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REPORT OF THE MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 15–17 September 2009

The newly elected OIE Biological Standards Commission met at the OIE Headquarters from 15 to 17 September 2009. Dr Kazuaki Miyagishima, newly appointed Head of the OIE Scientific and Technical Department, introduced himself and welcomed the Members of the Commission, Prof. Vincenzo Caporale, President, Dr Beverly Schmitt, Vice-President, Dr Mehdi El Harrak, Secretary General, and Dr Alejandro Schudel, Dr Chen Hualan, Dr Paul Townsend, members of the Commission, and as well as an observer, Dr Adama Diallo from FAO/IAEA¹.

Dr Bernard Vallat, Director General of the OIE, congratulated the elected and re-elected Members and wished them every success. He indicated that one of the main challenges facing the Commission was to ensure that the OIE Reference Laboratories and Collaborating Centres comply with the OIE standards of excellence and meet the demands of OIE Members. He urged the Commission to review the technical aspects of applications with this need for quality in mind and reminded the members of the OIE twinning initiative, the object of which is to provide support and capacity building to laboratories in developing and in-transition countries. The ultimate goal is to achieve a more balanced geographical distribution of the OIE Reference Laboratories and Collaborating Centres. Dr Vallat went on to stress the importance of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)*, the companion volume to the *Terrestrial Animal Health Code*. One of the Commission's main tasks is to ensure that descriptions of the best diagnostic tests and vaccine production methods are included in the *Terrestrial Manual*. Finally, Dr Vallat described the current and future activities of the OIE in the area of vaccine quality, test performance and veterinary medicinal drugs, including the designation of national focal points on this subject by the OIE Delegates.

The Agenda and List of Participants are given at [Appendices I](#) and [II](#), respectively.

1. Mandate and working procedures of the Biological Standards Commission

Dr Elisabeth Erlacher-Vindel, Deputy Head of the OIE Scientific and Technical Department, briefed the Commission and especially new members on the Terms of Reference (ToRs) and working procedures of the Biological Standards Commission. The Commission would have to collaborate with the Scientific Commission for Animal Diseases on a number of issues of common interest.

2. Declaration of conflict of interest of Members of the Commission

The Commission was informed that the Scientific Commission, while discussing the same subject matter at its meeting the week before, had produced a draft agreement of confidentiality and impartiality that could be useful for other OIE Specialist Commissions, Working Groups, *ad hoc* Groups and designated experts in carrying out specific tasks. The Biological Standards Commission noted that the matter should be approached

¹ FAO/IAEA: Food and Agriculture Organization of the United Nations/International Atomic Energy Agency

carefully in view of the status of the members of the Commission being elected by the General Assembly and the legal implications of signing a declaration. A view was expressed that such a declaration was irrelevant in the context of the Commission. The Commission did not pursue the matter further.

3. OIE Reference Laboratories and Collaborating Centres

3.1. New applications for Collaborating Centre and Reference Laboratory status

An application had been received from the Faculty of Veterinary Medicine, National Autonomous University of Mexico, Mexico for a Collaborating Centre for Animal Welfare and Sustainable Animal Production. Dr Vallat had requested advice from the OIE Working Group on Animal Welfare on the OIE policy for approval of new animal welfare collaborating centres. The Commission postponed a decision until a response has been received from the Working Group.

Regarding an application from National Institute of Animal Health (NIAH) and the National Veterinary Assay Laboratory (NVAL), Japan, for an OIE Collaborating Centre for Diagnosis and Control of Transboundary Animal Diseases and Evaluation of Related Drugs in Asia, the Commission decided to request clarification of the following aspects: for the proposed scope, which was considered very large; for the objective of the application to become a Reference Laboratory as opposed to a Collaborating Centre; and for documented evidence of experience in international cooperation in the field of the evaluation of medicinal products.

OIE Collaborating Centre for Animal Disease Surveillance Systems and Risk Analysis (Fort Collins, Colorado, USA)

The OIE Collaborating Centre had requested to extend its mandate to include epidemiological modelling such that its title would now be: OIE Collaborating Centre for Animal Disease Surveillance Systems, Risk Analysis and Epidemiological Modelling. Dr Cristobal Zepeda will continue to be the contact point. The Commission endorsed this request.

The Commission recommended acceptance of the following new application for OIE Reference Laboratory status:

OIE Reference Laboratory for Rabies

WHO Collaborating Center for Reference & Research on Rabies, Poxvirus & Rabies Branch, Division of Viral and Rickettsial Diseases, National Center for Zoonotic, Vector-borne, & Enteric Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Mail Stop G 33, Atlanta, Georgia 30333, UNITED STATES OF AMERICA

Tel: (+1-404) 639.1050; Fax: (+1-404) 639.1564; E-mail: cyr5@cdc.gov

Designated Reference Expert: Dr Charles Rupprecht.

The Commission reviewed other applications and decided to request additional information, resubmission of the application, or rejected the application.

Finally, the Commission agreed that in order to allow full evaluation of applications at its meeting, dossiers should be submitted, in principle, at least 4 weeks in advance of a meeting of the Commission and be circulated to the members of the Commission in good time. Incomplete dossiers should be filtered by the secretariat and applicants should be invited to resubmit before consideration by the Commission.

3.2. Updating the list of Reference Laboratories

The OIE has been notified of the following changes of experts at OIE Reference Laboratories. The Commission recommended their acceptance:

Foot and mouth disease and Vesicular stomatitis

Dr Rossana Allende to replace Dr Ingrid Bergmann at the Centro Panamericano de Fiebre Aftosa (PANAFTOSA), Brazil.

Contagious equine metritis

Dr Matthew Erdman to replace Dr Brenda Morningstar-Shaw at National Veterinary Services Laboratories (NVSL), Ames, Iowa, United States of America.

