Ref. Ares(2014)26483 - 08/01/2014

ANNEX 3

EU COMMENTS

ON THE PROPOSED CHANGES TO THE

OIE MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS

PRESENTED FOR COMMENTS IN OCTOBER 2013

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

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Glossary of terms

General comments

The EU can support this revised glossary of terms.

Specific comments

None

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

CHAPTER 2.1.3.: Bluetongue

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 154 (Table 1): "C-ELISA" should be changed to "Antibody detection ELISA" both in the table and throughout the text. Methods for C-ELISAs have been published, however in recent years laboratories have also been using Blocking (B-ELISA) and double recognition (sandwich) ELISAs for the detection of BTV antibodies. Pirbright as EURL for BTV run an annual ring trial. In the 2013 European ring trial, four laboratories used blocking ELISAs, eight used double recognition (sandwich) ELISAs and 29 laboratories used competitive ELISAs. All assays have been deemed fit for purpose with the correct results being returned routinely on an annual basis since 2009.
The double recognition ELISA was found to be more sensitive than the traditional C-ELISA for the detection of antibodies post vaccination with BTV-8 inactivated vaccine. The double recognition ELISAs detected antibodies in >50% of vaccinated sheep at 7dpv compared to the C-ELISAs which detected<10% (Oura *et al.*, Vaccine 27 (2009) 7326–7330). Hence was adopted by laboratories in Europe following the BTV-8 vaccination campaign in 2008.
The majority of European laboratories have accredited these assays to ISO/IEC 17025. The Pirbright institute have validated and accredited a double recognition ELISA and have shown that it detects antibodies to all known BTV serotypes and does not cross react with antibodies from other orbiviruses.
Although VN is a good test for antibody detection, especially for serotype specific antibody it is not really practical in all laboratories due to the requirement to have known reference strains of known titre, well characterised antisera and the difficulties with test interpretation, in areas where more than one serotype circulates.

LINES 428-429: The nested PCR does detect BTV-26, this has been shown in the EU ring trial of 2011 and 2012, when one laboratory reported detecting BTV-26 in the correct samples using this method.

LINES 462-491: i.e. the Hoffmann protocol for real time RT-PCR: the assay is not well described and there are errors:

LINE 470: The concentrations given are working concentrations of primers and probe not the final concentrations as described.

LINES 472-474: This should be reworded for clarity reasons, as follows: "A plate layout should be designed and loaded onto the real time PCR machine software. Using the layout as a guide, 0.5ul of each primer working stock (20pmol/ul) is added to each well of a PCR plate that will contain sample, positive or negative control RNA".

LINES 473-474: The words "4ul of distilled water […]" should be removed as it is not contained in the description of the Hoffmann assay.

LINE 480: After the words "being tested", please insert the following: "including 1ul of probe working stock (5pmol/ul) per sample". Indeed, at present the description does not indicate where to add the probe. The probe needs to be in the PCR master mix. Section F can be removed, as the 22ul of PCR mastermix can be added directly to the 3ul of heat denatured RNA plus primers.

LINE 765: The EU notes that both competitive and blocking ELISA are stated, however the prescribed test is a competitive ELISA. As discussed above perhaps the text should read "appropriate antibody detection ELISA" to allow for the B, C or double recognition to be used. The C-ELISA can still be described as an example, but the text will then allow laboratories to use any of the ELISAs on the market/published.

LINE 810: Again, please remove C-ELISA and describe as antibody detection ELISAs or ELISAs.

LINES 812-813: It should be noted that the labs across Europe are already using these commercial ELISAs to certify animals for trade. In the majority of labs across Europe these assays are accredited to ISO/IEC 17025, and have been through extensive in house validation.

LINE 1147: The EU suggests amending to "in ruminant animals with no/limited ECE or cell culture passes". It is not always possible to challenge with material that has only been passed in animals and hence recently animal experiments have used low KC passage for challenge with good success. Clinical signs and viraemia have been induced with BTV-8, 1, 4 and 26.

