

EU COMMENTS AND POSITIONS
On the proposed changes to OIE Manual of Diagnostic Tests and
Vaccines for Terrestrial Animals

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ON THE PROPOSED CHANGES TO OIE MANUAL OF DIAGNOSTIC
TESTS AND VACCINES FOR TERRESTRIAL ANIMALS

PRESENTED FOR ADOPTION IN NEXT MAY 2010 GENERAL SESSION

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Vaccines for Terrestrial Animals

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CHAPTER 1.1.7: THE APPLICATION OF BIOTECHNOLOGY TO THE DEVELOPMENT OF VETERINARY VACCINES

General comments

The EU can support the proposed changes. Nevertheless, in order to take into account all latest techniques and to have the clearest possible vocabulary referring to genes, the specific comments detailed below should be taken on board in the final revised version to be adopted in the next General Session or in the next meeting of the BSC.

Specific comments

LINE 32: There is no mention of "defective in second cycle" (DISC) vaccines in the section on reverse genetics. This is an exciting development which essentially involves the deletion of an open reading frame coding for a key viral protein involved in the replication or structure of a virus, then expressing the missing protein in trans – i.e. in the cell that is also replicating the defective virus. This way, one gets a virus which can only complete one round of replication, then is defective thereafter. It is more stimulatory than a killed vaccine but does not have the problems associated with a live vaccine. The EU suggests the BSC consider its inclusion.

Ref: Widman, D. G., I. Frolov, and P. W. Mason (2008), Third-generation flavivirus vaccines based on single-cycle, encapsidation-defective viruses, *Adv Virus Res*, 72, 77-126.

Zevenhoven-Dobbe, J. C., S. Greve, H. van Tol, W. J. Spaan, and E. J. Snijder (2004), Rescue of disabled infectious single-cycle (DISC) equine arteritis virus by using complementing cell lines that express minor structural glycoproteins, *J Gen Virol*, 85(Pt 12), 3709-3714.

LINE 30: add the words "or coding sequences" after the word "genes".

LINE 33: Reverse genetics does not only apply to RNA viruses; it is also used for DNA viruses, so the text here seems a little odd, as does the following paragraph. They should be rephrased or deleted.

LINES 38 and 129-150: The text sometimes uses the word "gene" inappropriately. This term should not be used for regions of the coding sequence of positive strand RNA viruses like the picornaviruses and flaviviruses where the genome essentially acts as an mRNA to encode a large polyprotein from a single open reading frame. There the word "gene" should be replaced by "coding sequence".

LINE 138 and ff: In this paragraph the text is correct in referring to "coding sequences" for specific proteins from the pestiviruses. However, in the paragraph beginning **line 144**, the text describes some of these coding sequences as "genes" from YFV and WNV. It is incorrect to describe these portions of +ve sense RNA genome as genes: they are parts of a single large open reading frame from an RNA which is analogous to an mRNA, i.e. a product of a gene.

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CHAPTER 2.1.7: JAPANESE ENCEPHALITIS

General comments

The EU can support the proposed changes. Nevertheless, the specific comments detailed below should be taken on board in the final revised version to be adopted in the next General Session or in the next meeting of the BSC.

Specific comments

LINE 33: The following sentence should be added at the end of the paragraph: "Cross-neutralisation between members of the JE serocomplex is common. For this reason, serological diagnosis should be a primary screen. Confirmatory diagnosis (preferably by virus isolation) is also required."

LINE 116: In order to be precise, it should read "Diagnosis requires a measurable significant" etc.

LINE 124: The word "perform" should be replaced by "undertake"

LINE 130: At the end of the sentence, the following words should be added to emphasize the reason when VN is the most specific: "especially if a 90% neutralisation threshold is required".

LINE 135: The word "virus" should be added after the words "West Nile" and after the words "Japanese encephalitis".

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CHAPTER 2.1.12: Q FEVER

General comments

The EU can support the proposed changes. Nevertheless, in order to take into account all latest diagnostic techniques such as fluorescent in situ hybridization, the specific comments detailed below should be taken on board in the final revised version to be adopted in the next General Session or in the next meeting of the BSC.

Specific comments

LINE 40: After the word "antibodies", add the words "by in situ hybridization with specific oligonucleotide probes".

LINE 220: Add the sentence: "Furthermore, fluorescent in situ hybridization using oligonucleotide probes specific targeting 16S rRNA may be used on paraffin embedded tissues especially on placenta samples (Jensen et al, 2007)."

LINE 582: Insert the following reference: "Jensen T.K., Montgomery D.L., Jaeger P.T., Lindhardt, Agerholm J.S., Bille-Hansen V., Boye M. (2007). Application of fluorescent *in situ* hybridisation for demonstration of *Coxiella burnetii* in placentas from ruminant abortions. *APMIS*, 115, 347-353."

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CHAPTER 2.1.13: RABIES

General comments

The EU wishes the Chapter to be improved by taking on board the numerous specific comments detailed below before the final revised version is adopted in the next General Session, or its adoption should be postponed for further examination in the next meeting of the BSC. Especially, the whole section on vaccines needs further work. There are no clear requirements like number of animals to be used, duration of studies, etc. As it is currently written it is very sketchy and often irrelevant. In particular, it seems that it deals less with oral vaccines than with oral vaccination strategy: this is not acceptable as it should be part of the Terrestrial Code, not the Manual. Moreover, vaccines for parenteral use hardly get any attention.

