

# DIAGNOSIS AND EPIDEMIOLOGY OF ANIMAL DISEASES IN LATIN AMERICA



XA9848639

A. COLLING

Joint FAO/IAEA Division of Nuclear Techniques in  
Food and Agriculture,  
International Atomic Energy Agency,  
Vienna

## 1. BACKGROUND

Support for scientists and their endeavours in developing countries by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture is provided through FAO/IAEA Co-ordinated Research Projects (CRP) and IAEA Technical Co-operation Projects (TCPs). Using these mechanisms the Animal Production and Health Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture aims to encourage and improve the capacity of national institutions in developing countries to identify and resolve problems connected with improving livestock productivity and health. In 1986, the Section introduced an animal health component into its Project. The initial support was for five years but in 1991 this was extended for a further three years and linked with the support available from the IAEA's Technical Co-operation Project through national and regional TCPs and ARCAL\* activities in Latin America dealing with diagnosis of animal diseases. Central to this overall project was the use of ELISA for the diagnosis and control of livestock diseases.

FAO/IAEA CRPs are developed around a well defined research topic on which between 15 and 20 national institutes collaborate — the topic itself being defined through consultation with national authorities in developing and developed countries and international agricultural research centers and organizations. The primary role of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in such programmes is to ensure that the inputs and efforts under these programmes are co-ordinated and that the results are published.

The studies being reported in this IAEA TECDOC were initiated in 1991 and whilst the focus was on three major disease affecting livestock in the region (foot-and-mouth disease (FMD), brucellosis and babesiosis) the approach taken by individual Research Contract holders was different and thus in some cases research concentrated on assay validation whilst in other cases the focus was on the disease itself and its importance within the country in question.

Although this publication contains details of research work conducted under two CRPs, the papers are essentially a compilation of data presented at final Research Co-ordination Meetings (RCM) of the two CRPs, held in Guadeloupe, Lesser Antilles, June 1994 and in Vienna, Austria, April 1997.

## 2. FAO/IAEA CO-ORDINATED RESEARCH PROJECTS ON DIAGNOSIS AND EPIDEMIOLOGY OF ANIMAL DISEASES IN LATIN AMERICA.

Both CRPs were concerned with supporting scientists in the Latin American region wishing to use ELISA for improving the diagnosis and control of animal diseases, such as FMD, brucellosis and babesiosis. Although the first CRP was funded primarily by SIDA and co-ordinated by staff of the Animal Production and Health Section, substantial additional support for the introduction and use of the ELISA was provided through the IAEA's Technical Co-operation (TC) Project in Latin America. This allowed for the provision of ancillary equipment through national and regional TCPs operating at institutes where individual research contract holders were located as well as the provision of experts to visit laboratories and training through fellowships and scientific visits. The follow-up CRP was funded through the regular budget of the Joint FAO/IAEA Division of Nuclear Techniques in Food and

---

\* ARCAL = Acuerdos Regionales Cooperativos para la Promoción de la Ciencia y la Tecnología Nucleares en Latino-América.

Agriculture but again activities were further supported through the Technical Co-operation Project of the IAEA.

## 2.1. Research Contracts and Agreements

### 2.1.1. Research contract holders

Under the CRP entitled Immunoassay Methods for the Diagnosis and Epidemiology of Animal Diseases in Latin America 1991–1994, 22 Research Contracts were awarded to scientists from Latin America (Figure 1). Three diseases e.g. brucellosis, FMD and babesiosis were covered by the project. Seven contracts were concerned with brucellosis, seven with FMD and eight with babesiosis.



FIG. 1. FAO/IAEA/SIDA Co-ordinated Research Project, 1991–1994, Immunoassay Methods for the Diagnosis and Epidemiology of Animal Diseases in Latin America.

A follow-up CRP (1994–1997) entitled: The Use of ELISA for Epidemiology and Control of foot-and-mouth Disease and Bovine Brucellosis in Latin America focused on the international standardization and validation of a competitive brucellosis ELISA and a FMD antibody liquid phase blocking ELISA. 10 Research Contracts (5 for each disease under study) were awarded to scientists in eight countries and 10 institutions (Figure 2).

Research Contract funds were used primarily to purchase ELISA equipment, reagents, FAO/IAEA ELISA kits and microtitre plates. In several cases a portion of the research grant was made available locally to provide funds for sample collection.