3.3. Review of twinning applications

The Commission recalled that since launching the OIE twinning initiative in 2007, thirteen twinning projects between OIE Reference Laboratories or OIE Collaborating Centres and candidate laboratories in developing or transitional countries had been approved and signed off, and were either underway or due to start imminently:

1. Italy (IZSve) and Russia	Avian influenza and Newcastle disease (project completed)
2. USA and Brazil	Avian influenza and Newcastle disease
3. Germany (FLI) and Egypt	Avian influenza and Newcastle disease
4. Italy and Cuba	Avian influenza and Newcastle disease
5. UK (VLA) and South Africa	Avian influenza and Newcastle disease
6. UK and Botswana	Avian influenza and Newcastle disease
7. UK (VLA) and China (People's Rep. of)	Classical swine fever and rabies
8. Italy (Teramo) and Eritrea	Brucellosis
9. UK (VLA) and Turkey	Brucellosis
10. Italy (Teramo) and Cuba	Epidemiology
11. Italy (Teramo) and Botswana	Contagious bovine pleuropneumonia
12. UK and Morocco	Bluetongue and African horse sickness
13. Germany and Turkey	Rabies

The Commission also recalled that two further projects had been cleared by the Biological Standards Commission and were awaiting budget clearance/ signatures:

1. Canada and Colombia:	Avian influenza and Newcastle disease
2. Australia and Malaysia:	Avian influenza and Newcastle disease

The Commission noted that nine proposals were tabled for this meeting and that some of them had been submitted late, and decided to consider them by electronic correspondence on an exceptional basis. The outcome of this consultation would be published as an appendix to this report (see [Appendix III](#)).

The Commission discussed the extent to which the twinning guide should be applied in the future assessments of candidate laboratories. A discussion with the Director General on the conditions for derogations to the twinning guide would also be useful. General aspects of the twinning initiative were examined and the Commission noted that a clear strategy had to be in place given that the final objective was not always to have new OIE Reference Laboratories but sometimes to significantly improve the capability of national laboratories so that they would have capability to provide support to other countries. The Commission noted that while twinning requests were driven by the initiatives taken between prospective parent and recipient laboratories, geographical distribution of the laboratories concerned as well as involvement of the same laboratories in twinning should also be considered when endorsing a twinning project on a case-by-case basis. The Commission encouraged prior coordination among institutions involved to ensure optimal benefits when a candidate laboratory is involved in several twinning projects. Finally the Commission recommended receiving the applications electronically at least four weeks before its meetings.

3.4. Follow-up from January – Reference Laboratories that did not provide a report or provided a poor annual report for 2008

The Commission was informed that a total of nine reference laboratories had shown little or no OIE-related activities in 2007/2008 and/or had not submitted the required annual report. The Commission recommended that these laboratories be given a short deadline to provide proof of their activities; if no answer is received before the deadline, they would be suspended from the OIE list of Reference Laboratories. The Commission will review the replies at its next meeting in January 2010. Unless sufficient proof of relevant activities was submitted, permanent deletion from the list would be confirmed.

3.5. Review of Terms of Reference for Reference Laboratories and Collaborating Centres

The Commission took note of the current ToRs for Reference Laboratories and Collaborating Centres. It proposed that new applicants should provide information on collaboration/networking with existing OIE Reference Laboratories or Collaborating Centres. If more than one Collaborating Centre is located at the same institute, the contact point should not be the same person. The difference between an OIE Reference Laboratory with expertise for topics (not diseases) and a Collaborating Centre needs to be clarified.

The Commission stressed the need to consult the Director General on the interpretation of its official ToRs in order to determine its role and relations with regard to the networks and networking of laboratories in the overall OIE context, given that some of the networks seemed to have gained certain operational autonomy from the Commission. The mechanisms for implementing an OIE network and its procedures for communicating with the Biological Standards Commission needed clarification.

4. International standardisation of diagnostic tests and vaccines

4.1. OIE standardisation programmes for diagnostic tests

The Commission noted written reports and made comments as follows:

Highly pathogenic avian influenza (HPAI) – Coordinator: Dr P. Selleck, Australian Animal Health Laboratory (AAHL), Geelong, Victoria, Australia

Dr Selleck had reported that a candidate reference serum for the avian influenza (AI) AGID² test had been prepared and sent to the other Reference Laboratories. He is currently evaluating their results for the purpose of submitting his final evaluation report in time for the January 2010 meeting of the Commission, with a view to adopting these sera.

Caprine and ovine brucellosis – Coordinator Mrs J. Stack, VLA Weybridge, UK

Mrs Stack had provided the data sheet of the International Standard anti-*Brucella melitensis* sera (ISaBmS). The sera are for use in the competitive ELISA³, the RBT⁴, the modified RBT and the FPA⁵ but not in the CFT⁶ so as to avoid confusion with the OIE International Standard Serum (OIEISS). The Commission recommended that a report on the preparation of the ISaBmS be published in the plurithematic issue of the OIE *Scientific and Technical Review*.

Porcine brucellosis – Coordinator: Dr K. Nielsen, Canadian Food Inspection Agency, Nepean, Canada

Dr Nielsen had reported that some of the OIE Reference Laboratories were experiencing problems finding the resources to test the candidate sera.

Dourine – Coordinator: Dr Noboru Inoue, National Research Center for Protozoan Diseases, Obihiro, Hokkaido, Japan

Dr Inoue and Dr Claes were working in collaboration to produce standard sera for dourine.

The Commission reviewed the list of currently approved OIE international standard sera and agreed that it would be useful to send a questionnaire to the laboratories concerned with the aim of ascertaining the quantity they have at their disposition, their production and distribution methods, and charging policies (free-of-charge, cost recovery basis, etc). This matter must be discussed with the Director General during the next meeting of the Commission. The Commission noted a view that the standardisation programme should also be expanded to include production of standard strains, in view of the importance of the latter for controlling the quality for diagnostic tests.

² AGID: agar gel immunodiffusion

³ ELISA: Enzyme-linked immunosorbent assay

⁴ RBT: Rose Bengal plate agglutination test

⁵ FPA: Fluorescence polarisation assay

⁶ CFT: Complement fixation test

4.2. International Evaluation Panel for use as Reference Standard for Assays that Detect Antibodies against NSP⁷ of FMD⁸ Virus in Cattle

The draft Guidelines for the use of an international reference standard panel (bovine serum panel), which had been prepared by Dr Ingrid Bergmann, would be given for final review to the *ad hoc* Group on Validation of Diagnostic Assays.