Specific comments

LINE 21: The EU does not agree. How can a less sensitive respective reliable method be used as a confirmatory test considering that other confirmatory tests are available?

LINE 23: The sentence “As a single negative test on fresh material does not rule out the possibility of infection, cell culture or mouse inoculation tests should be undertaken simultaneously” should be replaced by the sentence: “In cases of inconclusive results from FAT, or in all cases of human exposure, further tests (cell culture or mouse inoculation tests) on the same sample or repeat FAT on other samples are recommended.”

Considering the sensitivity and specificity of the FAT many countries even in the EU are wasting their capacities and limited financial resources in doing simultaneous cell culture or mouse inoculation; only FAT-inconclusive results or FAT-negatives results with a known human exposure should be subject of confirmatory testing.

LINE 39: The sentence “Results are expressed in International Units or equivalent units relative to an international standard antiserum” should be moved to line 37 after the first sentence in this paragraph.

LINE 39: This should only apply to VN assays.

LINE 52: The words “by mouse vaccination followed by intracerebral challenge” should be deleted.

LINE 59: The sentence should start by “Rabies is caused by neurotropic viruses”.

LINE 60: The first part of the sentence should be “As the viruses are”.

LINE 63: In the text, four new lyssaviruses are mentioned. It means that according to the present knowledge there are already 11 distinct genetic

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lineages of lyssaviruses. Therefore the EU suggests that the text would be amended accordingly: "Eleven distinct genetic lineages (species) can be distinguished within the genus".

LINE 67: Behind the words "(ABLV, genotype 7)." the following sentences should be inserted: "Four new lyssaviruses Aravan virus (ARAV), Khujand virus (KHUV), Irkut virus (IRKV), and West Caucasian bat virus (WCBV) have been isolated recently from Eurasian bats, and have been ratified as new lyssavirus species (Kuzmin et al., 2003, 2005). In addition, a newly identified bat lyssavirus (Shimoni bat virus) has been isolated from a bat in Kenya, Africa and is awaiting official classification (Kuzmin et al., 2010). Each of the four viruses: Aravan virus (ARAV), Khujand virus (KHUV), Irkut virus (IRKV) and West Caucasian bat virus (WCBV) can be considered as new independent species within the Lyssavirus genus (ICTV, 2009)."

LINES 76-83: As a consequence of the above and for more clarity, the four sentences starting with "Little or no cross protection" and ending with "(USA, unpublished data)." should be modified in order to read the following: "A reduced protection with pre-exposure vaccination and with conventional rabies post-exposure prophylaxis was observed against IRKV, ARAV, and KHUV (Hanlon et al., 2005) and all of the above mentioned lyssavirus species were assigned to phylogroup 1. Little or no cross-protection against infection with the members of phylogroup 2, MOKV and LBV is elicited by rabies vaccination and most anti-rabies virus antisera do not neutralise these lyssaviruses (Badrane et al., 2001,). WCBV does not cross-react serologically with any of the two phylogroups."

LINE 110: Behind the words "rabies diagnostic chain'." the following words should be inserted: "and should follow international guidelines."

LINE 133: The sentence "Bovine spongiform encephalopathy (BSE) should be considered in the differential diagnosis of most cattle that are considered to be 'rabies suspect'" should be deleted as it is not relevant to this chapter.

LINE 135: Behind the words "Sampling of brain specimens" the words "for both diseases" should be replaced by the words "in cattle".

LINE 136: Behind the words "tool' developed for" the word "BSE" should be replaced by "bovine spongiform encephalopathy (BSE)"

LINE 162: The word "saline" should be replaced by the word "PBS".

LINE 170: The sentence "Sensitivity may be lower in samples from vaccinated animals due to localisation of antigen, which is confined to the brainstem." should be deleted.

LINE 194: Behind the words "(Genovese & Andral, 1978[27]" the words "Lembo et al. 2006" should be inserted.

LINE 198: Behind the words "may be used on" the words "fresh brain tissue or" should be inserted.

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LINE 205: Insert “highly” after “(ATCC) are”.

LINE 210: Behind the words “(Rudd and Trimarchi, 1987)”, the following sentence should be inserted: “In general, three consecutive passages should be undertaken to confirm a negative result.”

LINES 214-221: The *suggested protocol* appears to be unclear and evokes some questions:

- Why is it suggested to use a 96-well plate? It would make the overall cell coated surface smaller than before. 24-well plates may be practical.
- The protocol does not tell how many wells should be infected.
- The suggested protocol tells to incubate the samples 24h. This time would be too long when testing samples which have not reached the laboratory in fresh state but could be already toxic to the cells. Incubation as short as 35min can be used, which is remarkably shorter than 24h mentioned in the suggested protocol.
- The number of passages required is unclear. The suggested protocol as described here using micro titre plates does not allow serial passaging! After original 4 days incubation further passages are recommended, not ordered. The text in *suggested protocol* should be rephrased in a more clear and unambiguous form.

LINE 237: Behind the words “mouse inoculation test” the words “whenever possible” should be added.

