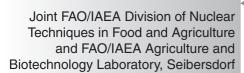
# ANIMAL PRODUCTION AND HEALTH



International Atomic Energy Agency Vienna







Issue No. 35	December 2001

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#### TO THE READER

Dear Colleague,

The events of 11 September will already have affected many of us, but for those involved in laboratory activities related to infectious agents. I believe there is a special significance brought about by the concept of bio-terrorism and the malicious release of anthrax in the USA and beyond. In the longer term, I see that this will significantly change the way we are able to work with such agents. Concepts of biosecurity and traceability of these agents will remove much of the freedom we currently enjoy in veterinary laboratories. In future, it is likely that we will need to keep detailed records of all potentially dangerous pathogens and account for their production, storage, and use in ways that will prevent, or at least, significantly reduce the risk of their use as bio-weapons. In the longer term, this will profoundly change the way we work in veterinary laboratories.

In the short term, there are however several issues that immediately arise. First and foremost, I see that we have a special role in informing all around us of the realistic risks and threats that exist. The media is having a fine time with this whole area, and there are many half-truths and inaccuracies being disseminated which can give rise to serious misconceptions. It should be our role as informed and knowledgeable people in this area to assist others in understanding what these pathogens can, and cannot do, in order to permit good science and common sense to prevail.

We must also be vigilant. Many of our laboratories have potential bio-weapons and the means to propagate these. At this time, we must ensure that we remain accountable for these agents and that we report any suspicious activities or concerns to the appropriate authorities. None of us can condone bio-terrorism no matter from what political or geographical area of the globe we are located, and we must make sure it is not our laboratories that provide a source of material for these activities.

The third issue is somewhat more upbeat. Whilst none of us would welcome the arrival of BSE, the outbreaks of FMD or CSF in Europe or the use of anthrax as a bio-weapon, the fact remains that there has been a new global focus on livestock disease. Whether this is related to poverty reduction, livestock trade, aspects of food safety, or the risk to human health, we are at present on the centre stage. There is a global recognition that the level of support for research into ways of

combating many of these diseases has been inadequate and that funds must now be allocated to redress this situation. A number of organizations including the World Bank, FAO, OIE, ILRI, IFRPI and national development Agencies such as the UK DfID have been looking at what are the priority areas and how best the problems should be tackled.

The World Bank, in particular, is contemplating a livestock "Challenge Programme" that aims to develop a new mechanism for funding livestock research with a focus on poverty reduction but including aspects of livestock trade and food safety. Linked to much of the excellent work undertaken by ILRI on behalf of DfID in identifying priority areas during the past six months, the support will be looking both at technology research (improved vaccines and diagnostics) and at ways of re-aligning policy and veterinary service delivery to ensure impact and change. You may rest assured that we in this Subprogramme will do our utmost to ensure that you. the scientists in developing countries, have an opportunity to become involved and benefit directly from these new endeavors. You, for your part, should use this opportunity to lobby at your level and in your countries for resources to assist you undertake research and to develop systems for the uptake of the outputs of the research. However unfortunate the circumstances, we may not get an opportunity of this nature again and we all need to make the most of this situation to help improve the well-being of livestock keepers and their beneficiaries throughout the rural areas of the developing world.

As usual, I would like to take this opportunity to highlight a few new areas in the Sub-programme. In overall terms, we are presently re-directing our programme of support towards the use of biotechnology and gene-based technologies for improving both livestock production and livestock health. I would like to draw your attention to the preliminary report in this Newsletter of an FAO/IAEA consultants meeting that we held in November on this very topic. This meeting was able to provide us with focus and direction within this general area and outlined two new FAO/IAEA Co-ordinated Research Programmes that we hope to start in late 2002 or early 2003. We will of course be describing these in more detail in the next edition of the Newsletter and asking you to submit research proposals. However, from this report you can already see the

areas where we see we are best able to provide support. As this process of re-direction gains momentum, we will be looking to our Symposium in 2003 to confirm the approaches we have already taken and provide perhaps new areas where we can assist. Again, I would like to draw your attention to the update provided by Harinder Makkar, on the progress being made in planning for this important meeting.

I would also like to mention activities at our laboratory in Seibersdorf that are supporting this re-direction. Under the guidance of Adama Diallo, we now have laboratories and the related equipment to undertake gene study and sequencing, to undertake gene expression and manipulation and to further develop technologies such as PCR for use in your countries. We have already identified a number of fellows who will spend six months to a year with us working on these technologies, and we would welcome further fellowship requests from those of you who

wish to benefit through training on these technologies within our own laboratories.

On the staff side, there have been few changes. Roland Geiger, who was a staff member of the Sub-programme for some 7 years, has returned for a six-month spell and has been assisting us develop distance learning programmes for use within the animal health programme.

All that remains for me then, is to take this opportunity to wish you all the very best for the coming year and hope that we continue to provide you with the support you need and deserve.

Martyn Jeggo

Head, Animal Production and

Health Section

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The Animal Production Unit, Seibersdorf, is a collaborating Center for ELISA and molecular technologies in animal disease diagnosis for both the OIE and WHO.

#### **B. FORTHCOMING EVENTS**

Second Research Co-ordination Meeting of the Co-ordinated Research Project on the Use of Non-structural Protein of Foot-and-Mouth Disease Virus to Differentiate Between Vaccinated and Infected Animals (D3.20.20)

Technical Officer: John Crowther

This RCM will be held from 4 to 8 March in

Geelong, Australia.

A detailed status report of this Co-ordinated Research Project is given on page 20 of this Newsletter.

# Project Review, Co-ordination and Planning Meeting of the AFRA III-2 Project on Improving and Increasing Milk and Meat Production (RAF/5/046)

Technical Officer: Oswin Perera

This meeting will take place from 4 to 8 March

2002 in Addis Ababa, Ethiopia.

It will review progress, plan future activities and identify inputs required from national sources and IAEA.

# First Research Co-ordination Meeting of the Co-ordinated Research Project on Integrated Approach for Improving Small-scale Market-oriented Dairy Systems (D3.10.23)

Technical Officer: Oswin Perera

This meeting will be held from 8 to 12 April 2002 in Vienna, Austria. It will review and refine work plans of Contract holders for the first phase of the project, provide information and training to facilitate the conduct of a participatory rural appraisal (PRA) and an

economic opportunity survey (EOS).

A test version of a computer database for recording, analysing and reporting data (Livestock Information Management Application – LIMA) will be demonstrated and discussed.

# RCA Workshop of National Consultants on Evaluation of Breeding Bulls and Semen Quality Control (RAS/5/035)

Technical Officer: Oswin Perera

This Workshop will be held from 22 to 26 April 2002 in Faisalabad, Pakistan. The meeting will consider the aspects stated below and arrive at a consensus on the best procedures and practices to be adopted to suit conditions and needs in RCA Member States:

Selection, management and health control of AI bulls.

- Semen technologies from collection through processing to storage.
- Delivery and follow-up of field AI services to farmers.

As a background document, it will use the guidelines for use in AFRA Member States under the AFRA III-2 project (RAF/5/046). The meeting will determine and undertake any modifications and additions required for RCA Member States.

# Regional Co-ordination Meeting under the Regional IAEA Technical Co-operation Project RAF/5/053 and within the Auspices of the OAU/IBAR PARC Programme

Technical Officer: Martyn Jeggo

This RCM will be held in Kenya from 22 to 26 April 2002. This meeting will involve heads of laboratories and project counterparts involved under this project.

The purpose of the meeting will be to:

Review the project achievements and exchange information amongst the participating countries.

- Discuss the problems experienced and the ways in which these can be remedied.
- Review progress made in the implementation of follow-up actions from the Regional Workshop on "Update on technologies for the Surveillance of Rinderpest Freedom"
- held in Dakar, Senegal in November 2001.
- Discuss links with IAEA's regional project RAF/5/053, the OAU/IBAR PACE programme and PACE national programme.
- Identify future needs for IAEA's inputs to Member States in support of PACE.

#### National Training Workshop on Livestock Disease Control (YEM/5/002)

Technical Officer: John Crowther

This three-week national training Workshop on

livestock disease control will be held in April 2002 in Sanaa, Yemen.

Workshop on SADC Veterinary Laboratory Capacities and Accreditation Procedures under RAF/5/053

Technical Officer: Martyn Jeggo

This Workshop will take place in April 2002 in one of the SADC countries and will bring together heads of national veterinary laboratories from SADC to discuss current capacities for the diagnosis of the major diseases affecting livestock trade in the region and to further consider progress in each laboratory towards international veterinary laboratory accreditation.

Fourth Research Co-ordination Meeting of the Co-ordinated Research Project on the Use of Nuclear and Colorimetric Techniques for Measuring Microbial Protein Supply from Local Feed Resources in Ruminant Animals (D3.10.22)

Technical Officer: Harinder Makkar

This meeting will be held from 6 to 10 May 2002 in Hue, Vietnam. Nine Research Contract holders, six Agreement holders and the Technical Officer will participate in this

meeting. The purpose of the meeting is to review the work conducted and to formulate conclusions and recommendations. This project will conclude with this meeting.

