IICA

Consultant Final Report
IICA/EMBRAPA-PROCENSUL II

"ELISA" FOR SEROLOGIC DIAGNOSIS OF EQUINE INFECTIOUS ANEMIA

ESCRITÓRIO NO BRASIL

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ENZYME-LINKED IMMUNOABSORBENT ASSAY (ELISA) FOR SEROLOGIC DIAGNOSIS OF EQUINE INFECTIOUS ANEMIA

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Jan Thorsen

Brasília, fevereiro de 1989

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA
EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA

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APRESENTAÇÃO

A reprodução e difusão dos Relatórios de Consultores, no âmbito restrito das Diretorias das Unidades do Sistema Nacional de Pesquisa Agropecuária, vinculado à EMBRAPA, tem como objetivo principal o de divulgar as atividades desenvolvidas pelos consultores e as opiniões e recomendações geradas sobre os problemas de interesse para a pesquisa agropecuária.

As atividades de consultoría são realizadas no âmbito do Projeto de Desenvolvimento da Pesquisa Agrtopecuária e Difusão de Tecnologia na Região Centro-Sul do Brasil - PROCENSUL II, financiado parcialmente pelo Banco Interamericano de Desenvolvimento - BID e a EMBRAPA conforme os contratos de Empréstimo 139/IC-BR e 760/SF-BR, assinados em 14 de mar)o de 1985 entre o Governo Brasileiro e o BID.

As opiniões dos consultores são inteiramente pessoais e não refletem, necessariamente, o ponto de vista do IICA ou da EMBRAPA.

A coordenação dos Contratos IICA/EMBRAPA agradeceria receber comentários sobre estes relatórios.

Horacio H. Stagno
Coordenador Contratos IICA/EMBRAPA

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
IICA/EMBRAPA CONTRACT

CONSULTANT FINAL REPORT

1. Consultant's full name: Jan THORSEN
2. Specialist in: Virologist
3. Title of IICA Project: 2.SB.3

4. EMBRAPA Program for which consultancy is provided:

Sanidade Animal (Bovinos)

IICA Project Activity Code: 2.SB.3.05	Administrative Code: R4847B1B 03105
Title of Activity of IICA Project corresponding to this consultancy	Cooperation with EMBRAPA on research and applications of genetic resources, biotechnology and biologic control of plagues, diseases and weeds.

CONSULTANT CONTRACT PERIOD	DUTY LOCATION (Center)
April 21 to June 5, 1988	CNPGC, Campo Grande and FUFMS
CONTRACT EXTENTION PERIOD (IF any)	DUTY LOCATION (Center)

5. Financial support: PROCENSUL II

6. ACTIVITIES UNDERTAKEN BY THE CONSULTANT AND RESULTS

6.1 RESEARCH DONE UNDER DIRECT RESPONSIBILITY OF THE CONSULTANT

FUFMS

The objective of activities at FUFMS was to develop propagation of equine infectious anemia (EIA) virus in cell cultures and to use the antigen thus produced in an enzyme-linked immunosorbent assay (ELISA) for serologic diagnosis of equine infectious anemia.

A cell culture-adapted strain of EIA virus, Wyoming strain, was imported with the approval of the Federal Ministry of Agriculture. Cultures of Cf2Th cells, a canine thymus cell line and ED cells, an equine dermis cell line, were also imported, also with the approval of the Federal Ministry of Agriculture.

Cultures of equine fetal kidney cells were initiated from a fetus obtained at UFMS and propagation of EIA virus in these cells was also undertaken.

Concentration and partial purification of cell-cultured EIA virus was attempted by differential centrifugation and polyethylene glycol precipitation.

The Cf2Th and ED cell lines unfortunately became contaminated with bacteria and fungi and were no longer suitable for propagation of EIA virus. The fetal equine kidney cell cultures appeared to contain a latent virus and thus could not be maintained for the long periods required for continuous cultivation of equine infectious anemia virus. Nevertheless, some propagation of EIA virus in cell cultures was achieved and stocks of cell culture-propagated virus were stored frozen, for future use as inocula in cell cultures.

Additional Cf2Th and ED cells will be ordered from the American Type Culture Collection, for shipment to UFMS. As well, it has been suggested that additional fetal equine kidney cell cultures be established, since this is probably the best cell culture system for propagation of EIA virus.

EIA virus, propagated in cell cultures, was concentrated by precipitation with polyethylene glycol. The resultant antigen was tested by ELISA with sera positive and negative for antibodies for EIA virus. Although the antigen was not sufficient to produce an effective, highly concentrated antigen, there was enough to indicate that the test would work for serologic diagnosis of EIA.

In summary, activities at UFMS resulted in the establishment of methods for cell culture propagation of EIA virus and for production of an antigen for serologic diagnosis by ELISA.