At each RCM each principal investigator provided a detailed report of the work carried out and an account of the workplan for the following year. The country reports, presented at the final RCMs in Guadeloupe, Lesser Antilles, and Vienna, Austria contained in addition a summation of these individual reports and constitute an account of the activities and results carried out between 1991 and 1997. For the



FIG. 2. FAO/IAEA Co-ordinated Research Project, 1994–1997, The Use of ELISA for Epidemiology and Control of foot-and-mouth Disease and Bovine Brucellosis in Latin America.

most part, these reports detail the introduction of the ELISA into a laboratory, the establishment of local "cut-off" values for the assay, the establishment of the test as routine and the collection and testing of samples initially for assay validation and subsequently as part of a national or regional survey on the occurrence of the disease in question and/or the monitoring control and eradication programmes.

## 2.2. Research co-ordination meetings

Under each Project 3 RCMs were held. The first of these was held at PANAFTOSA, Rio de Janeiro, Brazil in November 1991. The second meeting was held at ICA-CEISA, Bogota, Colombia in November-December 1992. The final meeting of the first Project was held at the IEMVT-CIRAD Guadeloupe, Lesser Antilles in June 1994.

The first meeting of the second Project was held in Buenos Aires, Argentina in November, 1994, the second meeting at PANAFTOSA, Rio de Janeiro, Brazil in September, 1995 and the final meeting in Vienna, Austria in April, 1997.

These meetings provided a platform for wide-ranging discussions on problems and experiences. Solutions to individual or common problems were thus shared amongst the group. During these meetings, Agreement holders were able to provide overall guidance and to offer advice at both the individual and group level. These meetings also offered the opportunity to provide training in both ELISA and in the use of computer software for data analysis and epidemiology.

## 2.3. Support activities

### 2.3.1. FAO/IAEA ELISA kits

Central to supporting the Latin American project was the development and introduction of standardized ELISA kits specifically suited to the types of conditions found in laboratories in this region.

A full report of the ELISA systems for the various diseases and their field validation is contained in these proceedings. However, crucial to their design was the use of a standard format and protocol and wherever possible standard reagents, thus ensuring that once a laboratory had established an FAO/IAEA ELISA kit for one particular disease, it would be a simple matter to introduce similar kits for the study of other epizootics. In principle the approach was to use an indirect assay utilizing Ortho-phenylenediamine (OPD) or 2,2'-azino bis (3-ethyl-benzthiazoline sulfonic acid) (ABTS) as the substrate, samples tested in 100µl amounts and in duplicate, a 96-well plate format, and one hour incubation steps at 37°C with three plate washes between each step.

The cut-off value for the assay was determined using 2–3 standard deviations of the mean value of the local negative population and in comparison to the local positive population. The internal controls included a strong positive (C++), moderate or weak positive (C+), a negative (C-) reference serum and a conjugate control (Cc) in quadruplicate. Robustness of the kit was considerably improved through the use of freeze-dried reagents.

The FAO/IAEA ELISA kits were designed to contain all the necessary reagents, be robust enough to withstand extremes of temperature and contain sufficient reagents to test 4000 sera in duplicate. ELISA plates were purchased in bulk to avoid batch to batch variation and shipped together with the kit. To ensure further standardization, the equipment supplied was primarily from the same manufacturer.

### 2.3.2. Training

Adequate and appropriate training was considered a key element in this Project. At the first RCM in Brazil a one-week course introduced the FAO/IAEA ELISA kits for the diagnosis of FMD, brucellosis and babesiosis to the Contract holders. During the following 12 months several of the Contract holders were visited by FAO/IAEA experts and thus further training provided at the national level.



19 TCP

FIG. 3. FAO/IAEA Technical Co-operation Projects 1993, Animal Disease Diagnosis in Latin America.

The second RCM of the project was preceded by a one-week FAO/IAEA/ARCAL regional training course in epidemiology and data analysis held at the ICA-CEISA, in Bogota, Colombia in November, 1992. Also scientists, who were not involved in this CRP were able to attend this training course. During the subsequent three years of this Project and the follow-on CRP further local training was provided through visits to the region by FAO/IAEA and PANAFTOSA experts and Agreement holders in the Project.