Third Research Co-ordination Meeting of the Co-ordinated Research Project on the Effectiveness of Vaccination Strategies Against Newcastle Disease and Gumboro Disease Using Immunoassay-based Technologies for Increasing Farmyard Poultry Production (D3.20.19)

Technical Officer: Martyn Jeggo

The third RCM of this CRP will take place in Quatre Bornes, Mauritius, from 6 to 10 May 2002. The meeting will review work undertaken since the last meeting in Morogoro, Tanzania, in September 2000. In particular, an examination of the effectiveness of the intervention strategies that have been undertaken at specific locations will be carefully evaluated to ensure that firstly, these are having the desired impact and do not require modification and secondly, that all the

necessary data to measure impact is indeed available. In this context, a standardized database has been circulated (in November 2001) and will be used at the meeting to ensure a co-ordinated approach to data collection and impact assessment.

Work plans under each Research Contract will be developed for the coming 18 months taking the above into account. This will provide a suitable framework to ensure that we can bring this CRP to a fitting conclusion at the next final RCM.

#### **Training Course on PCR**

Technical Officer: John Crowther

A training course on PCR will be held in May 2002 at the Onderstepoort Veterinary Institute, Applied Biotechnology Division, Private Bag X 05, Onderstepoort 0110, South Africa. The course organizer is Gerrit Viljoen (gerrit@moon.ovi.ac.za). Please contact him directly about the course contents. The course will be open for 25 participants and there is a strong chance that some funding for the course

will also come from the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Those interested should contact Gerrit Viljoen.

You might wish to consider applying for an IAEA TC fellowship training for the period of the course. If so, remember to obtain the application forms and send them through your Energy Ministry or Commission to the Department of Technical Co-operation, IAEA.

# AFRA Task Force Meeting to Update and Customize the AIDA Database for Integration with Existing Data Recording and Management Systems, for use by National AI Services (AFRA III-2, RAF/5/046)

Technical Officer: Oswin Perera

This meeting will take place from 13 to 17 May 2002 in Nakuru, Kenya. The objectives are to:

- Review the results and experiences obtained in the use of AIDA (Artificial Insemination Database Application) by participants.
- Consider the current systems of recording, analysing and reporting AI data in national AI programmes of five selected AFRA Member States.
- Review the structure and content of AIDA-Asia and SPeRM (Semen Processing Records Management) which were developed under the RCA project RAS/5/035, and determine features that are applicable to African conditions.
- Define a suitable "Minimum Data Set" (MDS) and individual cow record sheet to be adopted for AI recording in the field.
- Identify and document the modifications necessary for customising AIDA for routine use in AFRA Member States.

# RCA Training Workshop on the Standardized and Validated Nuclear-based Technologies, in Particular Those Based on Urinary Purine Derivatives, for Measuring Microbial Protein Supply in Ruminants (RAS/5/035)

Technical Officer: Harinder Makkar

This Training Workshop will be held from 10 to 21 June in Kuala Lumpur, Malaysia, in cooperation with the Government of Malaysia, MARDI and UPM. The objective of the course is to provide training on the standardized and validated nuclear-based technologies. particular those based on urinary purine derivatives, for measuring microbial protein supply in ruminant. The course will consist of lectures on the metabolism of nucleic acids, basis for the use of urinary purine derivatives as markers for rumen microbial protein, and estimation of rumen microbial protein supply to the animal from the measurement of xanthine allantoin, uric acid. and hypoxanthine. These lectures will be blended with demonstration and practical laboratory work on the methodologies, data analysis and interpretation of results. The course is open to groups already participating in the regional project RAS/5/035. Preference will be given to candidates having experience in working with the *in vitro* gas method, Tilley and Terry and/or the *in sacco* method. Participants are expected to have good knowledge of rumen fermentation, and biochemistry of protein and carbohydrate metabolism in ruminants. As the course will be conducted in English language, participants must have a good command of the English language.

#### National Training Workshop on Livestock Disease Diagnosis and Epidemiology (MON/5/011)

Technical Officer: John Crowther

This national Training Workshop will be held in Mongolia during the first three weeks of July.

# First Research Co-ordination Meeting of the Co-ordinated Research Project on the Development of Strategies for the Effective Monitoring of Veterinary Drug Residues in Livestock and Livestock Products in Developing Countries

Technical Officer: Andrew Cannavan

This RCM will be held in 2002 in Vienna, Austria (dates to be finalized). Work plans will be defined for the first phase of the project, the development and validation of methods. Specific compounds and matrices of

importance to each Contract holder will be selected and the most appropriate analytical techniques for each identified. The RCM will conclude with a one-week Workshop, held at the Agency's Laboratories, Seibersdorf, on basic immunoassay and HPLC techniques.

# Workshop on Establishing Quality Systems in Veterinary Diagnostic Testing Laboratories (INT/0/060)

Technical Officer: Axel Colling

This Workshop will be held in Bogota, Colombia, from 2 to 6 September 2002. The objectives of the Workshop are the following.

- 1. To have a clear picture of the status of implementation of the project with identified areas of constraints and conclusions and recommendation how to overcome these:
- 2. To have individual work plans in terms of expert services, further production of quality documents (Quality Manual, SOPs), EQC panel round or proficiency test round; and

3. To have an updated and improved version of the FAO/IAEA "Guidelines for Establishing Quality Systems in Veterinary Testing Laboratories".

At a later stage it is foreseen to extend these activities through interregional Workshops in which the quality assurance co-ordinators will discuss the adaptation and dissemination of training material within their laboratories, the status of implementation and the constrains encountered. Based on their experiences other laboratories in each region will be identified to implement quality systems on a larger scale.

# International Symposium on Application of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries

Technical Officers: Martyn Jeggo and Harinder Makkar

This Symposium will be held from 6 to 10 October 2003 in Vienna, Austria.

The basic structure of the Symposium will be:

- Plenary lectures
- > Theme-specific sessions
- Round table discussion/discussion forum

Detailed information on the Symposium is available at the Website.

 $\underline{http://www.iaea.org/programmes/nafa/d3/index-symp2003.html}$ 

Some important dates:

March 2002: Release of second notice; September 2002: Announcement letter inviting extended synopsis and grant applications; End of January 2003: Receipt of extended synopsis and grant applications.

No registration fee will be charged to participants.

Suggestions and comments on the Symposium should be sent to: H.Makkar@iaea.org

#### C. PAST EVENTS

# AFRA Task Force Meeting to Harmonize Procedures for Selection and Management of AI Bulls and Use of Semen Technology in African Countries (RAF/5/046, AFRA III-2)

Technical Officer: Oswin Perera

This meeting was held from 7 to 11 May 2001 in Arusha, Tanzania. Project counterparts from six AFRA Member States with expertise in technical aspects of managing AI bulls and semen processing participated, together with an IAEA expert (Dr. David Galloway from Australia) and the Technical Officer. Based on the presentations made, discussions and group work, a draft version of a "Manual of Recommended Procedures for the Cattle Artificial Breeding Industries in African Countries" has been prepared and is currently being finalized in consultation with all national Project Co-ordinators. The draft version has the following contents:

### 1. Selection, Health and Management of Bulls

- 1.1. Selection of bulls
- 1.1.1. Milk recording
- 1.1.2. Beef recording
- 1.1.3. Likeability
- 1.1.4. Reproductive efficiency
- 1.2. Genetic improvement
- 1.2.1. Cornerstones of genetic improvement
- 1.2.2. Cross breeding
- 1.3. Statutory requirements for disease testing and quarantine
- 1.3.1. Registration of premises
- 1.3.2. Approval of animals as donors
- 1.3.3. Keeping and care of animals
- 1.3.4. Technical activities at centres
- 1.3.5. Records to be kept at centres
- 1.4. Management of bulls
- 1.4.1. Housing
- 1.4.2. Feeding
- 1.4.3. Handling
- 1.4.4. Health
- 1.4.5. Records

### 2. Semen Technology and Field Practices

- 2.1. Semen technology
- 2.1.1. Collection area and facilities
- 2.1.2. Preparation of bulls
- 2.1.3. Artificial vaginas
- 2.1.4. Electroejaculators
- 2.1.5. The collector

- 2.1.6. Collection procedure
- 2.1.7. Evaluation of semen
- 2.1.8. Dilution and extension (deep-frozen, chilled and room temperature)
- 2.1.9. Packaging (deep-frozen, chilled and room temperature)
- 2.1.10. Preservation and storage (deep-frozen, chilled and room temperature)
- 2.1.11. Post packaging quality control
- 2.2. Field practices
- 2.2.1. Heat detection
- 2.2.2. Body condition score at calving and at insemination
- 2.2.3. Semen handling and insemination technique (deep-frozen, chilled and room temperature)

# 3. Delivery of Improved Genetics and Breeding Services to Farmers

- 3.1. Organization
- 3.1.1. Artificial insemination services (communication, transport)
- 3.1.2. Co-operatives and farmer organizations
- 3.1.3. Linkages, information, education and extension
- 3.1.4. Development of services and increasing numbers of animals inseminated
- 3.2. Genetics, product quality and marketing
- 3.2.1. Milk
- 3.2.2. Meat
- 3.2.3. Semen
- 3.3. Farmer services, records and economics
- 3.3.1 Standard of management and heat detection
- 3.3.2. AI technician, technique and remuneration
- 3.3.3. Diagnosis of pregnancy and nonpregnancy (manual, progesterone RIA)
- 3.3.4. Records and their use (AIDA, feedback to AI centre, technician and farmer)
- 3.3.5. Value of services (cost and benefits)
- 3.3.6. Herd health services in relation to AI

#### **ANNEXES:**

- Record of Examination of Bulls for Breeding Soundness
- 2. Statutory requirements for disease testing and quarantine
- 3. Application for approval of a bull for use in AI
- 4. Nutrition of bulls