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

CHAPTER 2.1.6.: Epizootic haemorrhagic disease

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 97 (Table 1): "C-ELISA" should be changed to "Antibody detection ELISA" both in the table and throughout the text. A C-ELISA for EHDV antibody detection was historically produced by Pirbright and used globally, however this has been withdrawn from sale and therefore reagents are no longer available. It is understood that in house assays are used in Australia and America; however reagents for EHDV are scarce. At present there is only one ELISA commercially available for the detection of EHDV antibodies and this is a Blocking ELISA. It has been used routinely in laboratories in Europe since 2011. The commercial B-ELISA was developed between Pirbright and formally Laboratoire Service International (France), now part of LifeTechnologies and has been validated and accredited to ISO/IEC 17025. Validation indicated the assay detects antibodies against all known serotypes of EHDV from as early as 9 dpi and the B-ELISA does not cross react with BTV antibodies.
Pirbright as EURL for BTV run an annual ring trial and frequently include EHDV samples. In 2011, an EHDV-6, 37dpi serum sample was included; in 2013 two EHDV serum samples (EHDV2 and EHDV6) were included. Laboratories participating in the BT ring trial, test for EHDV and report the correct results, no cross reactivity has been reported, all laboratories used the commercial B-ELISA.
It is therefore suggested that throughout the text C-ELISA is changed to antibody detection ELISA to take into account the Blocking and antigen capture ELISAs that are available for serological detection of EHDV. For example the heading of section 2.1
Although VN is a good test for antibody detection, especially for serotype specific antibody it is not really practical on a global scale i) as there are no agreed reference strains or reference sera and ii) antisera to EHDV is scarce. Pirbright have a large collection of EHD serum samples and have standardised an in house VN test, however the majority of sera have low antibody titres and in many cases also contains BT antibodies. Pirbright and ANSES, France (OIE EHDV reference laboratory) would not be in the position to supply reference antisera globally without generating new reagents.

LINE 118: Please add the following reference: C.A. Batten *et al*. Veterinary Microbiology 154 (2011) 23–28.

LINE 121: Please add the words "however a blind passage may be required" at the end of the sentence.

LINE 131: The EU suggests adding sonication as a possible alternative to water to lyse the cells.

LINE 299: Please insert the following at the end of the paragraph:
"Commercially produced real time RT-PCR kits based on genome segment 9 are now available. These commercial assays are routinely used in many laboratories across the world and detect all known serotypes of EHDV. The assay has been shown to detect viral RNA as early as 2 days post infection in infection studies (Batten *et al.*, 2012)."

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

CHAPTER 2.1.8.: Leishmaniosis

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 22: The use of the word "must" may lead to misinterpretations and it is maybe too strong in this context. Therefore, the EU suggests amending the sentence as follows:
"As there are very few morphological differences among various species, the identification of any isolated Leishmania organism relies on biochemical and/or molecular methods."

LINES 39-40: The sentence regarding vaccines should be completed as follows in order to better reflect the actual situation:
"A number of vaccines for use in animals are under evaluation, and one has been authorised in Europe for use in dogs. Beyond other issues to be evaluated, the use of these vaccines is posing present and future challenges to the fields of diagnosis, epidemiology and surveillance of the parasite, especially in countries where the parasite does not occur."

LINE 404: The EU suggests replacing the words "in countries of Southern Europe" by "in Europe", as this more accurately reflects the situation.

LINE 410: The EU suggests adding the following sentence at the end of this paragraph:
"Surveillance programs, especially those based solely on the detection of antibodies in dogs should be revised in order to take into account the role of the vaccine; following actions should be taken, especially in order to produce real epidemiologic data of the infection".

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

CHAPTER 2.1.9.: Leptospirosis

General comments

The EU can in general support this revised chapter and has a specific comment.

