and these have been usually accompanied by substantial costs and risks for the dosed animal. The Center has carried out studies using a low cost compound Isochinolin-Pyrazin-derivative ("Droncit") and finds that this is effective against both immature and gravid E. granulosus parasites at a dose rate of 5 mg/kg body weight. On the basis of these studies, field trials are now in operation in Flores, Uruguay, with good results. Based on this it is hoped that "Droncit" will be used in the two major pilot programs of Neuquen, Argentina, and SAIS Tupac Amaru, Peru. The results of the Center's experience with "Droncit" are summarized in Table VI and VII.

Other projects in progress at the Center are the immunization of dogs against E. granulosus using purified antigen, ovicidal and scolical activity of drugs, and testing of newer drugs in laboratory animals.

### Hydatidosis - Laboratory Studies

Though hydatid disease in man has been recognized for many years principally in the southern hemisphere, laboratory diagnosis has been less than satisfactory. The Center, therefore, has carried out extensive research into newer, more effective and simpler techniques which are now adopted as routine procedures in a variety of countries. The status of diagnostic capability which previously existed can best be expressed as shown in Table VIII.

Each technique as performed in a given laboratory, differed by several parameters, including technical conditions for performing each test and the criteria for test positivity. As a result, each laboratory had adopted a technique on the basis of available reports which often lacked sufficiently detailed methodological information. On many occasions the tests were based on personal communication with workers from other

laboratories employing one or more of these test variants. As a result, many laboratories were not using adequate immunodiagnostic tests for hydatidosis nor were they interpreting test results on a sound basis.

The nonspecificity of tests used in seroepidemiologic studies did not permit the differentiation of hydatid from non-hydatid cases. Thus, estimations of prevalence on the basis of these results were inaccurate.

Studies conducted at CEPANZO were aimed at selecting the most specific techniques. These were then improved to obtain optimal sensitivity and specificity and so render them adaptable to minimally equipped laboratories in endemic areas.

As a result of the hydatid immunology and immunodiagnosis research program at the Center, a number of serologic techniques of known merit but with certain limitations are presently available. Sufficient evidence has been accumulated to discard others. Figure II shows the optimal methodology presently used for hydatid immunodiagnosis in clinical situations.

The three techniques of choice have been modified to employ the same lyophilized antigen, thus greatly simplifying the work of both the diagnostic laboratory and the laboratories responsible for hydatid antigen production. The choice technique is the IEP test (no positives in non-hydatid cases). The test was rendered applicable to routine diagnostic work by shortening the time of the electrophoresis run and using non-lyophilized serum from patients without affecting its effectiveness.

The IHA and IA tests were extensively evaluated and standardized and the optimal positivity criterion was determined.

Immunodiagnosis now complements clinical, radiographic and sintillographic studies in the diagnosis of human hydatid disease.

The new approach for detecting cyst carriers was developed at CEPANZO and is being employed in South America for mass surveys of persons at high-risk of exposure in endemic areas (Figure III). This approach will detect liver and other extra pulmonary localizations missed in X-ray surveys (thus complements these). Surveys are being done in areas where X-ray trucks and equipment cannot reach, because of geographical difficulties of terrain.

CEPANZO has published a monograph on techniques for the Immunodiagnosis of Human Hydatid Disease. Clear descriptions are provided of the preparation of antigen from hydatid cyst fluid, the preparation of antiserum to hydatid cyst fluid in large animals, the preparation of antiserum to hydatid cyst fluid in rabbits and of the methods for carrying



out the immunoelectrophoresis test (IEP), latex agglutination test (LA) and indirect haemagglutination test (IHA). The value of these tests is discussed. For the confirmation of diagnosis in patients the IEP test is recommended, the presence of arc 5 being diagnostic. From a positive LA or IHA test or the presence of bands other than arc 5 in the IEP test, varying degrees of probability of the presence of hydatid infection may be inferred. For mass surveys of populations the LA test is recommended, all positives being re-examined using the IEP test. The LA test is also recommended for the determination of the prevalence of human hydatid disease in a geographical area or for measuring variations of incidence during a control programme.

The Center now looks forward to clarifying the similar complex immunodiagnostic field in cysticercosis, fascioliasis, trichinosis and onchocercosis.

### Leptospirosis

Research investigations in leptospirosis currently being carried out are directed toward the development of newer reliable diagnostic methods for detecting disease in man and animals, and prevalence and distribution studies to locate new hosts for the ever increasing number of Leptospira serotypes.