Activities were complemented by several IAEA TC national and regional training courses (Chile, 1991; Ecuador, 1991; Paraguay, 1992; Peru, 1992; Costa Rica, 1993; Cuba 1993; El Salvador, 1994; Mexico, 1994; Chile, 1994; Paraguay, 1994; Argentina, 1994; Chile, 1997).

### 3. CONCLUSIONS AND RECOMMENDATIONS

#### 3.1. General conclusions

##### 3.1.1. *Support activities*

Both CRPs and the various training activities associated with them were concerned with introducing, validating and using FAO/IAEA ELISA kits for the diagnosis and monitoring of animal diseases in support of studies undertaken at national research institutes in Latin America. This approach was augmented by inputs from a number of IAEA national TCPs and one regional TCP (ARCAL III, RLA/5/028). For the most part this approach proved highly successful and the ELISA was shown to be both an appropriate and effective system for diagnosing and monitoring these diseases. In the majority of countries the clear advantage of ELISA over existing methods was demonstrated.

##### 3.1.2. *Reagents*

The development and provision of standardized reagents and protocols for the ELISA offered considerable advantages, particularly with respect to international assay validation, inter-laboratory comparison of results, trouble-shooting and assessment of the reliability of results through external quality assurance.

##### 3.1.3. *Standardized approach*

Difficulties were highlighted that can be encountered both in introducing and maintaining an ELISA system. Nevertheless, it was clear that a standardized approach to the diagnosis and control of a particular disease was possible and enabled several countries in the region to undertake co-operative control Projects. Central to these was assurance that ELISA results from individual laboratories participating in such projects were reliable and comparable.

##### 3.1.4. *Establishing ELISA laboratories*

Under the CRPs and the linked national/regional IAEA TCPs, the necessary equipment and training to carry out the ELISA were provided to many national research institutes. Over 50 ELISA laboratories in Latin America were established and strengthened through these activities.

##### 3.1.5. *FAO/IAEA ELISA software program EDI (ELISA Data Interchange)*

Initially individual ELISA software programs (e.g. BREIA) were developed and distributed for each assay. The new ELISA software package EDI was found to be suitable for all FAO/IAEA ELISAs and is crucial for the adequate handling and storage of results. Through the eqstat.qc file, results were retrieved and easily copied on a diskette for further analysis e.g. for the EQA project to monitor internal

controls. Initial communication problems between ELISA reader and computer were solved using a "smart cable".

### **3.2. General recommendations**

#### *3.2.1. Further use of ELISA*

ELISA should be recommended as the test of choice for the diagnosis and monitoring of FMD, brucellosis and babesiosis in the developing country situation. The further development and routine use of internationally standardized and validated ELISA kits against the major epizootics should further be encouraged and supported.

#### *3.2.2. FAO/IAEA external quality assurance programme*

The FAO/IAEA external quality assurance programme needs to develop towards a generic veterinary diagnostic testing laboratory accreditation scheme with a focus on quality management and documentation of specific laboratory activities through standard operating procedures (SOPs). Participation in an EQA programme will assist in creating a quality management working environment, which will assist laboratories - especially from developing countries, which do not have a national accreditation body and thus bridge the gap between what they have now and formal national or international recognition of Quality Management and Technical Competence.

#### *3.2.3. Further supply of crucial reagents*

Mechanisms should be developed to ensure the long term supply of crucial reagents for use in the ELISA. For the majority of diseases, laboratories should be encouraged to use internationally standardized and validated kits. Support should not be provided for individual laboratories in the region to develop their own kits but a move towards regional laboratories providing specific kits should be encouraged.

### **3.3. General considerations and future activities**

The reports contained in these proceedings clearly indicate that the FAO/IAEA CRPs and Technical Co-operation activity succeeded in introducing into the participating Latin American institutes an ability to carry out epidemiological studies using ELISA technology.

The first goal of the programme was to evaluate the usefulness of ELISA methods for the diagnosis of three diseases as compared with existing conventional methods.

The second goal was to use these test, once validated, for the conduct of serological surveys of the incidence/prevalence of the diseases under consideration and to monitor the effectiveness of national programmes aimed at disease control e.g. through seromonitoring studies. ELISA can also subsequently be used as a tool for surveillance to demonstrate freedom and provide evidence for international recognition of eradication.