- 5. Body condition scoring scheme
- 6. Semen evaluation using Nigrosin Eosin stain
- 7. Semen diluents and extenders
- 8. Individual cow AI record (Minimum Data Set)
- 9. Liquid nitrogen safety precautions
- 10. Liquid nitrogen and semen dispatch form

# Third Research Co-ordination Meeting of the Co-ordinated Research Project on the Monitoring of Contagious Bovine Pleuropneumonia in Africa Using Enzyme Immunoassays (D3.20.18)

Technical Officer: Martyn Jeggo

The third RCM of the CRP entitled "Diagnosis Contagious Control of Bovine Pleuropneumonia (CBPP) in Africa" was held under the auspices of the OAU/IBAR PACE Programme in Nairobi, Kenya, from 18 to 22 June 2001. The meeting was attended by Research Contract holders from 10 African countries (Côte d'Ivoire, Ghana, Kenya, Mali, Namibia, Nigeria, Ethiopia, Uganda, Tanzania and Zambia), two Agreement holders from CIRAD/EMVT, France, and the Scottish Agricultural Institute, UK, staff of the OAU/IBAR PACE Programme Co-ordination Unit and the PACE Epidemiology Unit, an FAO Technical Officer, Dr. W. Amanfu, and a consultant expert on penside assays, Dr. J. March (UK). Dr. A. Diallo (Head, Animal Production Unit, NAAL) attended much of the meeting, providing technical support to the deliberations and ensuring co-ordination of support available through NAAL both to PACE and this CRP.

The meeting provided an essential coordinating link between the studies being undertaken under this CRP, the programmes of PACE (Pan African Control of Epizootics) and a large regional FAO project on CBPP in West Africa. The presentations from the PACE Programme Coordination Unit (PCU) provided detailed information on the activities of PACE both in a regional capacity and through the individual national PACE projects.

Presentations were given by each Research Contract holder on work undertaken to date under the CRP including details of IQC data and CBPP antibody detection assay validation activities. CBPP is considered the most important livestock disease in Africa, at present, when taking into account its effect both on cattle trade and cattle productivity. The current situation of CBPP in the participants' countries and the latest results on

research on CBPP vaccine and diagnosis were presented and discussed. On Wednesday, 20 June a Workshop was held at the Kabete Veterinary Laboratories during which issues with the ELISA were discussed and the group were shown a recently developed pen-side test both for the detection of *m.mycoides* (the causative mycoplasma of CBPP) in CBPP infected animals and for antibodies developed against CBPP.

#### **Recommendations and Conclusions**

#### 1. Diagnosis of CBPP

While previous work under this FAO/IAEA CRP has internationally validated the CBPP cELISA and is recognized as an OIE approved assay for CBPP, the need to improve application in the field was stressed. Recent assay internal quality control (IQC) data had shown that the CBPP ELISA kit (supplied from CIRAD-IEMVT, France) had developed problems with the assay antigen. These had been rectified, but the need was stressed to continually assess IQC data to ensure that such problems are recognized as soon as possible. It was agreed that all IQC data should be submitted through Dr. Karim Tounkara (an IAEA Technical Co-operation expert based within the PCU of PACE). It was further agreed that Dr. Tounkara should act as a coordinator for all laboratory activities of PACE and for the support being provided through this to national PACE CRP and **SADC** laboratories.

#### 2. Studies under the CRP

Given that the assay has now been validated, it was agreed that the primary activities for all Contract holders was to undertake prevalence studies on CBPP in their countries. It was foreseen that sera and related data would be collected in many instances through resources available under the national PACE projects but that testing and analysis would be undertaken by the Research Contract holders It was

recognized that there were no agreed guidelines on how to conduct such surveys for determining the prevalence of CBPP. The meeting, therefore, recommends that surveillance guidelines for CBPP be developed by the PCU of PACE.

Other studies will focus on validation of the penside assays, on evaluating antibody responses following CBPP vaccination and on abattoir surveys.

# 3. Relationship between CRP and PACE/SADC programme

Considering the diversity of CBPP control measures, it is imperative that countries harmonize and synchronize these on a regional basis. This should be facilitated through the existing structures, i.e. OAU/IBAR (PACE) and SADC whose objectives include delivery of improved veterinary and animal health services and enhancing national capacities to sustain epidemio-surveillance systems. Studies undertaken through this CRP should be incorporated into these activities, and the coordinating role of Dr. Tounkara will be critical to this.

#### 4. Areas of further research

PACE has resources available for conducting rinderpest and CBPP research. This meeting deliberated areas of importance outside the scope of this current CRP and recommended that PACE consider the following as priority areas for support in terms of the future control of CBPP:

- An improved understanding of the effects of treatment on CBPP infected animals.
- The development of CBPP vaccines that give longer immunity.
- The development of a test that differentiates CBPP vaccinated from infected animals.

#### 5. Vaccine and vaccine production

The meeting recognized that the current CBPP vaccine provides significant protection to cattle when properly applied. There is however a need to improve the potency of the vaccine and standardize its application. It was recommended that each country using CBPP vaccine should have the ability to monitor the titre of mycoplasma organisms in the vaccine at the time of administration.

#### 6. Training needs for the Contract holders

The meeting recommended training in epidemiology for Contract holders to strengthen the ability of laboratory scientists to manage and interpret surveillance data.

#### 7. The final RCM for this CRP

The meeting recommended that this should be held in Vienna, Austria, in June 2003. This meeting should be held in conjunction with the final meeting of the FAO Regional project on CBPP.

# Second FAO/IAEA Interregional Consultants Meeting/Workshop on Developing Standardized Training Material to Assist FAO/IAEA Member States to Establish Quality Systems for Veterinary Diagnostic Laboratories (INT/0/060)

Technical Officer: Axel Colling

The meeting was held from 16 to 20 July 2001 in Onderstepoort, Republic of South Africa.

The objectives of the Meeting/Workshop were:

- (a) to monitor the implementation of the project;
- (b) to develop individual work plans; and
- (c) to review the "FAO/IAEA Guidelines".

The Meeting was hosted by the Onderstepoort Veterinary Institute (OVI) and took place within the OVI's facilities. It was attended by six project counterparts from Côte d'Ivoire, South Africa, Malaysia, Philippines, Colombia and Peru, two consultants from Australia, U.K., one observer from Brazil, representatives from the Directorate of Veterinary Services, OVI, the South African Accreditation Service and the Technical Officer.

#### Expected results:

- To have a clear picture of the status of implementation of the project with identified areas of constraints and conclusions and recommendations how to overcome these.
- To have individual work plans in terms of expert services, further production of

- quality documents (Quality Manual, SOPs), EQC panel round or proficiency test round, suggested venue and dates for the next meeting.
- To have an updated and improved version of the FAO/IAEA "Guidelines for establishing Quality Systems in Veterinary Testing Laboratories" and a timeframe to translate this document into Spanish and French.

#### Development of the Workshop

As outlined in the Agenda the Workshop was divided in four sessions:

**Session I**: Quality Systems in Veterinary Diagnosis Laboratories: Status and needs.

**Session II**: Discussion and review of individual quality documents produced.

**Session III:** Review of document "Guidelines for Establishing Quality Systems in Veterinary Testing Laboratories".

**Session IV**: Individual work plans and meeting report.

#### **Summary**

During the first year of the project, over 50% of the activities under a Quality System were implemented. **Participants** started different levels and expert visits were undertaken prior to the Workshop to two laboratories in Malaysia and the Philippines. This was taken into account when evaluating the progress in different laboratories. One laboratory wishes to apply for accreditation by 2002. Based on the individual status, work plans were established for the remaining year. These include expert missions, further production of quality documents, proficiency test rounds and the plan for a meeting in 2002. The FAO/IAEA Guidelines were reviewed and the updated version will be translated into French and Spanish and made available on the FAO/IAEA webpage.

It was felt that there is a lack of proactive management commitment to establish a QS and the experiences of this project show that most of the initiatives for implementation had come in an "upstream" manner. It was concluded that the "blessing" of the management was not enough to implement a sustainable QS and ways of how to ensure better commitment were discussed. All participants and consultants plus one observer from Brazil attended the meeting.

#### **Conclusions and Recommendations**

#### **Project progress**

Conclusion: Very good progress in establishing QS was achieved in most participating laboratories during the first year of the project (over 50% during the first quarter of the TC project (2001/2002)). Counterparts started from different levels and expert visits were undertaken prior to the Workshop to two counterparts (Malaysia and Philippines). This was taken into account when evaluating the progress in different laboratories (see implementation rates).

Recommendation: It is recommended that the remaining activities, e.g. expert visits, EQA round, ongoing production of quality documents and a second consultants meeting during 2002 be implemented as stated in the work plans.

The second expert visits should include an informal (internal) audit to train counterparts.

#### **FAO/IAEA Guidelines document**

Conclusion: The "Guidelines" document is a useful guide to establish QS in veterinary testing laboratories. Two new elements, measurement uncertainty and occupational health & safety, were incorporated into the document and some corrections made.

Recommendation: The revised "Guidelines" should be made available on the web and translated into French and Spanish. Practical examples of a QM and SOPs would be helpful in setting up a QS in other laboratories.

#### Accreditation

Conclusion: Clarification about the progress of the OIE Standard regarding its acceptance with national/international accreditation bodies is crucial to veterinary laboratories intending to implement the OIE Standard.