4.3. Updating the booklet on the OIE Standard and Guidelines

The next edition of the OIE *Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases* would not be published until the *ad hoc* Group on Validation of Diagnostic Assays had updated the guideline on validation.

The Commission also noted that the issue of copyright needed be sorted out with ISO⁹ for publication in hardcopy and on Internet.

4.4. Evaluation of the need for guidelines (for trade) in cases where a validated diagnostic test is not available for a disease and/or a species (e.g. wildlife, certain domestic camelids, etc.)

This issue would be included in the ToRs for the next meeting of the *ad hoc* Group on Validation of Diagnostic Assays.

5. List of prescribed and alternative tests

5.1. Review of the current list

The Commission noted the list of tests for international trade.

5.2. Follow-up question on the virus neutralisation test for equine viral arteritis

The Commission noted an article that had been published in April 2009 questioning the validity of the current virus neutralisation (VN) method for equine viral arteritis (EVA) and recent correspondence with the author of the relevant section of the *Terrestrial Manual*. The Commission agreed that there was no need to revise that chapter of the *Terrestrial Manual* at this stage.

6. Expert, *ad hoc* and Working Groups

6.1. Meeting of the *ad hoc* Group on Vaccines in Relation to New and Emerging Technologies

The Commission took note of the report of the *ad hoc* Group. The Commission agreed to the proposal to retain the introductory chapter on biotechnology and to add a new chapter on vaccines derived from biotechnology, and to review the individual disease chapters where biotechnology-derived vaccines exist on a case by case basis. On the issue of food safety, some general information could be included in an introductory chapter. The Commission also took note of the recommendation that authors of the individual disease chapters consider the safety of animal products originating from animals vaccinated with recombinant vaccines, where relevant. The President of the Commission expressed his willingness to ask the Director General to attend the next meeting of this *ad hoc* Group.

6.2. Meeting of the *ad hoc* Group on Diseases of Honey Bees

The Commission reviewed the ToRs for this *ad hoc* Group that had been drafted by the Scientific Commission and added one point: to review and update the diagnostic methods included in the *Terrestrial Manual*.

⁷ NSP: Nonstructural protein

⁸ FMD: Foot and mouth disease

⁹ ISO: International Organization for Standardisation

6.3. *Ad hoc* Group on Diseases of Camelids

The Commission proposed that the ToRs for next meeting of this *ad hoc* Group should be revised to focus on priority subjects, and then submitted for approval to the next meeting of the Biological Standards Commission. The next meeting of the *ad hoc* Group should be held in the first semester of 2010.

6.4. Report of the Meeting of the *ad hoc* Group on Diagnostic Tests for Trypanosomoses

The Commission took note of the report of this *ad hoc* Group ([Appendix IV](#)).

6.5. Report of the *ad hoc* Group to Develop an OIE Network of Collaborating Centres to Reduce the Risk of Infectious Diseases at the Animal-Human-Pathogen-Ecosystems Interface

The Commission suggested that as this network comprised three OIE Collaborating Centres it could report to and be under the responsibility of the Biological Standards Commission. The Commission recommended that the *ad hoc* Group expand its membership to include experts from other OIE Collaborating Centres and Reference Laboratories that are active in the area of zoonotic diseases. The Commission noted and endorsed in principle the *ad hoc* Group's proposed future activities.

6.6. List of the *ad hoc* Groups under the auspices of the Biological Standards Commission

The Commission reviewed the list of *ad hoc* Groups that operate under its auspices. It decided to suggest to the Director General to convene an *ad hoc* Group on Quality, Biosafety and Biosecurity of Veterinary Laboratories could be convened; to propose a modification to the current *ad hoc* Group on NSP tests to include analysis of the field performance of diagnostic assays, beginning with FMD; and to propose to convene a Group on Vaccine Quality to revise the vaccine component of each disease-specific chapter in the *Terrestrial Manual*, beginning with FMD.

7. OIE Register of diagnostic tests

7.1. Presentation of the Procedure for the new Members

The Commission noted the presentation by the secretariat of the procedure for registering diagnostic test kits. Having discussed the status where few applications had been received, the Commission agreed that there was a need to propose the revision of the current procedure, analysing possible impediments and streamlining or improving the process, with a view to attracting more applications. Overall the OIE needs to convince kit producers that it was to their benefit to have OIE certified test kits; the OIE should also continue to encourage its Members to accept OIE-registered diagnostic tests.

7.2. Review of current applications and information on current contacts for future applications

The Commission was informed that there were currently two kits being evaluated: one on *Salmonella* and one on FMD.

8. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees)

For this agenda item, the Commission was joined by the Consultant Editor of the *Terrestrial Manual*, Prof. Steven Edwards.

The list of chapters for update this year (2010), along with the list of authors and reviewers, were examined and amended. Some technical questions regarding chapters that had already been received were addressed.

A request from two OIE Members for clarification of text in the vaccine section of the FMD chapter would be forwarded to the future *ad hoc* Group on Vaccine Quality.

It was agreed to update the chapter on antimicrobial resistance in the light of the outcome of the meeting of the Codex Task Force on Antimicrobial Resistance (October 2009). The Commission noted that the French Edition of the *Terrestrial Manual* would be published shortly.

9. Technical disease cards

The Collaborating Centre for Diagnosis of Animal Diseases and Vaccine Evaluation in the Americas, at Iowa State University, had drafted technical disease cards on 33 listed diseases. These had been reviewed by OIE experts. Prof. Edwards would ensure that the information in the cards does not contradict the information on diagnostic tests in the *Terrestrial Manual*. Once complete, the cards would be posted on the OIE Web site.

10. Follow-up from the General Session

10.1. Review of the Resolution on Rinderpest

The Commission reviewed the Resolution on Rinderpest that had been adopted by the World Assembly of OIE Delegates at the General Session in May 2009. It did not believe that the Resolution as such needs to be amended. Rather the Commission agreed to proceed, without delay, with the mandate given to it to develop guidelines for the controlled safekeeping of rinderpest virus strains and vaccine stocks. The Commission suggested that as a first step, information be requested from the WHO¹⁰ on its approach to restriction of access to smallpox virus to see if a similar approach could be applied to rinderpest. The Director General of the OIE will be requested to provide updated information on the official recognition procedure during the next meeting of the Commission.