CNPGC

The objective of activities at CNPGC was to develop the ELISA for serologic diagnosis of infections of cattle with *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale*. There are no methods for *in vitro* cultivation of *Anaplasma marginale*, and since *in vitro* cultivation of *Babesia bovis* and *bigemina* is not a routine procedure, the organisms could only be propagated in splenectomized calves. Diagnostic antigens from the three agents were available, prepared previously and stored frozen. The possible use of these stored antigens to prepare ELISA antigens was investigated.

Propagation of antigens in calves is time-consuming. Susceptible calves must be splenectomized, allowed to recover from the surgery and infected. Erythrocytes are harvested at the time of high level

parasitemia and antigens purified and concentrated from parasitized erythrocytes. Often, the organisms must be passaged into a second splenectomized calf before a high enough level of parasitemia is developed. Since there were no splenectomized calves available at the beginning of the consultancy, time constraints prevented the propagation of the organisms in vitro. Indeed, by the end of the consultancy period, the first organism had been repassaged into a second splenectomized calf and the animal was still not reacting severely.

The use of the frozen, concentrated antigens for preparation of an ELISA antigen was investigated. Unfortunately, since freezing and thawing ruptures erythrocytes, releasing some of the protozoal antigens contained in the erythrocytes, it was not possible to produce good ELISA antigens from this material.

Procedures for performing an ELISA for serologic diagnosis, including preparation of buffers, substrate solutions and standardization of enzyme-labelled antiglobulins were demonstrated. The antigens produced from the frozen antigen preparations were adequate for demonstration purposes. Personnel in the laboratory were instructed in the procedures and the materials required.

A useful technique for antigen preparation and purification is gel electrophoresis, to identify protein antigens and their state of purity. The equipment for this technique had been acquired by CNPGC over two years ago but never used. With the assistance of Dr. Fabio, from the Faculty of Medicine, UFMS, this equipment was put into operation and used for analysis of a mixture of proteins. Laboratory personnel at CNPGC are now familiar with the use of this equipment and the reagents required.

In summary, no freshly parasitized erythrocytes were available for antigen preparation and frozen material was not suitable. In spite of this, procedures for ELISA for serologic diagnosis were demonstrated to laboratory personnel. Additional supplies and equipment required for ELISA will be ordered for CNPGC. The technique of gel electrophoresis of proteins was demonstrated to laboratory personnel, using equipment acquired by CNPGC two years ago but not used.

Detailed protocols for the preparation of ELISA antigens from fresh parsitized erythrocytes were prepared for the three organisms. These procedures can be instituted when splenectomized calves with a high level of parasitemia are available.

6.4 IN-SERVICE TRAINING PROVIDED BY THE CONSULTANT

As mentioned under 6.1, in-service training in the methodology of ELISA for serologic diagnosis was provided to Dr. Anna Maria Sacco, the counterpart at CNPGC and Dr. Bartolomeo, from Rio Grande do Sul. The trainees should be sufficiently familiar with the techniques to be able to carry out serologic tests when they have suitable diagnostic antigens.

6.6 ACTIVITIES IN SUPPORT OF OTHER CENTERS AND UNIVERSITIES IMPROVING THE RESEARCH CENTERS' LINKS WITH ABROAD

A proposal for a collaborative research link between CNPGC, UFMS and the University of Guelph will be developed and submitted to the International Development Research Centre. The proposal will include provisions for training visits by CNPGC personnel to Guelph and for short term visits by Guelph faculty members to CNPGC. If the proposal is successful, it could greatly strengthen the research capabilities of CNPGC.

8. CONSULTANT'S SUGGESTIONS AND TECHNICAL OR INSTITUTIONAL RECOMMENDATIONS FOR THE IMPROVEMENT OF THE RESEARCH SERVICE

At UFMS, the Laboratory of Infectious Diseases is not equipped adequately for activities such as antigen production for diagnostic tests. Equipment requirements include roller apparatus for large scale cell culture and virus propagation and a good ultracentrifuge for purifying and concentrating antigens. It is my understanding that money has been allocated for the acquisition of some of this equipment, but not yet made available. If this is the case, the sooner funds are released and the equipment acquired the better.

At CNPGC, all of the equipment and supplies needed for large scale ELISA testing are not available. These include a spectrophotometer for reading ELISA plates and a wider selection of rotors for the ultracentrifuge at CNPGC, which is a good one.

The consultancy at CNPGC could probably have been more effective if more advance planning had been done. Since the objective was to develop ELISA procedures, prior consultation as to materials required, e.g. special plastic multiwell plates and multi-channel pipettors could have ensured that they would be there during the consultancy period. Also, propagation of the organisms in calves could have been initiated well before the beginning of the consultancy period, to ensure that a supply of fresh parasitized erythrocytes would be available as a source of antigen.