Recommendation: FAO/IAEA will contact OIE on this matter. If it is likely that the OIE Standard is not accepted, then it may be necessary for laboratories to consider alternatives, e.g. ISO IEC 17025.

#### **Proficiency test rounds**

Conclusion: Participation in proficiency test rounds is a key element in the OIE standard and found to be a useful exercise by all participants.

Recommendation: Participation in External Quality Assurance rounds should be continued.

#### Management

Conclusion: Strong management commitment is crucial for implementation of a QS. From the practical experiences during the first year, the conclusion was that a lot of upstream work had to be done and that the "blessing" of the management was not sufficient in itself to implement a sustainable QS. The workload and responsibility for one person was considered too much.

Recommendation: It is imperative that management becomes pro-active with demonstrable commitment to the QS initiative. The first important step would be to assign new tasks, e.g. appointment of a Quality Manager as indicated in the OIE Standard under 4.2.2.

#### Training

Conclusion: Further training of participants, e.g. in internal auditing, is needed.

Recommendation: This can be achieved through national accreditation bodies. The possibility to further assist in training, e.g. under the TC project and/or local funds, should be considered during the next meeting in 2002 or during expert visits.

#### Maintenance and calibration

Conclusion: Maintenance and calibration of equipment is likely to be a major obstacle in establishing a QS that complies with the requirements of the OIE Standard.

Recommendation: Assistance from the TC project and through management commitment should be investigated.

# SADC/FAO/IAEA TC Workshop on Diagnostic Kit Production and Related Quality Assurance Issues in South African Developing Countries (SADC), RAF/5/043

Technical Officer: Martyn Jeggo

This SADC/FAO/IAEA Workshop took place in Harare, Zimbabwe, from 23 to 27 September 2001 and was attended by participants from 12 countries in the region (South Africa, Zimbabwe, Lesotho, Malawi, Mauritius, Botswana, Namibia, Mozambique, Zambia, Tanzania, Swaziland and Angola). Each participant was employed in his respective national veterinary diagnostic laboratory and in most countries, was the person responsible for quality assurance. The overall aim of this SADC network of laboratories created through this Workshop is to create a harmonized and standardized approach to the diagnosis of the diseases affecting livestock in the region. The focus during this two-week Workshop was to introduce concepts of quality assurance with the aim of achieving international laboratory accreditation. A second objective was to start the process of producing and distributing quality assured and standardized diagnostic kits to the major diseases in the region, with individual laboratories in the network taking responsibility for one or a number of kits.

The first three days focused on the production, distribution, and quality assurance of diagnostic kits (with a focus on brucellosis).

This Workshop was facilitated by Martyn Jeggo. Subsequently, the focus was on quality assurance and the development of plans for a pathway to accreditation for each of the national laboratories attending the meeting. This component was overseen by Dr. Janusz Proweska from the Onderstepoort Veterinary Institute in South Africa.

One output from the meeting was a clear plan for each laboratory that marked their way towards laboratory accreditation. Whilst it is obvious that each laboratory will require its own time to reach certified accreditation, the process is similarly for all SADC laboratories and this workshop provided an ideal opportunity to develop this process for laboratories through the region.

We are indebted to the Government of Zimbabwe who provided considerable technical, logistical, and financial support for this Workshop. They provided an outstanding venue in Harare and all the necessary local support to make this meeting a resounding success. We would particularly like to thank Charles Katsende for his support throughout the Workshop, Dr. Ushewokunze-Obatolu for her organization and support and Dr. Chiteka, the Principal Director Agricultural Services, for the overall support for this meeting.

Third Research Co-ordination Meeting of the Co-ordinated Research Project to Develop and Validate Standardized Methods for Using Polymerase Chain Reaction (PCR) and Related Molecular Technologies for Rapid and Improved Animal Disease Diagnosis (D3.20.17)

Technical Officer: John Crowther

The third RCM was held at the IAEA, Vienna, from 22 to 25 October 2001. The meeting was attended by seven of the eight Research Contract holders and the four Agreement holders as shown in the Table at the end of this article. Dr. M. Hussain was training at the time of the meeting.

Papers were delivered by the Research Contract holders on the past developments using PCR to help diagnose rinderpest and peste-des-petits ruminants (PPR) and to differentiate viruses of the vesicular/stomatitis complex as well as in the diagnosis of CBPP. The Agreement holders also presented papers dealing with advances in PCR technology and the latest research using molecular techniques into rinderpest and PPR. The meeting considered the preparation of a manual dealing with the technology transfer and use of the PCR in developing countries, particularly aimed at those involved in the Global Rinderpest Eradication Programme (GREP). It was concluded that the manual will contain the sections stated below and will be modelled along guidelines from the OIE facilitating the standardization of PCR.

- 1. The aim will be to produce an educational and practical manual covering standardized protocols for rinderpest, PPR, BVD, IBR, MCF, CBPP and FMD.
- 2. A list of experts in each disease will be prepared.
- 3. The background science involved in performing the protocols will be shown as well as example SOPs.
- 4. The setting up of laboratories as well as GLP will be included.
- 5. Elements needed to allow accreditation will be included and attention to the guidelines being prepared through the IOE will be made

to allow the material to be used directly in any standardization data. This includes IQC, EQA, e.g. ring testing.

6. The role of PCR in diagnosis and differentiation of viruses, along with serological techniques will be addressed. This will be aimed at all levels of scientist and management to allow a better cost-benefit analysis of the PCR to be examined nationally.

#### Recommendations and conclusions

- 1. It was recommended that a manual as outlined be prepared by the end of February 2002.
- 2. It was recommended that the participants should be active in the preparation of the OIE guidelines for PCR through their experiences in technology transfer and expertise in performing the method.
- 3. It was recommended that a ring test should be made in the next year to allow comparison of PCR results in different laboratories and to act as a model for this testing to OIE.
- 4. It was concluded that commercial kits were not needed for PCR, but that blocks of reagents should be assembled and sent from single distribution points to avoid multiple clearance when sent to developing countries. It was recommended that the APU laboratories at Seibersdorf should play an active role for this purpose both for general reagents and for standardization of control samples.
- 5. It was recommended that the final document of the CRP should be published as an OIE supplement.
- 6. It was the conclusion of the meeting that it had proved feasible to transfer the PCR to developing countries and that the major problem was obtaining samples through lack of infrastructure in the field.

RC number	Country	Last name
9796/RB	Cameroon	Yaya Aboubakar
9358/RB	Côte d'Ivoire	Emmanuel Couacy-Hymann
9356/RB	Ethiopia	Abraham Gopillo
9360/RB	Kenya	Karim Tounkara

RC number	Country	Last name
9359/RB	Kenya	Henry Mathuko Wamwayi
10965/RB	South Korea	Nam Yong Park
11614/RB	Tanzania	Joram Josephat Buza
11819/RB	Pakistan	Manzoor Hussain
9362/CF	South Africa	Gerrit Johannes Viljoen
9357/CF	France	Adama Diallo.
9363/CF	Sweden	Sandor Belak
9365/CF	United Kingdom	Tom Barrett

# Regional Training Workshop to Train Trainers on Methodologies and Use of Information Communication Technology (ICT) Based Training Materials in Animal Reproduction and Health (RAF/0/013)

Technical Officers: Oswin Perera and John Crowther

The Workshop was hosted by the National Radiation Commission (NRC) and the National Artificial Insemination Centre (NAIC), Arusha, Tanzania, and was held from 6 to 10 November 2001 at the MS-Training Centre for Development Co-operation (MS-TCDC). It was attended by all 21 selected participants (Ethiopia 6, Sudan 3, Tanzania 6, Uganda 6), and was assisted by three IAEA experts and one Technical Officer.

This Workshop was held under the framework of the regional Technical Co-operation project "ICT-based Training to Strengthen LDC Capacity" (RAF/0/013) and comprised activities in three themes in animal production and health: (a) refresher training of artificial insemination (AI) technicians; (b) detection and reporting of rinderpest; and (c) tsetse/trypanosomosis control.

The training commenced with lectures on the general aspects of ICT (by Mr. Jouseph Aboulfaki), covering concepts, methodologies

and tools, and considerations in developing ICT-based teaching/learning materials. The three resource persons for the thematic groups (AI - Dr. Oswin Perera; rinderpest -Dr. Roland Geiger; and tsetse/trypanosomosis - Dr. David Bourn) then reviewed currently available electronic information (on the Internet, on CDs. etc), demonstrated some of the materials collected and/or developed by them, and outlined the activities to be undertaken by each thematic group for the remainder of the Workshop. Subsequently, the three groups worked separately with their respective experts, while the expert on general aspects of ICT assisted each group in turn as required. Field visits were made by each group to an institution relevant to their work. The groups came together on the final day to draft, and finalize conclusions discuss and recommendations.

A full report on the Workshop containing conclusions and recommendations of all three groups is available in the Africa Section and the Animal Production and Health Section

# Second RCM on the Use of Nuclear and Related Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Feeding Ruminant on Tanniniferous Tree Foliage (D3.10.22)

Technical Officer: Harinder Makkar

This RCM was held from 19 to 23 November 2001 in São Paulo, Brazil. Five Research Contract holders and four Agreement holders and Consultants participated in the meeting. The objectives of the meeting were to review

the work conducted and plan studies for the second phase of the project. A full report of the meeting will be given in the next issue of the Newsletter. As of January 2002, this report will also be available on our Website.