11. Liaison with other Commissions and Groups

11.1. Scientific Commission for Animal Diseases

The Commission noted the report of the OIE FMD mission to South America. The issue of NSP tests would be referred to the *ad hoc* Groups on Test Performance and Vaccine Quality.

The Commission also noted that the need to coordinate work with the Scientific Commission on African horse sickness.

11.2. Terrestrial Animal Health Standards Commission

Dr Sarah Kahn, Head of the OIE International Trade Department, presented some comments that the Code Commission had received from OIE Members on proposed chapters for the *Terrestrial Code*. The Biological Standards Commission provided advice on some of the issues.

12. Meetings, Conferences and Workshops

The Commission was briefed on a number of meetings that had been held since it last met.

The Commission decided to propose to the Director General to hold a 1-day meeting at the OIE Headquarters on 12 November to advise the OIE on the programme and list of speakers for the Second Conference for OIE Reference Laboratories and Collaborating Centres, to be held from 21 to 23 June 2010. Members of the Commission who could not attend would provide their input electronically before the meeting or by telephone on the day.

The Commission was informed of the initiative to hold training workshops for national focal points, on a rotation basis in each of the OIE regions. So far a training workshop had been held in Panama for the focal points on wildlife. Following the Workshop on Veterinary Medicinal Products in Dakar, Senegal, a second one would be held in Damascus, Syria, in December 2009.

Dr Schudel informed the Commission that the OIE was co-sponsoring a symposium on Practical Alternatives to Reduce Animal Testing and Quality Control of Veterinary Biologicals in the Americas, 18–19 February 2010, in Buenos Aires, Argentina.

10 WHO: World Health Organization

The Commission noted that a member of the Biological Standard Commission should participate, either as a speaker or as a BSC representative, in technical conferences/meetings that are important and relevant to the work of the Commission.

13. Any other matters

13.1. Update on OFFLU¹¹

The OFFLU secretariat updated the Commission on the activities of OFFLU.

Links within the OFFLU network and with WHO have been strengthened considerably. In response to the emergence of pandemic H1N1 2009 in humans, OFFLU made a significant change to its mandate so that it now covers all influenzas in animals rather than solely avian influenza. Throughout the current H1N1 pandemic, information exchange and collaboration between WHO and OFFLU at the human–animal interface has been most fruitful. There have been numerous technical consultations between OFFLU and the WHO influenza network. OFFLU has developed guidance on diagnosis and laboratory surveillance of pandemic H1N1 2009 in animals, and has encouraged the rapid deposition of genetic sequences from pandemic H1N1 2009 viruses found in animals into publicly available databases. WHO looks to OFFLU as a key source of information for influenza in animals. OFFLU appreciates the active and positive contribution made by all of its animal health influenza experts, and by WHO for promoting OFFLU in the human health sector.

Two OFFLU meetings were held in September 2009:

1. The 6 monthly meeting of the OFFLU Steering Committee (14 September)
2. The second OFFLU Technical Meeting (15–16 September). The objectives of this second meeting were to:
 - Provide a forum for networking and an open exchange of information and ideas;
 - Review and build upon actions that had been agreed at the previous technical meeting in March 2008;
 - Contribute to developing a work plan and direction for the future.

A note of both of these meetings will shortly be posted on the OFFLU website www.offlu.net

13.2. Antimicrobial Resistance – follow-up of Resolution No. 25 on Veterinary Products

The Commission took note of the Resolution and would consider how to address the issues raised in the near future.

13.3. Memorandum of Understanding with IFAH¹²

The Commission took note of the proposed amendments to the Memorandum of Understanding between FAO and IFAH that refers to the role of the OIE and Codex Alimentarius standards for ensuring the quality of trypanocidal drugs.

13.4. PVS¹³ Gap Analysis – linked to labs/biosafety?

The Commission expressed its readiness to propose improvements to the PVS Gap Analysis format with the view to improving laboratory quality, biosafety and biosecurity component and performance.

13.5. Biological Weapons Convention

The Commission noted the mission report of Dr Keith Hamilton who had attended the BWC meeting of experts in August 2009.

¹¹ OFFLU: OIE/FAO Network on Avian Influenza

¹² IFAH: International Federation for Animal Health

¹³ PVS: Performance of Veterinary Services

13.6. New work items: Pen-side tests

The Commission noted that pen-side tests were not being used as authoritative tests but the potential of pen-side tests was expanding rapidly owing to recent technological developments. The Commission therefore decided to develop a position paper on pen-side tests. Dr Adama Diallo would provide the names of experts who could develop this paper.

13.7. Dates of next Biological Standards Commission meeting

The Commission noted the tentative dates for its next full meeting: 26–28 January 2010.

../Appendices

MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION

Paris, 15–17 September 2009

Agenda

1. Mandate and working procedures of the Biological Standards Commission
 2. Declaration of conflict of interest of Members of the Commission
 3. OIE Reference Laboratories and Collaborating Centres
 4. International Standardisation of Diagnostic Tests and Vaccines
 5. List of Prescribed and Alternative Tests
 6. Expert, *ad hoc* and Working Groups
 7. OIE Register of diagnostic tests
 8. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
 9. Technical disease cards
 10. Follow-up from the General Session
 11. Liaison with other Commissions
 12. Meetings, Conferences and Workshops
 13. Any Other Business
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MEETING OF THE OIE BIOLOGICAL STANDARDS COMMISSION
Paris, 15–17 September 2009

List of participants

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REVIEW OF TWINNING APPLICATIONS

In accordance with its decision during the physical meeting, the Commission reviewed, by correspondence, nine twinning project proposals. As a result of this review, the Commission formulated a favourable opinion on the technical aspects of proposals for the following seven twinning projects:

1. VLA, UK and CVRL, Sudan for **brucellosis**
2. ANMV, France and LACOMEV, Senegal for **quality control of veterinary medicinal products**
3. National Research Centre for Protozoan Diseases, Hokkaido, Japan and National Centre on Equines, Hisar, India for **equine piroplasmosis**
4. IZSve, Padova, Italy and CVRL, Qatar for **avian influenza and Newcastle disease**
5. AFSSA, France and NIAH, Thailand for **brucellosis**
6. ARC, Onderstepoort, South Africa and NVRI, Nigeria for **rabies**
7. SENASA, Argentina and SENACSA, Paraguay for **foot and mouth disease**

Consensus was not reached on the remaining two proposals, and noting that these proposals involved Candidate Laboratories that were under existing twinning programmes (CENSA, Cuba and BNVL, Botswana, are under twinning with IZS, Italy), the Commission requested additional information or project reformulation to ensure the avoidance of possible duplication and confusion between the new and ongoing projects. It was stressed that when a Candidate Laboratory is involved in more than one twinning project or other capacity building project, it is important to achieve maximal benefits through effective coordination of activities and, where appropriate, integration.

The Commission recommended that new and revised twinning project proposals be received at least 3 weeks before the next physical meeting of the Commission to allow for full and in-depth review.

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON DIAGNOSTIC TESTS FOR TRYPANOSOMOSSES

Paris, 30 March–1 April 2009

The meeting of the OIE *ad hoc* Group on Diagnostic Tests for Trypanosomoses was held at the OIE Headquarters in Paris from 30 March to 1 April 2009.

The meeting was chaired by Dr Filip Claes, and Dr Zhao-Rong Lun acted as rapporteur. The Agenda and the List of participants are presented at [Appendices I](#) and [II](#), respectively.

1. Introduction

Dr Elisabeth Erlacher-Vindel, Deputy Head of the Scientific and Technical Department of the OIE, welcomed the members on behalf of the Director General of the OIE, Dr Bernard Vallat. She presented the provisional agenda and explained the Terms of Reference, emphasising that the *ad hoc* Group should focus on the diagnosis of Surra and Dourine caused by *Trypanosoma evansi* and *T. equiperdum*. She also emphasised the important work that had been accomplished by the former Group on Non-Tsetse Transmitted Animal Trypanosomoses (NTTAT).

During the meeting, several presentations were given by the participants. Dr Claes presented the biological and molecular relationship between *T. evansi* and *T. equiperdum*, Dr Mamadou Dia presented an overview on Surra in dromedary camels and Dr Lun presented differential diagnosis between *T. brucei/T. equiperdum* and *T. evansi* by PCR¹ based on the sequences of kinetoplast DNA (kDNA) maxicircles (ND5) of the subgenus *Trypanozoon*.

Dr Francesco Berlingieri, Deputy Head of the Animal Health Information Department, provided the Group with a brief presentation of WAHIS and an overview of the world animal health situation for Surra and Dourine based on the reports of the OIE Member Countries and Territories. Dr François Diaz, Scientific and Technical Department, presented the OIE international standards dealing with diagnostic tests, and the OIE procedure for validation and certification of diagnostic assays.

2. Definition of *Trypanosoma evansi* and *T. equiperdum* and diagnostic criteria for their identification

The Group discussed in detail the definition of the pathogens (trypanosomes) that cause Surra and Dourine and their diagnostic criteria.

The Group pointed out that there are currently no serological methods that can be used to distinguish *T. evansi* from *T. equiperdum*. Differentiation requires molecular methods based on the presence of kDNA maxicircles in *T. equiperdum* but not in *T. evansi* (only kDNA minicircles were found in *T. evansi*). It is known, however, that akinetoplastic strains can occur in naturally infected hosts, which may limit this approach for diagnostic purposes. In these cases, other molecular markers can be applied (VENTURA *et al.*, 2002).

Trypanosoma evansi

Trypanosoma evansi is a blood and tissue trypanosome that can infect large numbers of domestic and wild animals. It is morphologically similar to *T. brucei* and *T. equiperdum*, but is a monomorphic trypanosome (these three taxa belong to the subgenus *Trypanozoon*).

¹ PCR: Polymerase chain reaction

The Group agreed that *T. evansi* could be defined using the following criteria:

- Clinical signs of the infected animal: compatible with the general signs described for trypanosomoses;
- Morphology and morphometry: *Trypanozoon*-specific and monomorphic; the slender form of *T. evansi* is most commonly observed. It is a long, thin trypanosome (length: 25–34 µm, width: 2–3 µm), with a large undulating membrane, a free flagellum (6–8 µm), and a small subterminal kinetoplast situated at the posterior end of the body. The nucleus (2–3 µm) is relatively homogeneous, situated equidistant from the anterior end and the kinetoplast. In dis- and akinetoplastic forms, the kinetoplast is not visible or is absent.
- Culture characteristics: able to grow in various hosts; laboratory animals, such as mice and rats are easy to infect;
- Molecular characteristics: absence of kDNA maxicircles. Other traits are shown in Table 2.1.

Additional parameters (for confirmation) should also be considered:

- Epidemiological characteristics of the pathogen (geographical distribution, vectors and transmission dynamics, reservoirs, susceptibility of hosts: camel, equines, rodents, dogs, etc.);
- Absence of pathognomonic signs, though *T. evansi* is most often present in the blood;
- Diagnostic tools: CATT²/*T. evansi* and ELISA³/*T. evansi*, ITL⁴;
- kDNA: homogeneous minicircles.

The diagnostic criteria for the identification of *T. evansi* are:

- Epizootiological data: sample information (host species, country or origin, clinical signs, apparent mode of transmission, etc.);
- Morphological identification based on microscopic examination of Giemsa-stained preparations. However this method is only *Trypanozoon* specific;
- With fresh samples: mouse inoculation and mHCT⁵, mAECT⁶;
- Molecular diagnostics (see Table 2.1.):
PCR: TBR⁷ (*Trypanozoon*), RoTat 1.2. VSG⁸, TEPAN minicircle primers (PANYIM *et al.*, 1993; to be confirmed),
PCR ITS⁹-1 for differential diagnosis from other *Trypanosoma* spp., (DESQUESNES *et al.*, 2001),
LAMP¹⁰: PFR primers (*Trypanozoon*) (KUBOKI *et al.*, 2003);
- Optional: serological diagnostic tools such as CATT/*T. evansi* and indirect ELISA/*T. evansi* (VSG or soluble antigen).