LINES 245 – 249: For the sake of clarity, only the tests which can be recommended for primary diagnosis should be included in c), i.e. this paragraph iii) should be moved to d), as well as new paragraphs iv) and v) proposed below.

LINE 249: The following paragraphs should be inserted:

“ Detection of viral RNA and genome

Various molecular diagnostic tests, e.g. detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR), PCR-enzyme-linked immunosorbent assay (PCR-ELISA), hybridisation in situ and realtime PCR have been proposed as rapid and sensitive alternative techniques for rabies diagnosis (for review see Fooks et al., 2009). The principle of lyssavirus specific PCRs is a reverse transcription of the target RNA (usually parts of the N gene) into complementary DNA followed by the amplification of the cDNA by PCR. Although those molecular tests have the highest level of sensitivity, their use is currently not recommended for routine post-mortem diagnosis of rabies (WHO 2005). Such techniques can produce false positive or false negative results and therefore, require standardization and very stringent quality control. Nevertheless, they represent the only possibility to diagnose rabies ante-mortem in humans. In such cases, serial samples of fluids (e.g. saliva and urine) should be tested, owing to intermittent shedding of virus (WHO 2005).

other identification tests (characterization)

The tests above describe methods to accurately diagnose rabies and to isolate and identify the virus. Typing of the virus can provide useful

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epidemiological information and should be carried out in specialised laboratories (such as OIE or WHO Reference Laboratories). These techniques would include the use of MAbs, nucleic acid probes, or the polymerase chain reaction (PCR), followed by DNA sequencing of genomic areas for typing the virus (Bourhy et al., 1993[17]). These characterisations enable for instance a distinction to be made between vaccine virus and a field strain of virus, and possibly identify the geographical origin of the latter. "

LINE 257: Under this paragraph d (other identification tests), recently developed RIDT needs to be mentioned

LINE 274: Behind the words "(Turmelle et al., 2009)" the words "however, standardisation of modified versions for testing of bats is still missing" should be inserted.

LINE 340: Previous to the words "six 50 µl" the words "four to" should be inserted.

LINE 503: Behind the words "Control and Prevention is the" the word "first" should be replaced by "second".

LINE 504: The words in brackets "Montano Hirose et al. 1991" should be replaced by the words "Lynng 1994".

LINE 561: This part needs urgent reconsideration, and until then the ELISA test should not be considered a prescribed test for international trade. To avoid confusion and to set state of the art standards for the future the EU strongly suggest rewording the complete paragraph as follows: "Commercial kits are available for indirect ELISA that allow a qualitative detection of rabies antibodies in individual dog and cat serum samples following vaccination. In contrast to seroneutralisation assays, which detect specific (virus neutralising antibodies) and unspecific neutralising activity of a test serum, most ELISAs are based on the detection of binding antibodies to the rabies glycoprotein. ELISA methods are also useful for monitoring of vaccination campaigns in wildlife populations. ELISA methods provide a rapid (~ 4 hours) test that does not require handling of live rabies virus, to determine if vaccinated animals have sero-converted. The sensitivity and specificity of any kit used should be determined, and kits validated for the animal species under study. In accordance with the WHO recommendations (WHO Expert Committee on Biological Standards, 1985), 0.5 IU per ml of rabies antibodies is the minimum measurable antibody titre considered to represent a level of immunity that correlates with the ability to protect against rabies infection. However, since binding assays do not necessarily show the correct neutralizing activity of sera, by comparison with virus neutralisation methods ELISAs per se are not the correct test to be used as a standard method for measuring protection. If individual VNAs are required serum neutralizing assays are a prerequisite and any ELISA can only be used as a screening tool regardless of its design. ELISAs may be acceptable as serological tests for international movement of dogs or cats provided that a kit is used has been validated according to the specific needs (i.e. seroconversion) and adopted on the OIE Register as fit for such purposes

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(see http://www.oie.int/vcda/eng/en_VCDA_registre.htm?e1d9). Virus neutralisation methods may be used as confirmatory tests if desired. “

LINES 579-586: For an OIE document, this is rather additional information and could be omitted. Based on the experience in some countries such statement may be used as an excuse for the failure of a vaccination campaign in dogs or wildlife.

LINES 594-596: This cannot be stated in such a general term. It has been shown that not all live vaccines are effective in certain animal species, e.g. skunks.

LINES 601-602: It has been shown that some vaccines, especially live vaccines, offer protective immunity for a much longer period of time.

LINE 603: The word ‘they’ is confusing because it seems to refer to live vaccines but actually "all animal vaccines" is meant.

LINES 606-609: This sentence again is confusing. Vaccination of animals after exposure to a rabid animal is in most countries only permitted when animals have been previously vaccinated. Here it is stated in such a way that one could think that it is generally permitted but in case that the animal has been vaccinated previously it must also be observed for 45 days but previously non-vaccinated do not have to be observed.

LINES 705-706: This is no longer acceptable for oral rabies vaccines used for wildlife. At least it is not a workable approach and should be reconsidered.

LINES 710-712: This is not understandable. During a challenge study in the target species the minimum effective dose is to be determined. So what have suckling mice to do with this?

LINES 760-763: This should be reworded so as to be as precise as possible otherwise it results in an endless list of safety studies.