Specific comments

LINE 112 (Table 1): In the table, the EU suggests scoring the MAT with "++" instead of "-" for the purpose "Confirmation of clinical cases". Indeed, in the veterinary field, suspected cases based on clinical signs (which are sometimes not apparent) should be confirmed by the isolation of the agent or the detection of leptospiral DNA (via PCR). However, these methods require well-done sampling in order to be successful, and biological samples such as kidney tissue or urine are not always delivered to laboratories. Therefore, the MAT should still be considered as a suitable method for the confirmation of clinical cases in diagnosing acute infections, as high titers are usually available and, as stated in lines 339-342, a "*four-fold change in antibody titers in paired acute convalescent serum samples is diagnostic*". The MAT could also be useful to give an indication of the agent serogroup/serovar. Moreover, as stated in lines 413-414, only a small number of ELISA tests for animals have been validated, with certain limitations.

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

CHAPTER 2.1.11.: Paratuberculosis

General comments

The EU can in general support this revised chapter and has some specific comments.

In general, this OIE terrestrial manual chapter would benefit from being developed a bit further to take into account:

- the progress made in relation to the application of the more rapid and well-functioning PCR tests now also commercially available;

- the possibilities to provide better diagnostic reliability with a view to disease control. Albeit not sufficiently reliable when used at individual animal level (due to very low sensitivity), and as already partly outlined in the current manual chapter, diagnostic tests for paratuberculosis (serology, culture or PCR) can serve the purpose to differentiate between infected and non-infected populations when used repeatedly at herd level. Some countries around the world already apply herd testing to control and document the status in herds and regions. Based on this, adequate tools are available for differentiating between infected and non-infected herds also for documentation and risk-assessments.

Specific comments

LINE 10: As diagnosis is not always done on clinical grounds, i.e. when detecting subclinical infections at herd level, the EU suggests adding the word "usually" before the words "made on clinical grounds".

LINE 56: The EU suggests slightly amending the last part of the sentence as follows: "[…] on its association with clinical signs and defined laboratory findings, including PCR diagnostic test results".

LINE 62: The EU suggests adding the word "usually" before the words "are a slow progressive wasting", as occasionally an animal may die within a short period with limited wasting observed in the previous months.

LINE 72: The EU questions whether infection can ever be eliminated. Has this actually been shown? The EU would ask the OIE to provide a scientific reference to support the statement "Depending on the resistance of the individual, this infection is eliminated […]".

LINE 77: The EU suggests replacing the words "delayed-type hypersensitivity (DTH)" by "cell-mediated immune (CMI) responses", as this is the more accurate term. This should be done throughout the chapter.

LINE 87: For clarity reasons, the EU suggests adding the words "with whole cell vaccines" after the words "against paratuberculosis".

LINE 98 (Table 1): The EU is of the opinion that there are no tests available that can provide reliable individual animal freedom from infection. Therefore, the score for "Culture" for the purpose of "Individual animal freedom from infection prior to movement" should be changed from "+++" to "+". Likewise, the score for "Culture" for the purpose of "Population freedom from infection" and "Confirmation of clinical cases" should be changed from "+++" to "++".

At the same time, the score for "PCR" for the purpose of "Population freedom from infection" and "Confirmation of clinical cases" should be changed from "+" to "++". Indeed, there are several publications supporting that PCR is at least as sensitive as culture and there are well functioning commercial methods on the market (References: Leite "Comparison of fecal DNA extraction kits for the detection of *Mycobacterium avium* subsp. *paratuberculosis* by polymerase chain reaction". J Vet Diagn Invest. 2013 Jan;25(1):27-34, 2013; Hanifian S "Quantitative real-time PCR and culture examination of *Mycobacterium avium* subsp. *paratuberculosis* at farm level". Vet Microbiol. 2013 Feb 22;162(1):160-5; Logar K "Evaluation of combined high-efficiency DNA extraction and real-time PCR for detection of *Mycobacterium avium* subsp. *paratuberculosis* in subclinically infected dairy cattle: comparison with faecal culture, milk real-time PCR and milk ELISA". BMC Vet Res. 2012 May 2;8:49).