A differential diagnosis has to be performed with the other *Trypanosoma* spp. (*T. equiperdum*, *T. b. brucei*, *T. cruzi*, *T. vivax*, *T. congolense*, etc.) and pathogens causing diseases with similar clinical signs (*Anaplasma*, *Babesia*, *Theileria*, *Haemonchus*, etc.).

Trypanosoma equiperdum

Trypanosoma equiperdum is a typical tissue trypanosome and is only found in equines. It is morphologically similar to *T. evansi* and is also a monomorphic trypanosome (belonging to the subgenus *Trypanozoon*), but it differs from *T. evansi* in several traits.

The Group agreed that *T. equiperdum* could be defined using the following criteria:

- Morphology and morphometry: *Trypanozoon*-specific and monomorphic;

² CATT: Card agglutination test

³ ELISA: Enzyme-linked immunosorbent assay

⁴ ITL: Immuno-trypanolysis test

⁵ mHCT: Micro-haematocrit centrifugation technique

⁶ mAECT: Mini-anion exchange chromatography technique

⁷ TBR: Tris (bypyridine) ruthenium

⁸ VSG: Variant surface glycoprotein

⁹ ITS: Internal transcribed spacer

¹⁰ LAMP: Loop-mediated isothermal PCR

- Host specificity: only equines;
- Clinical signs in the infected animal: compatible with the general signs described for trypanosomosis associated with inflammation and oedema in the genital area and paralysis (paraplegia);
- Culture characteristics: it cannot infect mice and rats without a long adaptation period;
- Molecular characteristic: see Table 2.1.

Additional parameters (for confirmation) should also be considered:

- Epidemiological characteristics of the pathogen (geographical distribution, sexual transmission, etc.);
- *Trypanosoma equiperdum* is a tissue parasite that is mainly present in the genital area and can be collected in genital secretions. Note: classically described lesions of “dourous-like” plaques are not regularly observed, in particular in recent cases.

The diagnostic criteria for the identification of *T. equiperdum* are:

- Epizootic data: sample information (host species, country, clinical signs, etc.);
- The specimen collected should be isolated from genital secretions and tissues and, optionally, from blood (mHCT and mAECT);
- Morphological identification based on microscopic examination of Giemsa-stained preparations. However this method is only *Trypanozoon* specific;
- With fresh samples: mouse/rat inoculation is often unsuccessful;
- Molecular diagnostic (see Table 2.1.)
PCR: TBR (*Trypanozoon*), maxi-circle (nd5-PCR);
- Optional: Serological diagnostic test methods such as CFT¹¹/*T. equiperdum*, CATT/*T. evansi* and indirect ELISA/*T. evansi* (RoTat 1.2. VSG or soluble antigen) should be positive.

A differential diagnosis has to be performed with the other *Trypanosoma* spp. (*T. evansi*, *T. b. brucei*, *T. cruzi*, *T. vivax*, *T. congolense*, etc.) and pathogens causing diseases with similar clinical signs (*Anaplasma*, *Babesia*, *Theileria*, *Haemonchus*, etc).

Table 2.1. Primers for differential and diagnostic purposes

Primers	<i>T. brucei</i>	<i>T. evansi</i>	<i>T. equiperdum</i>	Comments
TBR	Yes	Yes	Yes	Subgenus <i>Trypanozoon</i> specific primers MASIGA <i>et al.</i> , 1992
TE2249/50	No	Yes Akinetoplastic strains: No	Yes To be confirmed	Minicircle primers, ARTAMA <i>et al.</i> , 1992; species specificity should be confirmed.
TEPAN1/2	No	Yes	Yes To be confirmed	PANYIM <i>et al.</i> , 1993; species specificity should be confirmed.
RoTat1.2	No	Yes Type B strains: No	? To be determined	Typical for <i>T. evansi</i> Type A strains.
ND5-PCR	Yes	No	Yes	Can be used to distinguish <i>T. evansi</i> from <i>T. equiperdum</i> and <i>T. brucei</i> . LI F.J. <i>et al.</i> , 2006

¹¹ CFT: Complement fixation test

3. Recommendations for diagnostic tests and proposals for the revision of the chapters in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)*

As a general issue and prior to the discussion on diagnostic tests, the Group emphasised the importance of the collection of the specimens (preferentially capillary blood for *T. evansi* and genital secretions for *T. equiperdum*) and of the collection of data on the epidemiological situation (geographical origin, host species, clinical signs, apparent transmission mode, etc.).

The Group stated that if the parasite can be fixed and stained with Giemsa, its morphology and morphometry will identify it as belonging to the subgenus *Trypanozoon*.

Direct microscopic examination of the blood, mHCT, mAECT and mouse inoculation should always be applied, when possible.

The next step in the identification can only be fulfilled using molecular tools. Differentiation of *Trypanozoon* and other subgroups and species is easy and reliable as cross reactions with the species-specific primers for *T. vivax* (TVW), *T. congolense* and its subtypes (TCS, TCF, TCK¹²), and *T. cruzi* have never been observed. As for species identification, several primers need to be confirmed (primers by ARTAMA *et al.*, 1992 and PANYIM *et al.*, 1993), but a combination of these primers and new ones (ND5) should lead to the differentiation of the three taxa as indicated previously. In conclusion, the species specificity of some primers remains to be confirmed with a larger range of referenced DNA.

Despite the high sensitivity of molecular techniques, infected animals may still often test negative. Thus, serological techniques are very helpful for detecting such animals.