LINES 784-787: There are strong objections to this statement because this is not realistic. To illustrate the current statement: Let us say there is a parenteral vaccine for dogs. It is just worldwide; does this mean to conduct challenge studies with all local strains circulating across the world? The EU proposes that as a guideline, the challenge virus used should kill at least 80% of the control animals.

LINES 811-812: All vaccines used for ORV induce adverse signs because they are all live replication competent viruses and can therefore induce disease especially in immunocompromised hosts. See two human cases with V-RG; these two cases are adverse events.

LINE 813: What does the term: ‘saliva should be checked’ mean? As it is written it seems unclear, e.g. check saliva after how many hours, days, weeks? In case it is present or not, what are the consequences? What about

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shedding of viable recombinant viruses in faeces in the environment by orally vaccinated wildlife?

LINE 815: The term “lowest residual pathogenicity” needs a definition as it is very relevant. It could be defined as no mortality in 10-day old suckling mice after i.c. administration, or 5-day suckling mice, or nude mice, etc. The EU suggests making it even more stringent: no mortality in RAG2 or SCID mice.

LINES 818-819: Such approach would need another type of safety studies. What to suggest, especially when the vector used is a human pathogen?

LINES 822-825: This sentence needs serious reconsideration. It clearly states that the vaccine producer has to do the challenge study with the vaccine baits, and if this study has been accomplished then the titre used in these baits is sufficient to protect the foxes. Why was another criterion added by stating that a 100% protective dose needs to be obtained by direct oral instillation, which would result in two challenge studies?

LINE 825: Behind the words “live rabies viruses,” the words “, such as the ERA strain” should be deleted.

LINE 826: Behind the words “Fehlner-Gardiner *et al.*, 2008” the words “Müller *et al.*, 2009” should be inserted.

LINES 825 - 827: Comment on maximal vaccine titre: There are strong objections to this statement as these cases have nothing to do with the high dose of the vaccine in the bait.

LINE 842: Behind the words “(Rosatte *et al.*, 2007)” the words “and therefore, complements aerial distribution.” should be inserted.

LINES 843 - 859: The EU wonders whether this kind of information actually belongs here as it relates more to vaccination strategy, than to vaccines.

LINES 843-846: While considering the comment above, the section should be rephrased as follows: “For wildlife in Europe, usually two campaigns are performed yearly in spring and autumn according to the biology of the target species. Taking into account the geographical and epidemiological conditions of the area, generally four campaigns (i.e. 2 years) are conducted after the last detected rabies case in an area.”

LINE 847: The sentence should be rephrased as follows: “The impact of vaccination on the wild host/vector population is evaluated in three different ways:”

LINE 850: Behind the sentence ending with the words “animals to be determined” the following sentence should be added: “Determination of the bait-uptake alone will result in an overestimation of the vaccination coverage as not all animals having consumed a bait will seroconvert.”

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LINE 853: Behind the sentence ending with the words “quality field specimens” the following sentence should be added: “Serological monitoring will only provide information on the achieved level of herd immunity after vaccination campaigns in the field (adequate vaccination).”

LINE 854: Behind the sentence ending with the words “in the vaccinated area.” the following sentence should be added: “This is the key factor for the evaluation of the success of oral vaccination campaigns independent of the bait-uptake rate and herd immunity. Therefore, adequate rabies surveillance is essential.”

LINE 859: Behind the word “Rabies” the word “monitoring” should be replaced by the word “surveillance”.

LINE 881: Behind the words “continue to be a concern” the words “(Rupprecht et al., 2001)”, should be inserted.

LINES 884-885: This sentence should be deleted as everyone is trying to improve its product and no vaccine strain should be explicitly mentioned.

LINES 886-889: This whole section needs reconsideration. Is it the aim to review all potential rabies constructs (baculovirus, CAV2, raccoon poxvirus) or all potential oral vaccine constructs (canine herpesvirus) here? MVA may be suitable but not just as an oral vaccine.

LINES 896 - 901: **The EU strongly recommends deleting this whole section. The information given is premature.** For instance CAV2 seems a dead end (not officially but even the people initiating this research have admitted it).

LINES 904-908: It would be good to set some minimum standards here, e.g. contamination of product with wild-type, shedding of virus in the environment, recombination with wild-type, stability of insert, etc.

LINES 948/949: Between the references to Fehlner-Gardiner and Genovese the following reference should be inserted:

“Fooks, A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C., McElhinney, L. M., Marston, D. A., Dastjerdi, A., Wright, E., Weiss, R. A. & Muller, T. (2009). Emerging technologies for the detection of rabies virus: challenges and hopes in the 21st century. PLoS Negl Trop Dis 3, e530. »

line 954/955: Between the references to Hooper and Knowles the following reference should be inserted: “Kang, B., Oh, J., Lee, C., Park, B. K., Park, Y., Hong, K., Lee, K., Cho, B. & Song, D. (2007). Evaluation of a rapid immunodiagnostic test kit for rabies virus. Journal of virological methods 145, 30-36.”

LINES 957/960:

Between the references to Knowles and Nadin-Davis the reference to Montano Hirose should be replaced by the following references:

“Kuzmin IV, Orciari LA, Arai YT, Smith JS, Hanlon CA, Kameoka Y, Rupprecht CE. (2003). Bat lyssaviruses (Aravan and Khujand) from Central

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Asia: phylogenetic relationships according to N, P and G gene sequences. *Virus Res.*, 97(2):65-79.