# Regional Training Workshop Update on Technologies for the Surveillance of Rinderpest Freedom Supported under OAU/IBAR/PACE programme RAF/5/053

Technical Officers: Mamadou Lelenta and Martyn Jeggo

This Training Workshop was held from 19 to 30 November 2001 in Dakar, Senegal, in cooperation with the Government of Senegal within the framework of regional Technical Co-operation project RAF/5/053 — Assistance

to OAU/IBAR PACE Programme for the Control and Eradication of Major Diseases Affecting Livestock.

A full report of the meeting will be given in the next issue of the Newsletter.

# Consultants Meeting to Discuss and Make Recommendations on Significance, Suitability and Potential Applications of Gene-based Technologies for Improving Livestock Production in Developing Countries

Technical Officers: Martyn Jeggo, Harinder Makkar and Oswin Perera

The meeting was held from 27 to 30 November 2001 in Vienna, and was attended by the five consultants, Dr. P. Cronje (Australia), Dr. A. Eggen, (France), Dr. J. Gibson (Kenya), Dr. C.S. McSweeney (Australia), Dr. A. Murray (New Zealand) as well as staff from the Animal Production and Health Sub-programme.

The objectives of the meeting were to:

- Identify the areas which could be addressed by the Animal Production and Health Sub-programme, both in a normative and operational capacity.
- Identify important collaborating institutes and organizations and consider methods of appropriate collaboration and co-ordination.
- Consider and develop modalities for support through FAO/IAEA CRP(s), through the IAEA technical cooperation programme and through other mechanisms, e.g. training courses.
- If a CRP is appropriate, describe in detail the objectives of the CRP and the activities that will need to be undertaken to reach these objectives.
- Identify possible areas of activity for undertaking within the Animal Production Unit, Seibersdorf.
- Consider and develop a framework for the FAO/IAEA International Symposium on "Application of genebased technologies for improving animal production and health in

developing countries" to be held from 6 to 10 October 2003 in Vienna.

#### Conclusions and recommendations

- 1) The consultants concluded that gene-based technologies had significance, suitability and applications for improving livestock production in developing countries.
- 2) It was recognized that almost all gene-based research is taking place in the developed world. The question of how these technologies can be transferred and applied to solve problems of the developing world is a major issue that needs to be addressed.
- 3) The model in the developed world tends to focus resources in large specialized institutes. An appropriate model needs to be found to harness this technology for developing countries, and should be discussed at the forthcoming FAO/IAEA symposium.
- 4) It was concluded that characterization of the gene pools of livestock, microbes and forages was an important first step. Information generated from these studies would have important applications for future livestock production in developing countries and there is a need to develop appropriate methods, tools and capacity to facilitate this.
- 5) The consultants felt that the Agency could play an important role in both characterization and application through its existing mechanisms using nuclear and related technologies.
- 6) It should be recognized that livestock production in developing countries is always a part of an integrated agricultural system,

and therefore, development strategies for gene-based applications should consider the total production system. Collaboration with other disciplines such as plant breeding and food safety should be fostered. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture having Sections dealing with these subjects is well placed to establish research and development projects with multi-disciplinary focus.

- 7) The consultants reviewed the plans already made for the Symposium to be held in 2003, made recommendations for improvement, and concluded that it would be important for identification of future research and development areas, and for forging collaborative links.
- 8) Specific areas identified for future research were the following:

#### Development and use of rumen molecular techniques for predicting and enhancing productivity

The overall objective of the project is to improve ruminant performance through a reduction in methane production. The specific objectives are to:

- Reduce the level of methane production by up to 50% in animals fed roughage diets;
- Increase microbial protein and energy supply through reduced methane production using approaches such as inhibitors of methanogens, dietary approaches (e.g., use of polyunsaturated fatty acids or ingredients containing these acids), supplementation strategies, etc.;
- Build in-country capacity to develop and use molecular techniques for studying rumen function;
- Develop molecular probes for quantifying populations of methanogens, fibre degrading bacteria, fungi and protozoa;
- Correlate methane production to methanogen numbers;
- Determine effects of reduced methanogen numbers on fibre degrading bacteria, fungi and protozoa; and

Identify naturally-occurring plant secondary compounds that inhibit methanogens.

#### Improvement of animal productivity in developing countries by manipulation of nutrition in utero to alter gene expression

The overall objective of the project is to improve animal productivity in developing countries by manipulation of nutrition *in utero* to alter gene expression. The specific objectives are to:

- Provide proof of the concept: Does plane of nutrition *in utero* alter gene expression of key metabolic hormones and enzymes for a long period after birth in cattle?
- Quantify the relative impact of pre vs. postnatal supplementation strategies in cattle; and
- Build in-country knowledge and capacity in the use of gene-related techniques for measuring geneexpression, physiological genomics and radioimmunoassay.

#### Gene-based Technologies in Livestock Breeding

a) Characterization of Small Ruminant Genetic Resources in Asia

The overall objective is to generate information and decision support systems for phenotypic and molecular genetic diversity enabling development and implementation of national, regional and global strategies for use and conservation of small ruminants. The specific objectives are to:

- Complete the databases of characteristics and status for a representative set of breeds of sheep and goats of Asia which will complement existing FAO and ILRI data for Africa and Europe;
- Develop capacity within the Asian region to use radio-isotopic micro satellite methods for genotype characterization of ruminants;
- Complete the analyses of regional and global genetic diversity of each species based on molecular data;
- Assess new technologies for diversity assay; and

Make recommendations on their future application for improving ruminant productivity.

# b) Genetic Resistance to Helminths in Sheep

The overall objective is the development of well adapted, productive and disease resistant sheep suitable for small scale market oriented production in the developing world. The specific objectives are to:

- Develop capacity within member states to apply gene-based technologies for improving animal production;
- Demonstrate the principle that phenotypic and molecular data on global livestock is essential for design of national livestock genetic improvement strategies; and
- Produce well adapted, productive and disease resistant sheep suitable for production in several locations in the developing world.

Improvement of tests for African Swine Fever diagnosis and molecular epidemiology analysis of the disease

The overall objective is to improve the effectiveness of national and international

ASF control/eradication campaigns through the use of molecular and serological methods. The specific objectives are to:

- Develop and validate diagnostic tests for the detection of tick infestation through an examination of pig sera (surveillance of the vector in the country);
- Develop and validate PCR test for the detection of the virus in clinical samples and also in the tick vector; and
- Build up a sequence data bank to ease tracing the evolution of the virus strain.
- 9. The collaboration of FAO, other international organizations and partners in advanced research laboratories that have the expertise in gene-based technologies will be necessary for effective implementation of the proposed CRPs.
- 10. The animal production unit can play an important role in the validation of selected techniques and in training of counterparts from developing countries.

As of January 2002, this report will also be available at our Website.

#### D. STATUS OF EXISTING CO-ORDINATED RESEARCH PROJECTS

# Use of Nuclear and Colorimetric Techniques for Measuring Microbial Protein Supply from Local Feed Resources in Ruminant Animals (D3.10.21)

Technical Officer: Harinder Makkar

This CRP is now in the last phase and will conclude in May 2002. It has a total of nine Research Contracts and six Research Agreement holders. It is aimed at developing a simple method, which can readily be used by extension

workers or farmer advisors to identify major problems of nutrition that result in a grossly inefficient rumen digestion of feed and a low level of microbial supply to the host animal. The final RCM will be held from 6 to 10 May 2002 in Vietnam.

# Use of Nuclear and Related Techniques to Develop Simple Tannin Assays for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage (D3.10.22)

Technical Officer: Harinder Makkar

The first phase of this CRP was concluded in November 2001. The main objective of the first phase was to identify a tannin assay or a battery of tannin assays that could predict the biological effects of tannin-containing tree or shrub leaves on ruminants. A Research Co-ordination Meeting was held from 19 to 23 November 2002 in São Paulo, Brazil, to review the work conducted and to develop future work plans. The full report of this meeting will be available at our Website as of January 2002.

Three to four new Research Contracts will be awarded in the second phase of the CRP.

Interested groups are suggested to contact the Technical Officer.

The main thrust in the second phase will be on using the validated tannin assays to evaluate strategies to detanninify tannin-rich tree leaves and browses using simple and economically viable approaches, and to exploit the full benefits of tanniniferous plants as animal feed supplements and as strategic feed reserves in situations of fluctuating nutrient supply. All participating groups in the second phase will undertake work on this common theme. High tannin forages of economic importance for the region should be selected for these studies.

# Standardized Methods for Using Polymerase Chain Reaction (PCR) and Related Molecular Technologies for Rapid and Improved Animal Disease Diagnosis (D3.20.17)

Technical Officer: John Crowther

The CRP has now Research Contract holders from Mali, Côte d'Ivoire, Ethiopia, Kenya and Korea.

The second RCM was held from 22 to 25 October 2001 in Vienna, Austria. A detailed meeting report can be found on page 14 of this Newsletter.

### The Monitoring of Contagious Bovine Pleuropneumonia in Africa Using Enzyme Immunoassays (D3.20.18)

Technical Officer: Martyn Jeggo

This CRP has eleven Research Contracts, three Research Agreements and one Technical Contract for penside test development. The main objective of the CRP is to validate, standardize and utilize the competitive ELISA for the detection of antibodies to contagious bovine pleuropneumonia

(CBPP) through field studies in different African countries. A full update of this project is given in a report on the RCM held in June 2001 in Kenya (see Section B). The next and final RCM will be held in Africa in 2003 in conjunction with a FAO CBPP programme in West Africa.