The Group indicated that serological examinations are highly sensitive and recommended that CATT/*T. evansi* and indirect ELISA/*T. evansi* be performed. Although these tests can cross react with other pathogenic trypanosomes, they have excellent predictive values, especially positive predictive values for the CATT/*T. evansi*, and negative predictive values for the ELISA/*T. evansi*. The CATT/*T. evansi* could be readily standardised according to the OIE standards, but more work is still necessary before the indirect ELISA/*T. evansi* can be standardised in the various host species. Work is in progress in this respect (DESQUESNES *et al.*).

Although they cross react at the species level, serological tests are specific for pathogenic trypanosomes and, so far, CATT/*T. evansi* and ELISA/*T. evansi* can detect all the pathogenic trypanosomes. During studies carried out in France, however, strong cross reactions were observed in several serum samplings from sheep bred in non-infected areas. This drawback could be overcome by the application of the RoTat 1.2 ITL in sheep when false positive results are obtained using the ELISA. The ITL should be evaluated further against the other previously described tests.

It is difficult to evaluate the performance of the CFT/*T. equiperdum* in the absence of recent and certified reference samples. To date, it appears that results can be different from one laboratory to another using the same serum samples. This variation may be due to different stocks used for antigen preparation or to sensitivity levels. The Group observed that like all the other serological tests, CFT/*T. equiperdum* should cross react with *T. brucei*, *T. evansi* and other pathogenic trypanosomes. It is therefore, not a species-specific test. Molecular confirmation should be made in cases of serological suspicion.

The Group strongly recommended that a coordinated research project on *T. equiperdum* be developed, based on a series of experimental infections in groups of ten horses with five different isolates of *T. equiperdum* in order to observe the evolution of the parasite in its natural host and to generate reliable reference samples for the evaluation of diagnostic tests.

The LAMP technique is still being evaluated, but seems to require a high level of technical expertise and laboratory staff training in its correct use. The necessity for penicillin tests has been emphasised.

¹² *Trypanosoma congolense* type savannah (TCS), *T. congolense* type forest (TCF), *Trypanosoma congolense* type Kilifi (TCK)

In conclusion, the Group agreed that the diagnostic tests available have limitations, but that combining tests can give satisfying results in terms of sensitivity and specificity. Priority can be given to one of the tests according to the purpose of testing. Proposals of protocols for the diagnostic test methods used according to the situation and of measures suitable for the control of the diseases (quarantine, surveillance, etc.) could be discussed and provided by this Group on request of the OIE.

Concerning the two chapters from the OIE *Terrestrial Manual*, all participants agreed that a large part of the information needs to be updated. Complete rewriting of these chapters was proposed under the coordination of Dr Desquesnes for Chapter 2.1.17. *Trypanosoma evansi* infections (including Surra) and of Dr Claes for Chapter 2.5.3. Dourine. They will begin to update the current English version of these chapters in editing-mode and will then consult the other members of the Group. This work should be completed within 3 months (1 month for initial rewriting, 1 month for consultation with other members and 1 month for final editing).

4. The current global situation regarding Dourine and Surra

The Group discussed in detail the current global situation regarding Dourine and Surra, and emphasised that it is difficult to define the 'true' global situation for these diseases because of the lack of comprehensive field surveillance data. The problem of differential diagnosis of Dourine and Surra in equines makes it difficult to clarify the global disease situation, in addition to the observed discrepancies between official data, scientific publications and other available expert information (e.g. from the former NTTAT Group).

As the epidemiology of the disease can be different depending on the host species, the Group defined the important host species of Dourine and Surra as follows:

- Dourine: equines (horses, donkeys and their crossbreeds). No wild intermediary host has been identified so far.
- Surra: two subgroups:
 - a) Highly susceptible animal species: camelids (*Camelus* spp.), equines and dogs;
 - b) Susceptible species: water buffalo (*Bubalus* spp.), cattle, deer, goats, pigs and sheep. In addition to the above-mentioned host animals for Surra, wild animals, such as capybara (*Hydrochoerus* sp.), coati (*Nasua* sp.), vampire bats (*Desmodus* sp.) in Latin America and game animals everywhere, should be considered as important reservoir hosts.

Dourine

Although clinical cases of Dourine have rarely been reported, at least during the past decades, because of the manner by which it is transmitted, the disease could occur all over the world, and local 'hot spots' still remain in several regions.

Proven clinical cases of Dourine were reported by Dr Claes in Ethiopia in 2008. The Group agreed that further characterisation of these 'new' *T. equiperdum* isolates should be carried out.

Confirmed and/or suspected Dourine cases have regularly been reported to the secretary general of the former *ad hoc* Group on NTTAT during the past 25 years. However, to confirm true disease status, the Group strongly recommends carrying out extensive epidemiological surveillance of Dourine (and Surra).

Russia, Asia and Southern Africa have reported cases. In Europe (Germany), suspected cases have been found in imported horses. No information is available for Latin America, but Dourine could be present.

Surra

Surra is mainly epidemic in subtropical and tropical areas where effective vectors, such as *Tabanus* and *Stomoxys*, exist. Although several epidemiological surveys of Surra are published every year, information is not sufficient to fully understand the worldwide situation. Bearing in mind the pandemic that progressively invaded South America since the 17th century and is still present in this subcontinent, the Group strongly recommends conducting extensive field surveillance of Surra.

As Surra is epidemic in a substantial number of countries, the names of countries or areas free from this disease are provided here: Australia, Japan, New Zealand, North America and South Korea. Europe was considered as free until 1998 (MOLINA *et al.*, 1999); since that time, some localised cases have been confirmed.

Transmission and epidemiological cycles

Although the mechanical transmission of *T. evansi* by biting insects (tabanids, stomoxes, hypoboscids, etc.) between herbivores seems to be well understood, as well as the peroral transmission from herbivores, including small ruminants or rodents, to carnivores, it was emphasised that the links between herbivores and wildlife reservoirs such as rodents is not known, as well as the ascendant link (should one exist) from wildlife reservoirs to livestock; these links should be investigated to better understand and control the risk of the disease becoming endemic.

Studying the potential vectors of *T. evansi* should also be encouraged.

General recommendations

- Update the distribution map for Dourine and Surra in collaboration with OIE.
- Conduct serosurveys in camels by CATT in Europe, especially in southeastern Europe.
- Publish a scientific review of the global situation regarding Dourine and Surra. Dr Desquesnes has initiated this task; other members are invited to participate as co-authors.