Kuzmin IV, Hughes GJ, Botvinkin AD, Orciari LA, Rupprecht CE. (2005). Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Res.*, 111(1):28-43.

Kuzmin IV, Mayer AE, Niezgodá M, Markotter W, Agwanda B, Breiman RF, Rupprecht CE. (2010). Shimoni bat virus, a new representative of the Lyssavirus genus. *Virus Res.*, Feb 6. [Epub ahead of print]

Lembo T, Niezgodá M, Velasco-Villa A, Cleaveland S, Ernest E, Rupprecht CE. (2006). Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerg Infect Dis.*, 12(2):310-3.

Lyng J. (1994). Calibration of a replacement preparation for the International Standard for Rabies Immunoglobulin. *Biologicals*, 22(3):249-55.

Markotter, W., York, D., Sabeta, C. T., Shumba, W., Zulu, G., Roux Le, K. & Nel, L. H. (2009). Evaluation of a rapid immunodiagnostic test kit for detection of African lyssaviruses from brain material. *Onderstepoort Journal of Veterinary Research* 76, 257-262.

Müller T, Bätza HJ, Beckert A, Bunzenthal C, Cox JH, Freuling CM, Fooks AR, Frost J, Geue L, Hoeflechner A, Marston D, Neubert A, Neubert L, Revilla-Fernández S, Vanek E, Vos A, Wodak E, Zimmer K, Mettenleiter TC. (2009). Analysis of vaccine-virus-associated rabies cases in red foxes (*Vulpes vulpes*) after oral rabies vaccination campaigns in Germany and Austria. *Arch Virol.*, 154(7):1081-91. “

Lines 973/ 974: Between the references to Rudd and Servat the following reference should be added:

“Rupprecht CE, Blass L, Smith K, Orciari LA, Niezgodá M, Whitfield SG, Gibbons RV, Guerra M, Hanlon CA. (2001). Human infection due to recombinant vaccinia-rabies glycoprotein virus. *N Engl J Med.* 23;345(8):582-6. Erratum in: *N Engl J Med* 2001 Dec 13;345(24):1784.”

LINE 933 (Insert):

Brookes S.M., Aegerter, J.N., Smith, G.C., Healy, D.M., Jolliffe, T., Swift, S.M., Mackie, I., Pritchard, J.S., Racey, P.A., Moore, N.P., and A.R. Fooks. (2005). Prevalence of antibodies to European Bat Lyssavirus type-2 in Scottish bats. *Emerging Infectious Diseases.* 11(4); 572 – 578.

LINE 940 (Insert):

Cliquet, F., McElhinney, L.M., Servat, A., Boucher J.M., Lowings, J.P., Goddard, T., Mansfield, K.L and A.R. Fooks. (2004). Development of a Qualitative Indirect ELISA Test for the Measurement of Rabies Virus-Specific Antibodies from Vaccinated Dogs and Cats. *Journal of Virological Methods.* 117(1); 1-8.

LINE 940 (Insert):

[Kuzmin IV](#), [Mayer AE](#), [Niezgodá M](#), [Markotter W](#), [Agwanda B](#), [Breiman RF](#), [Rupprecht CE](#). **Shimoni bat virus, a new representative of the Lyssavirus genus.** [Virus Res.](#) 2010 Feb 6. [Epub ahead of print]

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CHAPTER 2.1.17: TRYPANOSOMA EVANSI INFECTION (SURRA)

General comments

The EU commends the work of the OIE on this chapter, well written and referenced. However, little mention is made of infection or disease in dogs, which is becoming increasingly important with pet travel.

Specific comments (editorial)

LINE 53: The words "*T. cruzi*" and "*T. equiperdum*" should be in italics.

LINE 75-76: Use the plural for consistency: "buffaloses, cattle, llamases and dogses"

LINE 289: The word "subclinical" should be written "sub-clinical"

LINE 349: The words "for long" should be replaced by the words "for a long time".

LINE 367: "non-specific"

LINE 385: The word IFAT should be read: "Immunofluorescent Antibody Test (IFAT)".

LINE 390: Check the spelling of Baiyana or Bajyana. (See reference list.)

LINE 500: "There are a number of methods"

LINE 522: Check the spelling of Sasma or Sasmal. (See reference list.)

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CHAPTER 2.3.8: DUCK VIRUS HEPATITIS

General comments

The EU can support the proposed changes. Nevertheless, the specific comments detailed below should be taken on board in the final revised version to be adopted in the next General Session or taken into account in the next meeting of the BSC.

Specific comments

LINE 9: The words "Three genotypes and maybe" should be replaced by "Three genotypes that may also be".

LINE 26: The words "Reverse-transcriptase" should be replaced by "Reverse-transcription".

LINE 52: The words "thus DHAV-1 (the original DHV type I) should read: "thus Duck Hepatitis A virus type 1 (DHAV-1 - the original DHV type I)".

LINE 57: After the words "ICTV EC for consideration" should be added the words "re the classification of DHV type 1".

LINE 59: After the words "would be known", add the word "respectively".

LINE 75-76: Replace the words "they present similarly to DHAV-1 virus" by "the clinical presentation is similar to DHAV-1".