Moreover, the EU suggests changing the score for AGID for the purpose "Population freedom from infection" from "++" to "+", as that test – just as the CFT - has no documented diagnostic value for detection of infection.

Furthermore, the EU suggests deleting the column "Immune status in individual animals or populations post-vaccination" as there are no validated correlates of protection for paratuberculosis, so any results in that column would be misleading in terms of whether the animal is protected or not.

In addition, the EU suggests changing the score for ELISA for the purpose "Confirmation of clinical cases" from "+" to "+++", as it has a positive predictive value of 99.7% as decrinbed by Weber *et al.* 2009 (see www.ncbi.nlm.nih.gov/pubmed/19762098).

Finally, the EU suggests replacing "Gamma-IFN" by "INF-gamma" and changing the score for that test for the purpose "Population freedom from infection" from "-" to "++". Indeed, the IFN-gamma test is the only test that may be used to evaluate the level of exposure within a young population, as described by Jungersen *et al.* 2012 (see www.ncbi.nlm.nih.gov/pubmed/21616547).

LINE 104: "DHT" should be changed to "DTH" (same in the first column of table 1).

LINE 113: The EU suggests adding the following sentence at the end of this paragraph: "Similar test strategies with repeated herd level tests can also be applied within control programmes to estimate herd level probabilities of freedom of infection, and thus to identify so-called low risk herds in order to provide means for safer trade".

LINE 143: This statement is not correct. As the sensitivity of culture is approximately 20-30 %, it cannot provide a definitive diagnosis. The EU suggests that this be reflected in that paragraph.

LINE 148: There is no conclusive evidence to support that statement. Indeed, as only a relatively small proportion of infected cattle can be detected by that test, the statement is misleading.

Furthermore, the 30-40 % stated **in LINES 149-150** is not accurate. According to Whitlock *et al*. xxxx, that figure should be 23-29 % (see ADD REFERENCE).

LINE 152-154: There is no conclusive evidence to support that statement. According to published studies, average sensitivity among cattle with clinical disease is around 70%. The EU would ask the OIE to provide a scientific reference to support the statements that faecal culture will detect infected animals 6 months or more before they develop clinical signs, and that sensitivity approaches 100 % during the clinical stage.

LINES 213-217: The EU suggests mentioning PCR as a method to identify MAP from culture**.**

LINE 267: Freezing of tissue samples should be at the same temperature as faecal samples, i.e. at -70 °C (see **LINE 314**).

LINE 356-358: The EU suggests deleting that paragraph as the information is redundant and not really necessary, as PCR including commercial diagnostic PCR tests is common today. Furthermore, reference to a PCR method from 1990 in probably outdated, and its use is not just something that "has been reported".

LINE 364: The EU suggests clarifying that ELISA, not CFT, is the standard test for cattle, and has been since the 1990's. The OIE should consider removing the CFT from this Manual chapter, as it is inferior to modern ELISA tests.

LINE 417: "HPRO" should be replaced by "HRPO". As an alternative, the EU suggests using the more general term "enzyme-labelled anti-bovine immunoglobulin", as also other enzyme detection systems may be in use.

LINE 420: As the wavelength would depend on the enzyme-substrate combination used (see comment above on HRPO), the reference to 450 nm in the text should be indicated as an example. Perhaps it would be better to replace the parenthesis with a statement to that effect, e.g. "at the appropriate wavelength".

LINES 413-420: As an alternative to the two comments above, the OIE may consider deleting the entire paragraph could, as this is a very general description of ELISA that doesn't contain much specific information on paratuberculosis ELISA.

LINES 422-424: The EU suggests deleting that paragraph as that information is already provided previously in the text. Furthermore, it is very detailed and prescriptive, as also other enzyme detection systems may be in use.

LINE 430: The EU suggests deleting the words ", but less sensitive than blood test", as that statement is not correct. There are several studies pointing towards no difference (e.g. Sweeney *et al*. 1995; Nielsen *et al*. 2002; Lombard *et al*. 2006). The studies that point out otherwise include severe selection biases which already in the design phase favour blood tests.