10. CONSULTANT'S COMMENTS ON CIRCUMSTANCES WHICH AFFECTED THE CONSULTANCY WORK

At UFMS, problems with contamination of cell cultures seriously interfered with the propagation of EIA virus. Although the origin of the contamination is difficult to pinpoint, the inadequate facilities for cell culture propagation in the Laboratory of Infectious Diseases continue to impose constraints on virological activites. More glass and plasticware for cell culture propagation, better facilities for preparing glassware for virology and for filtering media, equipment for large scale cell culture production and a good ultracentrifuge are all required.

At CNPGC, as mentioned under 8, more consideration of the time required for propagating the organisms in calves and the supplies required for ELISA applications would have increased the effectiveness of the consultancy period.

Programa II. Geração e Transferência de Tecnologia

O Programa de Geração e Transferência de Tecnologia é a resposta do IICA a dois aspectos fundamentais: (i) o reconhecimento, por parte dos países e da comunidade técnico-financeira internacional, da importância da tecnologia para o desenvolvimento produtivo do setor agropecuário; (ii) a convicção generalizada de que, para aproveitar plenamente o potencial da ciência e da tecnologia, é necessário que existam infra-estruturas institucionais capazes de desenvolver as respostas tecnológicas adequadas ás condições específicas de cada país, bem como um lineamento de políticas que promova e possibilite que tais infra-estruturas sejam incorporadas aos processos produtivos.

Nesse contexto, o Programa II visa a promover e apoiar as ações dos Estados membros destinadas a aprimorar a configuração de suas políticas tecnológicas, fortalecer a organização e administração de seus sistemas de geração e transferência de tecnologia e facilitar a transferência tecnológica internacional. Desse modo será possível fazer melhor aproveitamento de todos os recursos disponíveis e uma contribuição mais eficiente e efetiva para a solução dos problemas tecnológicos da produção agropecuária, num âmbito de igualdade na distribuição dos benefícios e de conservação dos recursos naturais.

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA

O Instituto Interamericano de Cooperação para a Agricultura (IICA) é o organismo especializado em agricultura do Sistema Interamericano. Suas origens datam de 7 outubro de 1942, quando o Conselho Diretor da União Pan-Americana aprovou a criação do Instituto Interamericano de Ciências Agrícolas.

Fundado como uma instituição de pesquisa agronômica e de ensino, de pós-graduação para os trópicos, o IICA, respondendo às mudanças e novas necessidades do Hemisfério, converteu-se progressivamente em um organismo de cooperação técnica e fortalecimento institucional no campo da agropecuária. Essas transformações foram reconhecidas oficialmente com a ratificação, em 8 de dezembro de 1980, de uma nova convenção, que estabeleceu como fins do IICA estimular, promover e apoiar os laços de cooperação entre seus 31 Estados membros para a obtenção do desenvolvimento agrícola e do bem-estar rural.

Com um mandato amplo e flexível e com uma estrutura que permite a participação direta dos Estados membros na Junta Interamericana de Agricultura e em seu Comitê Executivo, o IICA conta com ampla presença geográfica em todos os países membros para responder a suas necessidades de cooperação técnica.

As contribuições dos Estados membros e as relações que o IICA mantém com 12 Países Observadores, e com vários organismos internacionais, lhe permitem canalizar importantes recursos humanos e financeiros em prol do desenvolvimento agrícola do Hemisfério.

O Plano de Médio Prazo 1987-1991, documento normativo que assinala as prioridades do Instituto, enfatiza ações voltadas para a reativação do setor agropecuário como elemento central do crescimento econômico. Em vista disso, o Instituto atribui especial importância ao apoio e promoção de ações tendentes à modernização tecnológica do campo e ao fortalecimento dos processos de integração regional e sub-regional.

Para alcançar tais objetivos o IICA concentra suas atividades em cinco áreas fundamentais, a saber: Análise e Planejamento da Política Agrária; Geração e Transferência de Tecnologia; Organização e Administração para o Desenvolvimento Rural; Comercialização e Agroindústria, e Saúde Animal e Sanidade Vegetal.

Essas áreas de ação expressam, simultaneamente, as necessidades e prioridades determinadas pelos própios Estados membros e o âmbito de trabalho em que o IICA concentra seus esforços e sua capacidade técnica, tanto sob o ponto de vista de seus recursos humanos e financeiros, como de sua relação com outros organismos internacionais.

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Responsáveis pela reprodução: Jadir José dos Santos e Murillo Sodré da Silva.

Interessados em receber mais exemplares deste o de outros Relatórios de Consultores poderão solicitá-los a:

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FECHA DE DEVOLUCION

Autor

Título Enzyme-linked immunoabsorbent assay (Elisa) for serologic diagnosis of equine infectious...

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