The highlights of investigations in progress include

The development of a simple to perform hemolysis-in-gel procedure which appears to be useful in differentiating pathogenic leptospire from the saprophytic soil and water leptospire.

Studies on the outer-envelope sheath material of leptospirae have been applied to diagnostic tests for leptospirosis. One component of the soluble outer-envelope reacts as a serotype-specific antigen in complement fixation tests for the detection of both serum immunoglobulins IgM and IgG. Another component of the outer-envelope complex is an erythrocyte sensitizing substance which is serogroup-specific and detects only IgM antibodies. Preliminary data indicates that this sensitizing substance is useful in detecting disease earlier than by agglutination tests.

Other related studies on the outer-envelope antigens of leptospirae are being applied to methods for the identification and classification of the different leptospiral serotypes, and as an immunogen against disease in man and animal.

Another investigation was undertaken to elucidate the cause of persistent and multiple non-specific leptospiral agglutinins that are frequently encountered in diagnostic tests. Immunological separation and characterization of a common broadly-reacting subsurface protein antigen

of a Leptospira biflexa strain was studied. It was shown that model animals inoculated with the common antigen produced both serum agglutinins and precipitins to pathogenic leptospirae. The findings suggest that the common antigen of saprophytic leptospirae can stimulate the production of cross-reacting antibodies to pathogenic strains in domestic animals.

Among the field investigations in leptospirosis, the Center has been actively participating in studies on leptospirosis in man and animals of Barbados, W.I. In other animal survey studies, the armadillo (Chaetophractus villosus) and the horse have been identified as new hosts for Leptospira hardjo which is an important serotype producing infections in man and cattle.

### Tuberculosis

The detection of reactors by means of tuberculin tests forms an indispensable tool in the overall program of control and eradication of this disease from the continent. Studies have been underway at the Center to determine the sensitivity of the tuberculin test in animals naturally infected with M. bovis in Argentina. Of particular importance are those aspects of the test concerning different qualities of PPD, the various methods of application of the test and the variety of interpretations that can be made on the results obtained.

In the test carried out by the Center, a whole herd (113 animals) known to be very heavily infected with tuberculosis, was submitted to tuberculin tests using bovine PPD, two human PPD's and two avian PPD's of various potencies.

After slaughter of the animals, the presence of tuberculous lesions was noted; samples of ganglia from the respiratory tract and mediastinum were processed at the laboratory and cultured. An analysis was made of the results of the tuberculin tests carried out in the group classed as "definite infected" (51 animals) from whose ganglia M. bovis had been previously isolated.

The results obtained confirm the superior sensitivity of PPD produced from M. bovis, as compared with that prepared from M. tuberculosis. It was shown that potency testing was essential in the maintenance of a high tuberculin sensitivity. An analysis was made of various different methods of interpretation of the test results, and its incidence and relevance to the sensitivity of the test. These results showed the importance of taking into account the degree to which the tuberculin test may err, and the effect of such interpretation failures, within a tuberculosis control program.

Continuing in this area of interpretative response, the Center is embarked on studies to determine if repetitive vaccinations for other diseases in cattle can affect the allergic response of the individual

animal to the tuberculin tests. These studies were carried out initially in guinea pigs, and later, in cattle.

A comparison was made between the tuberculin test response in guinea pigs which had been previously sensitized with BCG, and then vaccinated with foot-and-mouth disease vaccine, with rabies fixed virus, or with brucellosis strain 19 vaccine. Results obtained to date show that the FMD vaccine reduces the response level of tuberculin for a period. This reduction is also observed when the animal has been vaccinated with rabies fixed virus. On the other hand, no such change was noted when strain 19 vaccine was used.

Similar studies have now begun in cattle.

Studies continue on the differentiation of varying types of mycobacteria, with special reference to the reducing nitrate activity of M. tuberculosis, M. bovis and BCG. Different reduction characteristics are found in each type. From the results obtained it can be concluded that BCG may be considered to be a different mycobacterial species.

In addition to the Pasteur strain, other strains are being studied.

### Food Microbiology

The number and frequency of food-borne disease outbreaks is a subject of increasing concern to public health authorities. Among the diseases most commonly reported, those concerning food hygiene occupy a prominent place. The Center has developed a series of analytical techniques for microbiological control of foods, and has encouraged the use of standard techniques and criteria throughout the Hemisphere. We have developed a simple effective technique for determining the contamination with pathogenic microorganisms of surface working areas and carcasses in slaughterhouses. By means of this method, a variety of surfaces may be sampled for later culture at the laboratory. This technique can also be used in food-borne outbreaks for samples from suspected surfaces at the site of preparation or handling of the food.