LINE 80-82: Replace the sentences "The clinical disease is characterised by lethargy and ataxia. Ducklings lose their balance, fall on their sides and kick spasmodically prior to death. At death the head is usually drawn back in the opisthotonos position. "

By

"The clinical disease is characterised by lethargy and ataxia followed by opisthotonos and death. Ducklings lose their balance, fall on their sides and kick spasmodically prior to death."

LINE 85: Replace "distinct punctate" by "distinct petechial".

LINE 141: Replace "transcriptase-PCR assay virus" by "transcription-PCR (RT-PCR) assay".

LINE 152: After the words "It is based on primers specific to amplify a region of the 3D gene", add the words "of DHV type 1".

LINE 168: Do "washing buffers A and B" need to be described?

LINE 214: Why DHAV type 1 here, rather than DHV type 1 infection?

LINE 453-455: Is this paragraph in the right place here?

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LINE 453: Breeder ducks primed with live DHV type I: what is the frequency/age interval for live vaccine here?

LINE 454: Editorial: replace Wookcock by Woollcock.

LINE 470: Breeder ducks primed with live DHV type I: what is the frequency/age interval for live vaccine here?

LINE 472: Editorial: replace Wookcock by Woollcock.

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.3.13: MAREK'S DISEASE

General comments

The EU can support the proposed changes.

Specific comments

LINES 370 - 371: The EU proposes that this now be better placed under the following (new) section (c) Requirements for authorisation, for example as a new (iv). It seems to be more appropriate there rather than remaining where it currently sits under "Final product batch tests" - especially as it basically says there is not requirement to do duration of immunity batch test.

EU COMMENTS AND POSITIONS
On the proposed changes to OIE Manual of Diagnostic Tests and
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CHAPTER 2.4.4: BOVINE BABESIOSIS

General comments

The EU can support the proposed changes. Nevertheless, the specific comments detailed below should be taken on board in the final revised version to be adopted in the next General Session or taken into account in the next meeting of the BSC.

There is notably one recurring error: throughout reference is made to Bock et al 2008 but in reference the list is Bock 2006 – which is correct? All scientific names should be in italics in the introduction.

Specific comments

LINE 31: Editorial: “non-specific”

LINE 54: Editorial: “re-infection”

LINE 93: What is the definition of warmer weather? The text should ideally provide a minimum temperature.

LINE 163: Bock et al 2008 not in reference list

LINE 182: Editorial: “re-suspended”

LINE 187: Editorial: “re-suspended”

LINES 234 ff: Details on these tests should only be included if the materials (recombinant antigen, monoclonal antibody) needed to perform the assays are available. If so, the source, from which they can be obtained, should be clearly mentioned in the text.

LINE 282: The word ‘value’ should read ‘values’ since there are two predictive values (one for positive and one for negative diagnoses).

LINES 282 ff: ‘The specificity, sensitivity and predictive value of these competitive ELISAs have been calculated’: If estimates for these values exist, they should be explicitly mentioned in the text.

LINE 271: Editorial: “non-fat”

LINE 290: Editorial: “re-suspended”

LINE 332: definitions of high parasitaemias for *B. bovis* and *B. bigemina* have been deleted, though they would be useful.

LINES 365-366: There are no references for dot ELISA, slide ELISA and card agglutination tests (deleted in editing).

LINE 374: Bock et al 2008 not in reference list

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LINE 394: Bock et al 2008 not in reference list

LINE 406: Bock et al 2008 not in reference list

LINE 416: Bock et al 2008 not in reference list

LINE 424: “sub-inoculation”

LINE 435: Bock et al 2008 not in reference list

LINE 439: Bock et al 2008 not in reference list

LINE 447: Bock et al 2008 not in reference list

LINE 532: Bock et al 2008 not in reference list

LINE 544: Bock et al 2008 not in reference list

LINE 573: Editorial: *Babesia* in italics.

LINE 580: Bock et al 2008 not in reference list

LINE 598: Bock et al 2008 not in reference list

LINE 649: Bock et al 2008 not in reference list

LINE 662: Bock et al 2008 not in reference list

LINE 672: Bock et al 2008 not in reference list

LINE 685: Bock et al 2008 not in reference list

LINE 688: Bock et al 2008 not in reference list

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.4.6: BOVINE SPONGIFORM ENCEPHALOPATHY

General comments

The changes proposed are generally welcomed by the EU. However, some specific comments detailed below should be taken into account for the final revised version to be adopted in the next General Session.

Specific comments

LINE 13: The words "and possibly spontaneous" should be added as follows: "... suggesting that earlier, undetected indigenous and possibly spontaneous cases may have occurred."

LINE 31: The EU would argue for the re-instatement of the deleted phrase [*before, or without, the recognition*] since fallen stock in particular could be showing some clinical signs which went unrecognised. As written, it applies more to the active screening of the healthy slaughter population.

Line 228: Replace: "All currently recognized forms of BSE (C, H and L-Type) are detectable by these methods." with: "Classical BSE is recognized by all these methods, while a complete evaluation of the approved BSE rapid tests on atypical forms (C, H and L-Type) was never carried out".

Line 500: Editorial change: 'trail' should be 'trial'.

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER: CHAPTER 2.4.14.LUMPY SKIN DISEASE

General comments

The EU can support the proposed changes.