The third objective was to generate the data necessary to enable international acceptance of test reagents and protocols in order that these may subsequently be used in other countries and sectors of the world to improve the diagnosis and control of diseases considered to be of importance in the region.

It is clear though that the task of establishing these techniques in most countries in the region and for these three epizootics is almost complete. Over 50 ELISA laboratories were established in Latin America. FAO/IAEA ELISA kits for these three diseases are now available and for the most part have now been standardized and fully validated. Sustainability of utilizing ELISA-based systems can be assured through cost-benefit studies, which have shown that ELISA kits are only a minor component in the overall expenditure of animal health activities. Future support in the Animal Production and Health Sub-programme of the Joint FAO/IAEA Division will move away from kit development and standardization to the use of the ELISA system to carry out epidemiological studies on the occurrence of

such disease and the monitoring of control and eradication programmes. Central to the use of ELISA will be participation in the FAO/IAEA external quality assurance programme.

## **Specific Conclusions and Recommendations**

### **3.4. Indirect Brucellosis ELISA**

#### **3.4.1. Conclusions**

##### *3.4.1.1. Cut-off determination*

Considerable problems were experienced in initially establishing a universal “cut-off” for this assay and it became clear that it is necessary to undertake this exercise for each defined cattle population.

##### *3.4.1.2. Use of indirect brucellosis ELISA*

The indirect ELISA has been fully validated and standardized through the studies conducted. It has clearly been shown that it does not separate vaccinated from naturally infected animals but that variations in the “cut-off” can be used to alter sensitivity and specificity in a defined manner.

#### **3.4.2. Recommendations**

##### *3.4.2.1. Selection of an appropriate cattle population for cut-off determination*

At a minimum each national laboratory should establish a set of known brucellosis antibody negative sera and use these to determine its own “cut-off” value for the indirect brucellosis ELISA. Care should be taken that this set of sera are typical of the national cattle population. Studies may be necessary to ensure that a national “cut-off” is appropriate.

##### *3.4.2.2. Appropriate cut-off for different stages of brucellosis campaign*

The FAO/IAEA standardized ELISA is a valuable addition to the diagnosis and control of brucellosis. By altering the “cut-off” it can be used in a fully defined manner through the various stages of brucellosis control and eradication.

## **Competitive brucellosis ELISA**

### **3.4.3. Conclusions**

#### *3.4.3.1. ELISA in comparison with other diagnostic techniques*

In general ELISA was more accurate than the conventional tests e.g. buffered plate antigen test (BPAT), Rose bengal test (RBT) and confirmatory tests e.g. complement fixation test (CFT), 2-mercaptoethanol, (2-ME) and Radial immunodiffusion (RID). This study included 30 000 individual samples that were tested in seven different assays. The total number of individual tests performed was more than 200 000. Furthermore, the sera tested were from throughout the continent from various countries and different bovine breeds. This individual work has been the largest serological study ever done involving testing for brucellosis in the Americas.

#### *3.4.3.2. Performance of C-ELISA II*

Competitive ELISA II (which uses LPS as antigen and monoclonal antibody Mab 84 as competing reagent) is preferred because the LPS antigen is relatively simpler to prepare, the antibody is

directed against a defined epitope and possesses high affinity. In addition the test has high sensitivity and specificity, is useful for differentiating infected from vaccinated cattle and resolves cross reactions due to infections with *Yersinia enterocolitica*. Even though the competitive ELISA I (O-chain antigen) also performed well, it has the disadvantage that the antigen and conjugate preparation are more troublesome.

#### *3.4.3.3. Versatility of C-ELISA*

One of the major potentials of the competitive ELISA is that it can be used for diagnosis in different species of animals, including humans.

#### *3.4.3.4. Limitations of ELISA in general*

One of the limitations of all the current ELISA techniques is that they are not suitable for testing animals on the farm since they require adequate laboratory facilities and trained personnel for their use.

#### *3.4.3.5. Restrictions of indirect ELISA*

Although the indirect ELISA possesses good sensitivity and specificity, it is useful only for testing a limited number of species and does not distinguish vaccinal antibody from that due to infection.

#### *3.4.3.6. Cut-off selection*

Cut-off values must be established for the different countries and regions, and may depend on factors such as prevalence and vaccination status.