One of the principal health problems is concerned with the dissemination of salmonellosis. The Center has been engaged in determining the ecological relationships between salmonella isolated from animals, surface waters and food stuffs. New concepts in our understanding of the spread of salmonella are now being clarified.

A major obstacle in the control of sanitary status of foods is the lack of appropriate information to establish microbiological criteria. In this regard, the Center studied a variety of situations and is now in a position to make recommendations regarding minimal acceptable sanitary standards, for foods such as dairy and meat products.

A new and very sensitive technique for determining the presence of botulinum toxin in foods and in faeces and sera of patients is now being developed at the Center.

Of increasing interest to physicians is that aspect of the problem related to multiresistant strains of microorganisms, particularly enterobacteria C. We have carried out studies on enterobacteria isolated from animals and foods of animal origin and have attempted to identify the resistance transfer factors concerned. The findings are then related to the use of antibiotics with non-therapeutic uses of antibiotics in animal rearing establishments. The results of these studies carried out in collaboration with Food and Drug Administration of U.S.A., assisted in the development of new regulations to control the use of antibiotics in animal husbandry.

### Pathology

The unique biological characteristic of the armadillo have attracted the attention of workers interested in the use of experimental models in different fields of biomedical research. The nine-banded armadillo (Dasypus novemcinctus) has been known since 1910, through Newman and Patterson's description of its embryological development and the production of homozygous quadruplets. In 1971, scientific attention focussed on the armadillo after Storrs and Kirchheimer, and Binford proved it to be an excellent model for leprosy research. These authors were able to reproduce for the first time experimental infection of generalized leprosy through the inoculation of Mycobacterium leprae from lesions of human lepromatous leprosy. The armadillo has been used in many other biomedical investigations, in such fields; as ambriology, genetics, teratology, organ transplants, immunochemistry and endocrinology.

The significance of these studies led the Pan American Zoonoses Center (PAHO/WHO) to start the formation of a colony of the species Dasypus hybridus, known in Argentina by the name of "mulita". At present, the Center has its own colony of D. hybridus which, though small, has proved to be satisfactory for its intended purposes: the use of the species D. hybridus as a new experimental model in biomedical research; the provision of armadillos of this species to workers interested in the use of this new model, and to attempt its reproduction in captivity.

Prior to the research endeavour of the Center, little information was available on this animal. Research in progress has provided new insights into the anatomic and physiological characteristics of the "mulita", its habitat, their adaptation to captivity, and mating and reproduction factors. We are glad to inform that the Center is presently rearing these animales in captivity and have achieved striking success especially in their reproduction.

The development of the armadillo as an experimental laboratory animal has given added importance to the pathological examination of these animals. This research continues, with special studies on interstitial nephritis in the seven-banded armadillo and its association with infections caused by several types of leptospiral serotypes.

An Atlas on the histology of the seven-banded armadillo has been prepared at the Center. At the recent workshop on Armadillos in Biomedical Research held in Caracas, 23-27 May 1977, the Center was requested to proceed with the immediate publication of the text.