Assessment of the Effectiveness of Vaccination Strategies against Newcastle Disease and Gumboro Disease Using Immunoassay-based Technologies for Increasing Farmyard Poultry Production in Africa (D3.20.19)

Technical Officer: Martyn Jeggo

The Second Research Co-ordination Meeting took place at the Sokoine University at Morogoro, United Republic of Tanzania, from 4 to 8 September 2000, and the results are being published by Backhuys Publishers by the end of

this year. Please contact us directly if you would like a copy.

There are currently twelve Research Contract and five Agreement holders. The next RCM will take place from 6 to 10 May 2002 in Mauritius.

### The Use of Non-structural Protein of Foot-and-Mouth Disease Virus (FMDV) to Differentiate between Vaccinated and Infected Animals (D3.20.20)

Technical Officer: John Crowther

Kits

Three commercial kits now exist to measure antibodies. These are:

- a) The Indirect ELISA kits from United Biomedical Incorporated (UBI) for pigs and cattle, sheep and goats. These are based on plated pre-coated with synthetic peptides.
- b) The Indirect ELISA kits from S. America (PANAFTOSA) validated for cattle mainly (3ABC antigen from *E.coli*).
- c) The Indirect ELISA kits from Intervet based on the existing IAH, Pirbright, UK, and IZS, Brescia, Italy, developments (October 2001) (3ABC purified antigen).

It is hoped that these can be supplied and compared in the CRP participating laboratories.

Developments in other laboratories

a) Vet. Inst. For Virus Research, Lindholm, Denmark

Competitive ELISA based on competition for pretitrated reaction between guinea pig serum and baculo expressed 3A 3B. Kits have been examined by various laboratories.

b) USDA-Plum Island

Some testing of reagents has been made in Thailand and Philippines, to obtain validation

c) CISA-INIA Spain

SDS gel eluted NS proteins, Indirect ELISA, with national use only. Not tested in CRP.

Future developments

Geelong/IAEA/Vienna Veterinary University

A competitive ELISA is being developed with baculo expressed 3ABC and non-mammalian system. Estimated to be ready for field validation within next nine months.

Conclusions on comparative work

Tests need more validation with better IQC control and have an EQA element. There should be agreement on the validation factors (defining sensitivity and specificity) and documentation needed to establish a test. Robustness of reagents needs to be comnsiderd. There are differences in the relative analytical sensitivity and diagnostic sensitivity/ specificity of the assays. Some of the Indirect ELISAs suffer from problem of the antispecies conjugate and individual serum samples backgrounds. There is too much test-to-test variation in controls for the assays. There is little data on sheep/goat sera. Competitive assays should be developed rather than Indirect assays to allow any species to be tested. Vaccinated and recovering animals pose major problems. The sampling frames in various epidemiological situations has to be better considered. Reference sera are needed for all species. The most validated and used assay is from S. America where it has been used in the phase of vaccination and attention to this assay must be made along with strategies devised and interpretation of the results. Vaccines should be assessed for NS protein contamination. There should be a Central Reference Center for EQA management. Estimation of the performance of the tests in SE. Asia, Africa, Middle East, Russia and ex CIS countries should be made.

The next RCM is being arranged from 4 to 8 March 2002, in Geelong, Australia.

# Developing, Validating and Standardizing Methodologies for the Use of PCR and PCR-ELISA in the Diagnosis and Monitoring of Control and Eradication Programmes for Trypanosomosis (D3.20.21)

Technical Officer: John Crowther

The general objective is to improve livestock production through effective control and eradication of livestock diseases. More

specifically, the CRP aims to introduce molecular biological techniques for more effective diagnosis and surveillance of trypanosomoses.

At the first RCM in March this year, focus was put on:

### a) Standardization of methods and the role of PCR

It was agreed that clear ideas as to the role of the PCR must be worked out to establish whether the PCR is, in fact, the most useful and appropriate test to be used.

#### b) Methods and protocols

It was agreed that each PCR protocol (based on methods and primers used) should be written and that a standard format should be used. Model protocols from Dr. Peter-Henning Clausen have been received and passed on to participants. It was agreed that some basic background information should also be included with regard to reagent formulation, and Dr. G. Viljoen has sent this material which has been distributed to participant.

#### c) DNA Bank

It was agreed that a reference bank of DNA should be set up at the FAO/IAEA Laboratory, Seibersdorf. The DNA should be from trypanosomes, control DNA from various livestock and insect species and DNA from relevant organisms likely to complicate the PCR diagnostic potential with regard trypanosomoses. The strains selected would be well characterized and "reliable" in terms of their pedigree.

#### d) GLP

It was agreed that the setting up of a PCR laboratory was fundamental. It was agreed that each laboratory would examine closely their set up and report on any deficiencies. A good laboratory practice (GLP) document for PCR has been provided by Dr. Viljoen and distributed.

#### e) Primers

Extensive discussions on which primers could be used to obtain products for use in various aspects of the study of trypanosomoses were made.

#### f) Comparative testing and sensitivity/specificity

There was discussion on sensitivity and specificity of all tests including PCR. There is a

need to define sensitivity in terms of both analytical and diagnostic potential of the PCR.

#### g) Antibody assays (ELISA)

The indirect ELISA developed at Seibersdorf was outlined. Kits are now available for five participants requesting these.

### h) Kit technology transfer-Ketri-production and distribution

It was hoped that the technology transfer could be made to allow Ketri to produce and distribute kits as developed at Seibersdorf on a cost recovery basis. This includes training of Keri staff at Seibersdorf though TC programmes.

#### i) PCR-ELISA

The need to develop such methods was discussed following a paper by Dr. te Pas. It was concluded that there was no direct benefit from the approach in most laboratories and that the advantages of high throughput were not really required. There were discussions on the sensitivity aspects of the methods.

#### **Activities since the RCM**

Most Research Contract holders have now renewed their Contracts.

Equipment and reagents have been sent to Contract holders on request.

The plasmid vector for assessing the PCR on start up has been deposited in Seibersdorf.

Defined protocols for various trypanosomes have been sent to Seibersdorf and distributed to Contract holders.

There were no submissions of the trypanosomal DNA samples agreed in the first RCM.

The laboratory design has been improved in some laboratories based on SOP and advice from Agreement holders.

Indirect ELISA kits to detect antibodies against *T. vivax* and *T. congolense* have been prepared at the Seibersdorf Laboratories for distribution.

Extra funds were provided to prepare and sequence universal primers (Pan try).

#### E. NEW CO-ORDINATED RESEARCH PROJECTS

#### **Integrated Approach for Improving Small Scale Market Oriented Dairy Systems (D3 10.23)**

Technical Officer: Oswin Perera

Over 30 proposals for Research Contracts were received for this inter-regional project and ten have now been selected for awards, together with four Research Agreements and one Technical

Contract. The first Research Co-ordination Meeting will be held in April 2002 (see forthcoming events). The full list of participants will be given in the next Newsletter.

# The Development of Strategies for the Effective Monitoring of Veterinary Drug Residues in Livestock and Livestock Products in Developing Countries (D3.20.22)

Technical Officer: Andrew Cannavan

After a postponement pending the appointment of a new Technical Officer, this CRP is now under way. More than 40 proposals have been evaluated and from these 12 have been selected for awards as Contract holders. Three Research Agreements have also been awarded. The CRP will focus on the development of sampling procedures, the application of screening technologies such as ELISA and RIA, post-screening methods such as HPLC, and approaches towards laboratory accreditation. The first RCM will be held in late April/early May 2002 (date to be finalized). A full list of participants will be published in the next issue of the Newsletter.

#### Development and Use of Rumen Molecular Techniques for Predicting and Enhancing Productivity

Technical officer: Harinder Makkar

1. IntroductionThe world's livestock sector is amidst a massive transformation, fuelled by high demand for meat and milk, which is likely to double over the next two decades in developing countries. The major driving force behind this soaring demand for livestock products is a combination of population growth, urbanization and income growth, especially in developing countries. The challenge is to enhance animal productivity without any adverse effects on environment.

The major limitation to ruminant production in many tropical regions of Africa, Asia and Latin America is poor nutrition. The productivity of animals is restricted by the low nitrogen and high fibre content of the native grasses and crop residues, which form the basis of the diets in these regions. Chemical treatment of fibrous feedstuffs, supplementation of tropical roughages with leguminous fodder trees and shrubs (FTS) and low-cost nitrogenous sources, and use of agricultural by-products are promising methods to alleviate nutrient deficiencies associated with these basal diets. FTS often contain secondary compounds (e.g., tannins, saponins, phenolic glycosides), which can affect discrete populations of microorganisms in the rumen.

A large proportion of the global ruminant population are located in tropical environments,

where animals feed predominantly on low quality fibrous forages. Recent studies in respiration chambers have confirmed that methane emissions from ruminants fed on fibrous diets are higher than outputs from better quality temperate forages. The excretion of methane from the rumen can represent a loss of 8-10% of the digestible energy depending on the type of diet. Therefore, reducing methane production could benefit the ruminant energetically provided the efficiency of ruminal metabolism is not compromised. Animal trials involving agents that specifically inhibit microbial enzymes associated with methane production probably provide the most reliable data for interpretation of the effects of inhibition of methanogenesis on digestive and animal performance parameters. This data indicates that a reduction in methanogenesis in the rumen can be associated with improvements in feed conversion efficiency without affecting intake. Furthermore, any attempt to reduce methane emissions from livestock is unlikely to be adopted unless production efficiency is at least maintained if not enhanced. The challenge therefore is to devise strategies, which reduce methane emissions from ruminants and improve production efficiency.