Specific comments

LINE 296: “according to Karber (1931)”: this reference is not recorded in the reference list.

LINE 385: The word “batches” should be replaced by the word "batch".

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.5.1: AFRICAN HORSE SICKNESS

General comments

The EU can support the proposed changes. Nevertheless, the specific comments detailed below should be taken on board in the final version.

Specific comments

LINE 198: The EU proposes to replace "Spleen homogenate: approximately 2 cm with 3ml of MEM (minimal essential medium)" by "Spleen homogenate: 1 g of spleen in 2 ml of diluents".

LINE 207: Between steps vii) and viii) another washing step of five times should be included.

LINE 209: The EU proposes to replace "100µl of SDS by "50µl of SDS"

LINES 267-271: The paragraph should be substituted by: "Several agarose gel-based RT-PCR assays for the specific detection of AHSV RNA have been described targeted at viral segments three (Aradaib, 2009; Sakamoto et al., 1994), eighth (Bremer et al., 1998; Stone-Marschat et al., 1994) or seven (Laviada et al., 1997; Zientara et al., 1994). The most widely used method employs primers corresponding to the 5' end (nucleotides 1–21) and 3' end (nucleotides 1160–1179) of RNA segment seven amplifying the complete viral segment."

LINES 272-277: The paragraph should be substituted by: "Real-time RT-PCR (RRT-PCR) methods for a highly sensitive and specific detection of AHSV RNA have been recently developed based on the use of a pair of primers and a Taqman probe from conserved sequences of viral segment five (Rodriguez-Sanchez et al., 2008) or seven (Agüero et al., 2008; Fernández-Pinero et al., 2009). Although both gel-based and real-time RT-PCR procedures can detect reference strains from the nine virus serotypes, RRT-PCR provides advantages over agarose gel-based RT-PCR method, such as speed of analysis, higher sensitivity, suitability for high-throughput and capability for automation."

LINES 629: The EU proposes to replace "30-100 TCID₅₀" by "30-300 TCID₅₀".

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.6.2: RABBIT HAEMORRHAGIC DISEASE

General comments

The EU can support the proposed changes in general. Nevertheless, the specific comments detailed below should be taken on board in the final revised version to be adopted in the next General Session and taken into account in the next meeting of the BSC. Moreover, as the changes are very important and the time for comment more than limited, the EU might send more comments to the OIE.

Specific comments (given the fact that the text lines are not numbered, the comments below make reference to the paragraph and the line(s) within the paragraph)

SUMMARY LINES 11 - 12: After the words “interfere with HA and” and “ELISAs” the word “some” should be added. After that sentence the following sentence should be added: “The detection of calicivirus particles in liver homogenates or VLPs by electron microscopy is also possible.”

INTRODUCTION

LINES 8 – 12: The name of the expert is “Schirrmeyer”

LINE 24: After the words “monoclonal antibodies (MAbs)” the words “most of the” should be replaced by “outer an conformational epitopes, respectively”.

LINE 27: after the words “(PCR)” the words “4-6 weeks post” should be replaced by the words “up to 4 months after”

B. DIAGNOSTIC TECHNIQUES

1. C) ELECTRON MICROSCOPY - LINE 6: The words “due to the lower sensitivity of the drop method it is advisable to ultracentrifuge the sample” should be replaced by the words “In most acute running cases a concentration of the samples is unnecessary because the particle concentration is higher than 10⁷ particles per homogenate. If required an ultracentrifugation can be performed”

1. E) IMMUNOSTAINING - LINE 15: After the words “fixed in methanol” the words “or acetone” should be added.

C. REQUIREMENTS FOR VACCINES

1. A) RATIONALE AND INTENDED USE OF THE PRODUCT – LINE 27 F: Contrary to what is written here, most manufacturers recommend a singular basic vaccination and a yearly revaccination. Depending on infection pressure a boost after 2-4 weeks can be useful.

1. A) RATIONALE AND INTENDED USE OF THE PRODUCT – LINE 28: After the words “ in the neck region” the words “or intramuscularly” should be inserted.

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.8.7: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

General comments

The EU can support the proposed changes in general. Nevertheless, the comments below should be taken on board in the final revised version to be adopted in the next General Session or taken into account in the next meeting of the BSC.

1. The International Committee for the Taxonomy of Viruses has now designated the terms Type I and Type II for the European and North American genotypes of PRRS. The chapter should be updated to reflect this.
2. Mention should be made of the emergence of a reportedly highly virulent strain of type II PRRSV in the Far East, characterised by a discontinuous 30 aa deletion in the nsp2 region.
3. The spread of the disease by the airborne route is increasingly recognised as a significant factor in pig-dense areas (Dee et al 2009). Some mention should be made of this. Refs: Dee, S., Otake, S., Oliveira, S., and Deen, J. (2009). Evidence of long distance airborne transport of Porcine reproductive and respiratory syndrome virus and *Mycoplasma Hyopneumoniae* - Art. No. 39. *Veterinary Research* **40**, 39.

EU COMMENTS AND POSITIONS
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CHAPTER 2.8.8: SWINE INFLUENZA

General comments

The changes proposed are generally welcomed by the EU. However, the specific comments detailed below should be taken into account for the final revised version to be adopted in the next General Session.