#### *3.4.3.7. Other important serological diagnostic techniques*

BPAT and RBT are useful diagnostic tests for screening sera, specially in laboratories where the capability to effectively use the ELISA has not yet been developed.

### **3.4.4. Recommendations**

#### *3.4.4.1. OIE Approval*

OIE approval should be sought for ADRI/ELISA, C-ELISA I and C-ELISA II as OIE prescribed tests for the diagnosis of brucellosis and the separation of vaccinated from naturally infected animals.

#### *3.4.4.2. Publication of results*

The compiled validation data generated by all the participant countries should be published in a refereed scientific journal.

### **3.4.5. Recommendations for future projects**

#### *3.4.5.1. Alternative diagnostic techniques*

Evaluation of the fluorescence polarization assay (FPA) and radial immunodiffusion (RID) test for the field diagnosis of brucellosis and comparison of the performance, characteristics of these assays with the standardised ELISAs, in particular the C-ELISA should be undertaken.

#### *3.4.5.2. Further investigation*

Evaluation of the competitive ELISA II and other assays in calfhood, adult and revaccinated herds.

#### 3.4.5.3. *Milk-ELISA*

Validation of the I-ELISA(ADRI) for the detection of antibodies (anti-*brucella* LPS) in milk.

#### 3.4.5.4. *C-ELISA II*

Application of competitive ELISA II for the diagnosis of brucellosis.

#### 3.4.5.5. *Quality Assurance*

Any country that uses FAO/IAEA ELISA kits should participate in the FAO/IAEA external quality assurance programme.

### 3.5. **FMD group**

#### **Antigen liquid phase blocking ELISA**

##### 3.5.1. **Conclusions**

###### 3.5.1.1. *Sensitivity*

The FMD antigen detection ELISA is a more sensitive test than CFT for the primary diagnosis of FMD.

###### 3.5.1.2. *Standardization*

Reagents for the ELISA can be more easily standardized and stored than those for the CFT.

###### 3.5.1.3. *Monitoring*

Monitoring of field strains using ELISA provide valuable data for epidemiological surveillance and assessment of suitable vaccine strains.

##### 3.5.2. **Recommendations**

###### 3.5.2.1. *Superiority of ELISA*

The higher detection rate of the ELISA over the CFT and the ease of performance strongly recommends its use for fast and reliable diagnosis of FMD.

###### 3.5.2.2. *Standard Tests*

The ELISA and tissue culture isolation should be the OIE prescribed tests for primary diagnosis of FMD.

#### **Antibody liquid phase blocking ELISA**

##### 3.5.3. **Conclusions**

###### 3.5.3.1. *Interlaboratory comparison*

One of the central observations was that the intra- and interlaboratory variation of the test was too high and that the range for the C<sup>++</sup> (=strong positive control serum) and (Ca) antigen control were

too narrow. In Brazil and Venezuela the values for the C++ sometimes exceeded the upper control limit (too high) or were below the lower control limit (too low). In Argentina and Colombia only too high C++ values were observed in some cases. In Paraguay the general tendency was towards too low C++ values with the exception for the antigen of serotype A, where both too low and too high C++ values were observed. The reason for these differences may be due to different pipetting techniques which may become important when glycerinated antigen is used. Nevertheless the predictive value of the test was good.

#### *3.5.3.2. Reproducibility*

The evaluation of reproducibility of the liquid phase ELISA for FMD revealed that the C++, C+, and C- controls were reasonably consistent between laboratories.

#### *3.5.3.3. Upper and lower control limits (UCL, LCL)*

The pre-determined UCL and LCL values for the C++ were too narrow (3 PI units) to be useful in making decisions about this parameter. These limits were recalculated using data from all laboratories.

#### *3.5.3.4. Antigen control*

The antigen control (Ca) proved to be variable between laboratories. The accuracy of the assay (Ca values falling within control values) was lacking in some laboratories; either the data exceeded the upper control limits or were less than the lower control limits. The precision of the assay within a laboratory also varied between laboratories.

#### *3.5.3.5. Accuracy*

The accuracy for the Ca was assessed in relation to the UCL and LCL as originally defined by PANAFTOSA. These limits were too narrow. The UCL and LCL were thus re-defined based on the data collected for the Ca control from all laboratories.