#### 2. Rationale

Current approaches to the evaluation of digestibility and nutritive value of feed resources using conventional *in vitro* feed evaluation and animal studies have resulted in a large body of

information about nutrient composition, digestion kinetics and digestibility. However, these techniques are unable to describe the mechanisms involved in ruminal digestion, and are unlikely to result in the development of innovative technologies to improve animal productivity from available feed resources.

Gene-based technologies have the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and contain antinutritive factors. Until recently our knowledge of rumen microbiology was primarily based on classical culture based techniques (isolation, enumeration and nutritional characterization) which probably only account for 10 to 20% of the rumen microbial population. New gene-based technologies are being employed to examine microbial diversity through the use of 16S rDNA analysis and to understand the function of complex microbial ecosystems such as the rumen. These technologies have the potential to revolutionize our understanding of rumen function and will enable us to overcome current limitations in rumen biotechnology, which include isolation and taxonomic identification of strains important to efficient rumen function. The future of rumen microbiology research is dependant upon the adoption of these research technologies. However the challenge is how we utilize these technologies to improve ruminant production through a better understanding of microbial function and ecology.

These molecular based ecology techniques are likely to provide insight into the interactions between methanogens and the other rumen microorganisms, which should lead to strategies improving production bv reducing methanogenesis. The impact of reduced methane production on rumen fermentation has not been clearly elucidated, although it appears that the degree of inhibition of methane production is an important determinant of the associated effects on feed intake, feed digestibility and animal production efficiency. A consequence of inhibiting methanogens is the accumulation of H<sub>2</sub> in the rumen that is a major metabolic end product of forage digestion. The management of H<sub>2</sub> accumulation in the rumen under these circumstances is a critical factor, which will determine the efficiency of digestion and animal performance. When hydrogen accumulates in the rumen, bacteria shift their fermentation pattern to acetate from more reduced end products such as propionate. The adaptive changes in rumen microbial ecology to inhibition of methanogens is relatively unknown although enhanced propionate production is a consistent response consequence of disruption to interspecies

hydrogen transfer. One strategy to prevent  $H_2$  accumulating in the rumen is to provide dietary substrates that are precursors for propionate production by fermentative bacteria. Increase in the efficiency of microbial protein synthesis has also been observed with decrease in methane production.

#### 3. Overall Objective

To improve ruminant performance through a reduction in methane production.

- 4. Specific Research Objectives
- 4.1 Reduce the level of methane production by up to 50% in animals fed roughage diets.
- 4.2 Increase microbial protein and energy supply through reduced methane production using approaches such as inhibitors of methanogens, dietary approaches (e.g. use of polyunsaturated fatty acids or ingredients containing these acids), supplementation strategies, etc.
- 4.3 Build in-country capacity to develop and use molecular techniques for studying rumen function.

Develop molecular probes for quantifying populations of methanogens, fibre degrading bacteria, fungi and protozoa.

Correlate methane production to methanogen numbers.

Determine effects of reduced methanogen numbers on fibre degrading bacteria, fungi and protozoa.

Identify naturally-occurring plant secondary compounds that inhibit methanogens.

- 5. Expected research outputs
- Feeding strategies and or supplements that reduce methane production and improve productivity in ruminants on tropical diets.
- Development and application of molecular probes and techniques for studying rumen microbial ecology and effects of novel feed additives and dietary approaches.
- Increased capacity of NARS to integrate molecular rumen techniques into research programmes on ruminant nutrition.
- Improved knowledge of ecology of rumen microorganisms, particularly methanogenic archaea and their interaction with predominant rumen microorganisms.
- Published and disseminated research results.

#### **Proposals**

Scientists working in countries in Africa, Asia and Latin America where novel feeding strategies are being evaluated for improving ruminant production are requested to submit research proposals using the appropriate forms ('Research Contract Proposal'). The number of participants will be between eight and ten. The proposals should be submitted by 30 June 2002. It is hoped to start this CRP in early 2003.

institutes should those apply participation in the new CRP who already have (1) expertise in rumen microbiology and sound knowledge of biochemistry (2) sufficient (or access to) laboratory equipment and capability in the areas of anaerobic microbiology techniques molecular such as PCR. electrophoresis and genetic based <sup>32</sup>P fluorescent dye probing techniques. Capacity to measure methane production and <sup>15</sup>N enrichment is desirable but not essential. Evidence for availability of the expertise and facilities should be provided in the proposal.

Proposals should describe the experimental design (for example number of samples, number of animals, geographical area, parameters, sampling techniques, experimental animals, etc.) for evaluation of feeding systems and approaches to improve production and reduce methane. In addition, the expected output and benefits (for the laboratory, the farmers and the country) should be indicated.

Research Contract proposal forms can be obtained from IAEA, National Atomic Energy Commissions, UNDP offices or by contacting the Technical Officer. The form can also be downloaded from the Website: http://www.iaea.org/programmes/ri/uc.html

Such proposals need to be countersigned by the Head of the Institutions and sent directly to the IAEA. They do not need to be routed through other official channels unless local regulations require otherwise.

#### General information applicable to all Co-ordinated Research Projects

#### **Submission of Proposals**

Research Contract proposal forms can be obtained from IAEA, National Atomic Energy Commissions and UNDP offices. Such proposals need to be countersigned by the Head of the Institution and sent directly to the IAEA. They do not need to be routed through other official channels unless local regulations require otherwise.

#### Complementary FAO/IAEA Support

IAEA has a programme of support through national IAEA Technical Co-operation Projects (TCP).

These are concerned with aspects of animal production and diagnosis of animal diseases. Through such projects, additional support may be provided for the activities planned under the individual Research Contracts. This would provide further equipment, specialized training through IAEA training fellowships and the provision of technical backstopping through visits by IAEA experts for periods of up to one month. Such support is available to IAEA Member States.

#### F. TECHNICAL CO-OPERATION PROJECTS

#### **Operational Projects in 2001/2002**

(Number, Title, Technical Officer)

**ARG/5/010**, IMPROVING TUBERCULOSIS DIAGNOSIS IN RUMINANTS USING PCR, John Crowther

BGD/5/023, DEVELOPMENT OF AGROFORESTRY-BASED LIVESTOCK PRODUCTION SYSTEMS, Harinder Makkar

**BKF/5/002**, DEVELOPMENT OF A VETERINARY MEDICINE TO COMBAT THE FOWL POX IN POULTRY FARMING, Martyn Jeggo

**BOL/5/014**, DIFFERENTIAL DIAGNOSIS OF FOOT-AND-MOUTH DISEASE, John Crowther

CMR/5/009, NUCLEAR TECHNIQUES FOR IMPROVING LOCAL RUMINANT PRODUCTIVITY, Harinder Makkar

COL/5/020, USE OF PROTEIN BANKS FOR IMPROVING PORK PRODUCTION, Harinder Makkar

**CPR/5/014**, INCREASING PRODUCTIVITY OF CROP-LIVESTOCK PRODUCTION SYSTEMS, Harinder Makkar (Associate Staff)

CYP/5/019, ACCREDITATION OF LABORATORY FOR CONTROL OF FOODS OF ANIMAL ORIGIN, Andrew Cannavan

ETH/5/012, INTEGRATING SIT FOR TSETSE ERADICATION, Martyn Jeggo

**ELS/5/009**, IMPROVING CATTLE PRODUCTION AND QC FOR MONITORING OF ANIMAL DISEASES, Oswin Perera, Axel Colling

INS/5/029, SUPPLEMENTARY FEEDING AND REPRODUCTIVE MANAGEMENT OF CATTLE, Oswin Perera, Harinder Makkar

INS/5/032, IMPROVING BEEF AND DAIRY CATTLE PRODUCITON IN YOGYAKARTA, Oswin Perera

MAL/5/025, FOOD SAFETY MONITORING PROGRAMME FOR LIVESTOCK PRODUCTS, Andrew Cannavan

MAT/5/003, SURVEILLANCE OF PROGRAMMES FOR CONTAMINANTS IN FOODS OF ANIMAL ORIGIN, Andrew Cannavan

MEX/5/036, IMPROVING THE REPRODUCTIVE PERFORMANCE OF PELIBUEY SHEEP IN TROPICAL MEXICO USING LOCAL FEED RESOURCES, Harinder Makkar

MON/5/011, INTEGRATED APPROACH FOR FIELD MANAGEMENT OF ANIMAL PRODUCTION AND HEALTH, John Crowther, Harinder Makkar

MOR/5/027, MONITORING OF VETERINARY DRUG RESIDUES, Andrew Cannavan

MYA/5/011, DEVELOPMENT OF SUPPLEMENTARY FEEDING STRATEGIES BASED ON LOCAL FEED SOURCES, Harinder Makkar

MYA/5/012, DIAGNOSIS AND CONTROL OF SWINE VESICULAR DISEASE AND SWINE BRUCELLOSIS, John Crowther

NAM/5/006, MONITORING OF VETERINARY DRUG RESIDUES IN LIVESTOCK, Andrew Cannavan

PAK/5/041, SETTING UP IMMUNOASSAY AND MOLECULAR-BASED METHODS TO MONITOR AND SURVEY RINDERPEST DISEASE, John Crowther

POL/5/010, INCREASING PIG PRODUCTIVITY THROUGH RADIOIMMUNOASSAY TO DETERMINE METHODS FOR ADVANCING PUBERTY IN GILTS, Oswin Perera