FIGURE I

# NEUTRALIZING ANTIBODIES IN BOVINES VACCINATED WITH SMB WITH ADJUVANT

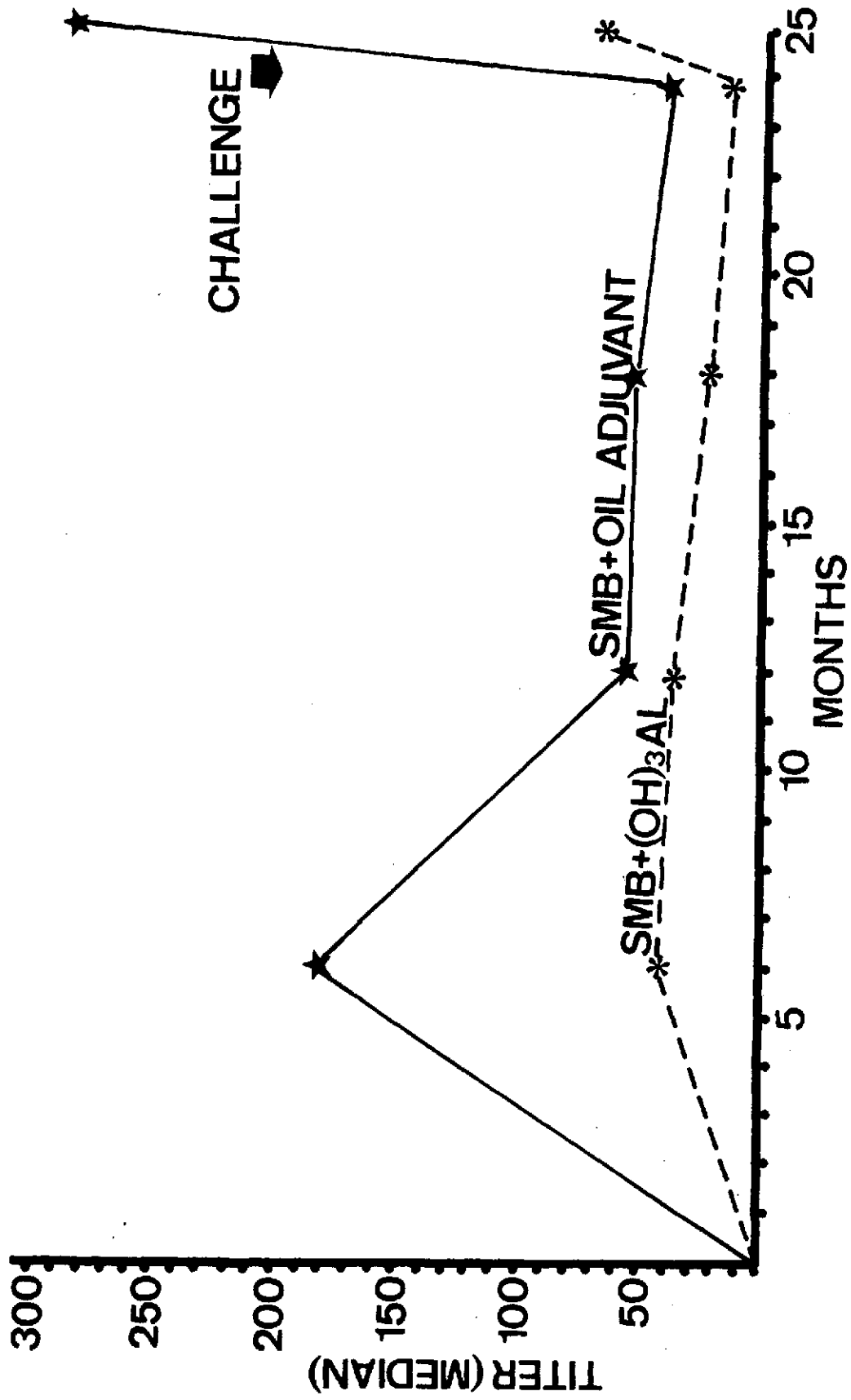


FIGURE II

Hydatid immunodiagnosis in clinical situations

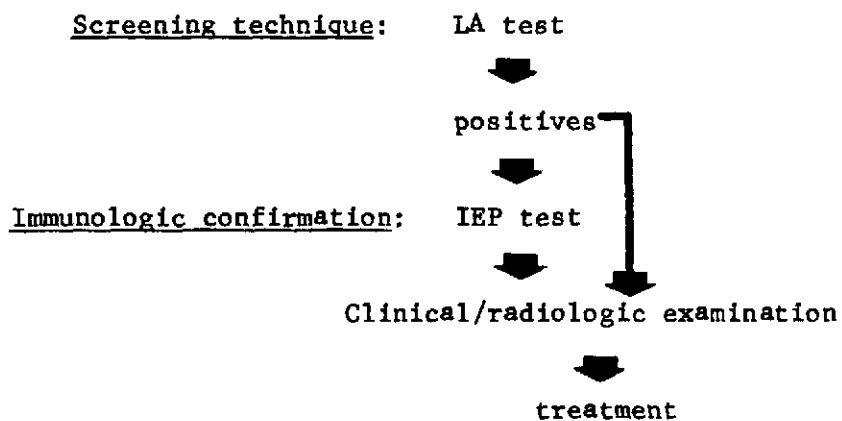
	Standardized antigen		
	IA test	IHA test	IEP test
False positives	2 per 1000	None at diagnostic titers	None with arc 5 none with more than 3 bands

Applications:

- 1) Patients with clinical/radiologic signs compatible with hydatidosis
- 2) Post-operative serologic monitoring

FIGURE III

Serologic surveys for the detection of hydatid cyst carriers



Applications:

- 1) Collective medical examinations
- 2) Determination of prevalence
- 3) Measuring variations of incidence during control programmes



Table I

CHALLENGE TESTS IN VACCINATED BOVINES,  
24 MONTHS AFTER VACCINATION

Vaccine	Challenge *	
	Dead/ Total	% mortality
SMB + (OH) AL	1/23	4.3
SMB + oil adjuvant	1/24	4.2
Non-vaccinated controls	5/8	6.3

\* The animals received 85,000 mouse LD<sub>50</sub>  
IC of DR-19 virus

Table II

CORRELATION OF CE AND SN TESTS

- HUMAN SERA -

Sera	Total	SN+		SN-	
		CE+	CE-	CE+	CE-
Immunized	47	37	3	6	1
Non-immunized	27	0	0	0	27

Table III

CORRELATION OF CE AND SN TESTS  
- ANIMAL SERA -

Sera	Total	SN+		SN-	
		CE+	CE-	CE+	CE-
Immunized	77	71	4	2	0
Non-immunized	70	0	0	0	70

Table IV

PERCENTAGE OF DOGS HAVING SN ANTIBODY  
TITERS OF 1:5 OR GREATER AT INTERVALS  
AFTER VACCINATION

Vaccine	1 week	4 weeks	1 year	2 years
LEP-CEO	47.5	97.5	70.0	73.0
LEP-CFTC	65.0	97.5	72.5	83.0
LEP-HKTC	97.5	100.0	87.5	90.0
CVS-HKTC	95.0	100.0	13.0	17.0
HEP-CKTC	95.0	100.0	62.5	73.0
ERA-PKTC	100.0	100.0	72.5	80.0
SMB	100.0	100.0	67.0	55.0
PRV	100.0	100.0	95.0	88.0
PV-BHK-EI	100.0	100.0	100.0	100.0

Table V

RESULTS OF CHALLENGE TEST IN DOGS  
VACCINATED WITH PV-BHK-EI

	Months post-vaccination		
	2	12	25
Vaccinated	0/10 *	0/10	0/10
Controls	7/	2/5	7/8

\* Dead/Total challenged

TABLE VI

Results obtained 7 days after Isochinolin-Pyrazin-Derivative ("Droncit")

against immature *E. gramulosus*

Treatment	WITH FOOD				WITHOUT FOOD			
	Number of dogs examined	Number of dogs positive	Number of parasites recovered in each dog	No. of dogs examined	No. of dogs positive	Number of parasites recovered in each dog	No. of parasites recovered in each dog	
Control	10	9	105890; 43906; 29137; 8920; 4366; 3010; 173; 28; 15; 0	10	10	20576; 14200; 14006; 13500; 10271; 7399; 4622; 4345; 1626; 824		
5.0 mg/kg body weight	10	1	2*; 0; 0; 0; 0; 0; 0; 0; 0; 0	10	0	0; 0; 0; 0; 0; 0; 0; 0; 0		

\* Worms were recovered in disintegrated condition.

TABLE VII

Results obtained 5 days after Isochinolin-Pyrazin-Derivative ("Droncit")  
treatment against gravid E. granulosus

Treatment	No. of dogs examined	No. of dogs positive	No. of parasites recovered in each dog
Control	10	10	28000;12000;8700; 2000;1680;1447; 832;430;23;15
5 mg/kg body weight (with food)	10	0	0;0;0;0;0;0; 0;0;0;0

TABLE VIII

Techniques for the immunodiagnosis of human hydatidosis (up to 1972)

Test	Technical variants (minimal nos.)	Percentage sensitivity (average)	Maximal nonspecificity
Intradermal (Casoni)	10	71.8	45.4 (a)
Complement fixation	4	69	28.0 (a)
Bentonite flocculation	2	85.4	27.8 (a)
Indirect haemagglutination	4	84.9	15.5 (a)
Latex agglutination	4	71.9	9.1
Immunoelectrophoresis	4	83.7	17.9
Double diffusion in gel	4	37.3	20.9
Counter-electrophoresis	3	79.9	48.1
Indirect fluorescent antibody	6	88.0	5.5

(a) Also employed in seroepidemiologic studies.

TABLE IX

Mulitas brought in from the wild and number of mulitas born in captivity

Year	Captured	Capture followed by death for different causes	Females that had young in captivity	Number of litters alive	Sent to other laboratories
1974	102	90	5	None	9
1975	59	48	5	1	2
1976	33	13	8	5	5
1977 (up to March)	-	-	-	-	6