#### *3.5.3.6. Pipetting*

Because of the general consistency of value for all Ca samples within and between plates in a run, it was concluded that the probable cause of the problem was with pipetting of the glycerinated antigen stock solution.

### **3.5.4. Recommendations**

#### *3.5.4.1. Consistency of Ca values*

The laboratories involved should retest the serum panels using a more explicitly defined procedure for pipetting the glycerinated antigen. It is essential that this reagent (Ca) gives uniform, reproducible, and accurate results, because it is used for calculation of the PI values for all control and test sera.

#### *3.5.4.2. Field use*

Application of the assay for routine diagnosis will require resolution of this problem.

#### *3.5.4.3. Training*

Once these problems are resolved, training will be needed for interpretation of FMD ELISA results in control and eradication campaigns.

#### 3.5.4.4. *FAO/IAEA external quality assurance programme (EQAP)*

An external quality assurance programme is essential to assess proficiency in conducting the FMD ELISA within and between laboratories.

#### 3.5.4.5. *Laboratory accreditation*

An international harmonized set of principles for the quality management of veterinary diagnostic testing laboratories and a process for monitoring compliance with these principles is needed to establish a common ground for understanding and evaluating the reliability of the management, operations and outputs of these laboratories.

#### 3.5.4.6. *Interregional comparison*

The reproducibility and repeatability of the data should be compared with the same data from an FAO/IAEA Co-ordinated Research Project on FMD diagnosis in Asia CRP (D3.20.14). Reagents and protocols for these two FMD assays differ: the current study was done with PANAFTOSA-specific reagents while the reagents for the Asian study are from Pirbright laboratories, UK. Such an analysis may clarify which assay format is most appropriate for use in developing countries.

#### 3.5.4.7. *FAO/IAEA ELISA software*

The FAO/IAEA ELISA software program “EDI” rejects a plate when the value of a control serum is a border line value. EDI should be modified so that border line values are still regarded as “within limits”.

#### 3.5.4.8. *Use of antibody ELISA*

The FMD virus antibody ELISA should be routinely used for surveillance, import/export testing and for assessing protection in vaccinated populations. It can also be used in combination with cattle challenge for measuring FMD vaccine potency.

#### 3.5.4.9. *Non-structural-protein antibody ELISA (NSP)*

Research should be undertaken preferably through a CRP to adapt the FMD ELISA for detection of antibodies to non-structural proteins for separating vaccinated from infected animals and a CRP.

### 3.6. **Babesiosis group**

#### 3.6.1. **Conclusions**

##### 3.6.1.1. *Use of ELISA*

The ELISA kit for the determination of antibodies to *Babesia bovis* proved to be appropriate for use in Latin American countries although the “cut-off” point needed to be determined for each country. This assay is particularly appropriate for epidemiological studies to determine conditions for establishing enzootic stability and the most appropriate control/intervention strategy.

##### 3.6.1.2. *Epidemiology*

Surveys carried out by the Contract holders showed that the transmission of *Babesia bovis* varied not only from country to country but also from regions within most countries and depended on the geography, climate, breed of cattle, acaricide treatment and infection rate of *Boophilus microplus*, the vector of *Babesia bovis*. Some Contract holders had difficulties in obtaining or selecting sample sub-populations of cattle that were truly representative of the whole population.

### 3.6.1.3. *Standardization*

These studies highlighted the need for standardization and international validation of such assays if conclusions are to be meaningful.

## 3.6.2. **Recommendations**

### 3.6.2.1. *Promotion of a standardized assay*

The use of an internationally validated and standardized assay and protocol to obtain international recognition for the ELISA as a standard assay for the diagnosis of babesiosis should be promoted.

### 3.6.2.2. *Establishing a serum bank*

Every effort should be made to expand national banks of sera by sampling regions where *Boophilus microplus* is present. In addition to serum collection, each serum should be identified according to the following criteria: age, sex, breed, geography, climate, acaricide treatment and *Babesia bovis* vaccination status. When analyzing data all parameters should be assessed in determining the risk of babesiosis outbreaks. On this basis meaningful recommendations for appropriate babesiosis control strategies can be made to national authorities.

### 3.6.2.3. *ELISA for control programme*

The use of ELISA to improve control programmes against *Babesia bovis* should be promoted.