RAF/5/046, INCREASING AND IMPROVING MILK AND MEAT PRODUCTION, Oswin Perera

RAF/5/053, ASSISTANCE TO OAU/IBAR PACE PROGRAMME FOR THE CONTROL AND ERADICATION OF MAJOR DISEASES AFFECTING LIVESTOCK, Martyn Jeggo, Mamadou Lelenta

RAS/5/035, BETTER MANAGEMENT OF FEEDING & REPRODUCTION OF CATTLE (RCA), Oswin Perera, Harinder Makkar

RLA/5/046, SUSTAINABLE ANIMAL PRODUCTION ON LANDSCAPES OF VENEZUELAN-COLOMBIAN ORINOQUIA, Harinder Makkar

**SRL/5/035**, MONITORING AND CONTROL OF RESIDUES IN LIVESTOCK PRODUCTS, Andrew Cannavan

**URT/5/021**, LIVESTOCK DEVELOPMENT IN ZANZIBAR AFTER TSETSE ERADICATION, Oswin Perera, Harinder Makkar, Martyn Jeggo

**VEN/5/021**, SUSTAINABLE ANIMAL PRODUCTION, Harinder Makkar

**YEM/5/004**, IMRPOVING THE DIAGNOSIS OF ANIMAL DISEASES, John Crowther

### G. ACTIVITIES OF THE ANIMAL PRODUCT UNIT (APU) AT THE FAO/IAEA AGRICULTURE AND BIOTECHNOLOGY LABORATORY

Activities in the Animal Production Unit in the FAO/IAEA Laboratory (Seibersdorf) are moving towards gene-based technology in animal health and production research areas. To cope with this change, important work on the renovation of the laboratory was started in June. This renovation is now completed and new equipment was purchased. Activities are starting with gene sequencing and PCR test development for disease diagnosis, recombinant antigen production in both

bacteria and baculovirus systems. It is expected to start animal genotyping in a year for the characterization and identification of traits that are interesting for animal production.

Dr Bodjo, from Côte d'Ivoire has been awarded a grant from IAEA for a one-year training in the FAO/IAEA Laboratory. He will be working on the production of PPRV antigen in the baculovirus vector for the improvement of the PPR cELISA.

#### **African Swine Fever Kits**

#### **Technical Contract 11294 (D3.00.00)**

# Production and distribution of Indirect ELISA kits for the detection of antibodies against African Swine Fever (ASF) virus

The kits have been developed by Mariame Diop, Institut Sénégalais De Recherches Agricoles ISRA, Laboratoire National de l'Elevage et de Recherches Vétérinaires (LNERV).

Indirect ELISA kits for the detection of antibodies against ASF have been prepared. The kits

comprise of antigen and control sera from CISA – INIA, 28130 Valdeolmos, Madrid, Spain and protein A conjugated to horse radish peroxidase. Validation data for the ASF kit is available. The kits will be distributed to selected laboratories and can be also purchased from the Senegal laboratory by arrangement with M. Diop. This is an encouraging landmark in the sustainable supply of kits in Africa.

#### Foot-and-Mouth Disease (FMD) Kits

#### Iran

A TC project (IRA/5/012), originally listed as Footnote A (awaiting funding), has now been funded by the Government of the Islamic Republic of Iran. This project will involve the preparation of FMD kits for the detection and serotyping of FMD viruses, as well as developing antibody detection kits for epidemiological screening and serum titration. The kits will be available to all and costed as commercial items ensuring continuity of supply and sustainable development.

#### **SE Asia**

There is every prospect that a Regional TC project (RAS/5/041) will be funded in 2003. This also allows development of kits for FMD for supply to all countries in the SE. Asian region as part of the project, but also aims to produce a sustainable source of reagents and kits.

In both cases, the APU will provide oversight and OA to these activities.

#### H. QUALITY ASSURANCE PROGRAMMES

# QUALITY ASSURANCE PROGRAMMES FOR THE DETERMINATION OF PROGESTERONE IN SKIM MILK AND PLASMA BY RADIOIMMUNOASSAY

The following is a summary of EQA rounds with the RIA for the determination of progesterone in milk and serum/plasma samples.

The 24<sup>th</sup> External Quality Control (EQC) exercise for progesterone RIA, which is a component of the FAO/IAEA External Quality Assurance programme (EQAP), has recently been concluded. EQC samples for milk progesterone determinations were distributed to 22 laboratories, and for plasma/serum determinations to 11 laboratories.

A report has been prepared from the results returned by 15 laboratories that processed milk EQC samples (68.2% response rate) and 7

laboratories that processed the serum/plasma EQC samples (63.6% response rate). In the present exercise, the results for milk progesterone values for laboratories using self-coating RIA (Sc-RIA) kits have also been analyzed, and provide an opportunity for participants to compare these with values obtained using DPC kits.

The 25<sup>th</sup> EQC exercise for milk and plasma was sent in July 2001, and we are still waiting to receive all the results. In order to complete the analysis of the 25<sup>th</sup> EQC exercise, we would appreciate receiving the remaining results as soon as possible.

#### I. GEOGRAPHICAL INFORMATION SYSTEMS

A number of activities were undertaken in providing GIS support to a variety of Subprogramme and Divisional activities. These included the following:

- 1. Attendance at a regional Workshop from 2 to 6 July 2001. Nine participants from four countries attended the course (Egypt, Jordan, Ghaza, Israel). This course was initiated under IAEA TC-Projects ISR/5/010 and JOR/5/009. The following topics were presented: Introduction to GIS; using GIS for data collections; building access database; use of ArcView. The lecturers were Mr. Patrick Alkcors (LDPA,SA), Ms. Anita Erkelens and Mr. Zowinde Koudougou.
- 2. Data processing under the project "Integrated control of animal trypanosomosis through creation of a tsetse fly free zone" (MLI/5/017). Maps of the distribution and apparent density of tsetse fly in the peri-urban and rivereine areas around Bamako, Mali were made. Total drainage linear patterns length were derived from the false composition image in order to estimate total number of traps required.
- 3. Generation of disease occurrence maps for Rift Valley Fever in Yemen. Maps of the spatial repartition of abortions of sheep and goats; distribution of number of animals at RVF antibody positive; distribution of percentage of RVF antibody positive were prepared. These

- maps were presented at a regional WHO meeting on Haemorrhagic Disease held in Cairo, Egypt in September.
- 4. The development of GIS based maps for a sampling strategy to conduct a survey for rinderpest in China. Using bovine density and land use/cover rastermaps, four strata were defined in each map. Three maps have were produced on one sheet showing a general view of China in terms of farming systems, bovine density and land use/cover; two sheets showing georeferenced sampling prints according to bovine density and land use/cover.
- 5. Attendance at the 36<sup>th</sup> International Scientific Council for research and trypanosomosis control at Ouagadougou from 13 to 18 October 2001.
- 6. A visit to FAO Headquarters, Rome from 5 to 9 November 2001 to discuss the overall use of GIS in FAO. Different GIS tools, particularly the spatial data management, available at FAO headquarters were demonstrated including new GISD software. Following this visit, a new GIS software, ARCGIS, will be introduced in the Section next year which will add considerably to the range of studies that can be undertaken.

#### J. PUBLICATIONS

#### **Published:**

Radioimmunoassay and related techniques to improve artificial insemination programmes for cattle reared under tropical and sub-tropical conditions (2001) IAEA-TECDOC-1220.

The FAO/IAEA Guidelines to Establish a Quality System in Veterinary Testing Laboratories in Developing Countries.

Performance indicators for rinderpest surveillance (IAEA-TECDOC-1261).

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#### K. WEBSITES

- The web page of the Section is in the process of being updated. Please feel free to look at the web pages and make comments. http://www.iaea.org/programmes/nafa/d3/index.html
- Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Homepage: http://www.iaea.org/programmes/nafa/
- FAO Homepage: <a href="http://www.fao.org/">http://www.fao.org/</a>
- FAO/IAEA Guidelines for Establishing Quality Systems in Veterinary Diagnostic Testing Laboratories http://www.iaea.org/programmes/nafa/d3/public/guidelines.pdf
- Web-based interactive programme about ISO/IEC 17025 <a href="http://www.aplactraining.asn.au">http://www.aplactraining.asn.au</a>
- International Symposium on Application of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries, 6–10 October 2003, Vienna, Austria. <a href="http://www.iaea.org/programmes/nafa/d3/index-symp2003.html">http://www.iaea.org/programmes/nafa/d3/index-symp2003.html</a>
- A livestock research portal has been developed: This allows users to easily access the websites of 30 or so different livestock research donors and implementing organisations. The portal also hosts the electronic version of the proceedings of the first interagency meeting (December 2000) and will host the proceedings of the second interagency meeting (these should be in place by the end of December 2001).
  - The address for the portal is: <a href="http://lri.virtualcentre.org">http://lri.virtualcentre.org</a>
- It is hoped to start a web page designed to deal with test validation. It is envisaged that the site will be interactive in some sections allowing data to be examined and discussed, as well as presenting validation data for kits as supplied, as well as allowing continuous data on the field performance of the kits to be examined. Validation data of tests involving rinderpest, PPR, FMDV, CBPP, ASF, trypanosomoses, brucella and NDV will be considered. It is hoped that this will lead to better quality of kits and reagent sets and help define the state of play on a more continuous basis.

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