5. Exchange of views on whether Dourine should remain on the OIE list

Although cases of Dourine have been rare in the last 20 years, the Group agreed by general consensus that at this time, the disease should still remain in the OIE list. The major arguments for this current position are:

- the fact that there are still clinical cases of Dourine found in different parts of the world and that some regions are still considered to be endemic for Dourine;
- the fact that so far no definite differential diagnostic test exists;
- the current rules and regulations are different for Surra and Dourine, more specifically: (i) there is a chapter in the OIE *Terrestrial Animal Health Code (Terrestrial Code)* for Dourine but not one for Surra; (ii) there are very different regulations regarding treatment of these diseases (i.e. treatment is recommended for Surra and stamping out/slaughtering is recommended for Dourine); (iii) the host species are different (Surra is a multispecies disease while Dourine is described only in equines).

The Group discussed the fact that in the future, Dourine could be considered as an equine trypanosomoses (including Surra and Dourine), if the following preconditions are taken into account:

- implementation of experimental studies with parasites causing well defined Dourine infections during which the efficacy of drug treatment for Dourine could be demonstrated periodically with diagnostic tests;
- to change the policy of the OIE *Terrestrial Code* regarding Dourine. If drug efficiency was proven in Dourine cases (as it is now for Surra), there would be no need for a stamping-out strategy and thus the treatment for both Surra and Dourine would be the same.

In this case, *Trypanozoon*-detection tests (a combination of serology and PCR) would be enough to identify an infection with equine trypanosomoses and would serve as sufficient control tool. Differentiating *T. evansi* from *T. equiperdum* would remain a scientific matter, but this would not affect the control measures.

Some Group members suggested that as *T. equiperdum* has specific hosts (equines), it should be considered as a different species from *T. evansi* that can infect multiple hosts. The multiplication of *T. equiperdum* in different animal species (e.g. camelids, goats, etc.) should be studied.

6. Any other business

General recommendations

- Examine the potential immunosuppressive effects of *T. evansi* and their impact on vaccination and secondary infections (HOLLAND *et al.*, 2001, 2003).
- Apply and develop validated models to assess the socio-economic impact of Surra as was recently done in the Philippines (DOBSON *et al.*, 2009).
- Consider the addition of a chapter on Surra to the *Terrestrial Code*.
- An EU Dourine reference laboratory has been recently established (AFSSA, Dozulé, France); it is recommended that OIE contact this laboratory for more information and to coordinate activities.
- Establish a list of laboratories working on *T. evansi*/*T. equiperdum* to create a worldwide network.
- Maintain the annual meeting of the former NTTAT Group, which generates an unrivalled gathering of information and exchange between researchers and epidemiologists concerned by these diseases and draws attention to a disease that was – and still remains – insufficiently explored since the discovery of the first pathogenic trypanosome in 1880. It is wished that further meetings be held in regions where NTTAT occur or could be considered as potential hazards and to this end that the necessary material support be found.

Other *Trypanosoma* species

During this first meeting, the *ad hoc* Group focused on *T. evansi* and *T. equiperdum* as recommended in the terms of reference. However, as it is concerned with the diagnostic tests for trypanosomoses, discussions could be extended in future meetings to other *Trypanosoma* species. It was emphasised that in various circumstances, other *Trypanosoma* species have to be considered, at least for differential diagnosis, depending on the hosts and geographical areas, with *T. brucei*, *T. vivax*, *T. congolense*, *T. simiae* and *T. cruzi*.

Strategy for the control of Surra and Dourine

The Group agreed that although the rigid sanitary regulations regarding the control of Dourine (with the final outcome of castration or slaughter) contributed to the considerable reduction in the occurrence of this plague, at the same time they prevent, to some extent, reporting of the disease. It seems that, if the possibility of treatment – duly checked and controlled – existed, regulations could be less severe and reporting would improve. The experience gained during the eradication of Dourine from countries in North Africa and Europe, which was reported to the OIE (severe sanitary measures combined with the treatment of ‘valuable’ horses with neoarsphenamine), proved that this method could be applied with success. Currently, neoarsphenamine has been completely discontinued, but another arsenic derivative, melarsomine¹³ – specific for the treatment of camel Surra – demonstrated in the laboratory good activity with regard to *T. equiperdum* and would be of merit in field trials. The Group suggested that this treatment could be evaluated during the experimental infection of horses in Ethiopia. Should a chapter on *T. evansi* be added to the *Terrestrial Code*, the Group strongly suggested that the regulations for Surra should not be as strong as those for Dourine, as the lack of information from the field would result in a spread of the disease. Surra is treated with appropriate doses of melarsomine administered intramuscularly. The dose of the drug must be adapted to the circumstances and the host species (for example: double dose prior to introduction from infected to non-infected area).

Melarsomine in cattle

For the time being, melarsomine is only registered for use in camels. According to several previously published works, a normal dose of melarsomine for cattle and buffalos is 0.5 mg per kg of body weight, administered intramuscularly (DIA *et al.*, 2008), and is very expensive for farmers. It would be opportune to ask the drug manufacturers if melarsomine could be registered for bovines, and if the price could be adjusted to make it affordable to cattle farmers.

.../Appendices

¹³ Brandname: Cymerlarsan®

**MEETING OF THE OIE AD HOC GROUP
ON DIAGNOSTIC TESTS FOR TRYPANOSOMOSES
Paris, 30 March–1 April 2009**

Agenda

1. Introduction
 2. Definition of *Trypanosoma evansi* and *T. equiperdum* and diagnostic criteria for their identification
 3. Recommendations for diagnostic tests and proposals for the revision of the chapters in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)*
 4. The current global situation regarding Dourine and Surra
 5. Exchange of views on whether Dourine should remain on the OIE list
 6. Any other business
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Appendix II

**MEETING OF THE OIE AD HOC GROUP
ON DIAGNOSTIC TESTS FOR TRYPANOSOMOSES
Paris, 30 March – 1 April 2009**

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