Specific comments

LINE 64: After the words “influenza viruses can” the word “occasionally” should be inserted.

LINE 65: After the words “influenza virus can” the word “occasionally” should be inserted.

LINE 319-320: The words "reducing the risk of exposing swine caretakers and producers to this zoonotic virus" should be replaced by: “minimizing the risk of virus exposure to swine caretakers.”

EU COMMENTS AND POSITIONS

On the proposed changes to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.9.6: HENDRA AND NIPAH VIRUS DISEASES

General comments

The EU can support the proposed changes. Nevertheless, the Chapter does fail to mention two significant publications on serological test development for Nipah (Tamin et al 2009; Kaku et al 2009) which utilise non-infectious pseudotype virus constructs. There is also an important publication on a PCR utilising a conserved region of the henipavirus genome, which could be cited (Feldman et al 2009). Moreover the specific comments below should be taken into account in the final revised version to be adopted in the next General Session or in the next meeting of the BSC.

Specific comments

LINES 449 AND 454: Regarding the mention of the potential value of vaccines for felines and canines, there has been no evidence that these animals passed disease on, so the need for vaccines in these species is very questionable

LINES 454 - 455: "In addition, a vaccine for wildlife (e.g. targeting vermin) may assist in outbreak control": it is highly unlikely that vaccines would be used for rats and mice. The use of the word "vermin" is moreover not adapted for wildlife reservoirs of Nipah and Hendra, since there is also a danger of implying that bats are vermin, which would be very undesirable.

References to insert

Feldman, K.S., Foord, A., Heine, H.G., Smith, I.L., Boyd, V., Marsh, G.A., Wood, J.L., Cunningham, A.A. and Wang, L.F. (2009) Design and evaluation of consensus PCR assays for henipaviruses. *Journal of Virological Methods* 161(1):52-7.

Kaku Y, Noguchi A, Marsh GA, McEachern JA, Okutani A, Hotta K, Bazartseren B, Fukushi S, Broder CC, Yamada A, Inoue S, Wang LF. (2009) A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *J Virol Methods*. 2009 Sep;160(1-2):7-13. Epub 2009 May 9.

Tamin A, Harcourt BH, Lo MK, Roth JA, Wolf MC, Lee B, Weingartl H, Audonnet JC, Bellini WJ, Rota PA. (2009) Development of a neutralization assay for Nipah virus using pseudotype particles. *J Virol Methods*. 2009 Sep;160(1-2):1-6. Epub 2009 Mar 9.

EU COMMENTS AND POSITIONS
On the proposed changes to OIE Manual of Diagnostic Tests and
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CHAPTER 2.9.9: SALMONELLOSIS

General comments

The EU can support the proposed changes. Nevertheless, the specific comments below should be taken into account in the final revised version to be adopted in the next General Session or in the next meeting of the BSC.

Specific comments

LINE 203: The following sentence should be added: "Recently a monophasic variant of *S. Typhimurium* (S. 4, 5, 12: i: with resistance to ampicillin, streptomycin, sulphonamides and tetracycline) has emerged in pigs and caused outbreaks of salmonellosis in humans in several countries worldwide (Soyer *et al.*, 2009)".

LINE 268: Replace the word "bacteriosis" by "bacteriocins".

LINES 290-291: The statement: "novobiocin may be added to suppress gram-negative organisms" should be corrected as novobiocin is more effective against gram-positive bacteria and only to some extent against gram-negative bacteria. It should read: "...novobiocin may be added to suppress most gram-positive organisms and the gram-negative bacteria *Proteus* or specific etc."

LINE 313: The word "organisms" should be replaced by "tissues".

LINE 369: *arizonae* should be in italics.

LINE 452: *S.* (in italics) Enteritidis.

LINE 490: Replace the word "diarizona" by "diarizonae".

LINE 561: S. Enteritidis-infected flock.

LINE 737: replace "is" by "if".

LINE 754: *Salmonella* in italics.

LINE 762: *S.* (italics) Gallinarum.

LINES 812-813: Administration of gut flora preparations before or after parenteral administration of inactivated *Salmonella* vaccines might potentiate the effect of the vaccine. However, any administration of gut flora preparations before oral administration of live *Salmonella* vaccines will not "potentiate the effect of vaccines" but considerably reduce the protective effect of live vaccines. It should be stressed that the efficacy of live *Salmonella* vaccines is only promoted when the gut flora preparations are administered before or simultaneously with the live *Salmonella* vaccine and definitely not when the live "*Salmonella* vaccine is given subsequently" to the flora preparation as

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stated in line 813. (Methner, U.; Barrow, P.A.; Berndt, A.; Steinbach, G. 1999. Combination of vaccination and competitive exclusion to prevent Salmonella colonization in chickens - Experimental studies-.Int. J. Food. Microbiol., 49 35-42)

LINE 983 (Insert):

Soyer Y; Switt AM; Davis MA; Maurer J; McDonough PL; Schoonmaker-Bopp DJ; Dumas NB; Root T; Warnick LD; Grohn YT; Wiedmann M (2009) Salmonella enterica Serotype 4,5,12:i:-, an Emerging Salmonella Serotype That Represents Multiple Distinct Clones. Journal of Clinical Microbiology 47(11): 3546-3556