LINE 458: The EU suggests adding the following sentence, which clarifies the relation between CMI vs. serological testing: "In infected populations, a much higher number of animals are expected to react in tests for CMI compared to antibody-based tests as the CMI test indicate exposure while antibodies indicate progression of disease".

LINE 459: The EU suggests replacing "Gamma-interferon" by "Interferon-gamma release". Indeed, these tests are now commonly referred to as "interferon-gamma release assays" (IGRAs) in diagnosis of human TB, whether they are based on ELISA (Quantiferon-TB) or ELISPOT (T-SPOT.TB) format.

LINES 462, 463 and 472: For reasons of consistency, please replace "gamma interferon" by "IFN-γ".

LINES 475-492. As the skin test is of limited value, the OIE should consider deleting it from table 1 and giving it less attention here.

LINE 616: The statement that live vaccines are not available is in conflict with the introduction paragraph in section C1.1. (**LINES 496-497**).

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

CHAPTER 2.2.2.: American foulbrood of honey bees

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINES 107-110: The brood should preferably be sampled by sending whole frames. Cut out pieces could be deformed during the transportation and it is difficult to make the cultivation of the right place.

LINE 119: The EU suggests adding the following reference:
BZDIL J. (2007): "Detection of *Paenibacillus larvae* Spores in the Debris and Wax of Honey Bee by the Tween 80 Method". Acta Vet. Brno, 76, 643-648.

LINE 138-145: The EU suggests addin the following to this paragraph:
"Hive debris and wax should be wrapped in a paper bag, plastic pots covered with a paper lid or in paper tubes covered with a plastic lid. Secondary packaging consists of a plastic bag as a protection against cross-contamination. In the case of bulk samples, samples may be stored even in tertiary packaging (large cardboard boxes) that protects samples from mechanical damage".

LINES 147-208: The EU invites the OIE to consider adding the following method to section 1.4.1.:
"**Cultivation by Method Tween 80 (Bzdil, 2007):**
One gram of debris or 1g of wax is put to the test tube with airtight seal. Larger pieces of wax should be cut by sterile instruments into very small pieces (ideally up to 3mm in size). Wax pieces contained in debris do not have to be further cut because they are usually very small. The smaller the pieces, the easier and faster is the process of homogenization. Dry material prepared this way should be stirred thoroughly and diluted with 8.5ml of sterile distilled water. The resulting suspension is then supplemented with 0.5ml of Tween 80. Approximately 30 minutes before pipetting, required volume of Tween 80 should be withdrawn from the original container, put into another sterile container with airtight seal and immersed in hot water bath (70±2°C) in order to reduce viscosity of Tween 80 and facilitate its pipetting. The suspension of debris, water and Tween 80 is shaken thoroughly and the test tube is placed in hot water bath (70±2°C) for 30 minutes. If the wax dissolves slowly or there are pieces of wax larger than 5mm, the test tube could be left in water bath for up to 1 hour. While warming the sealed tube in water bath, it should be thoroughly shaken in longitudinal direction at least three times (or better, in several 5 to 30 second cycles 5 to 10 minutes apart). Such a careful homogenization results in development of homogenous greyish brown pulpy material which can harden as it cools down. Afterwards, tubes are removed from water bath and let cool down to room temperature at which they should be stored for 2 - 4 hours until a sufficient amount of liquid is separated at the bottom of tubes. Then 2 - 5ml of this liquid is withdrawn with disposable balloon pipette and mixed with the same volume of distilled water in another

sterile sealable tube. Again, the resulting mixture should be shaken thoroughly in longitudinal direction for at least 5 minutes and put to hot water bath (90±2°C). After 10 minutes, tubes with stand are removed from the bath, let cool down to room temperature and shaken again. Then the material is inoculated in 0.2ml doses to 3 - 5 plates of MYPGP with nalidixic acid and at least one plate of blood agar serving as a control. Before culturing, the plates should be dried in thermostat at 37±1°C. Drying time is selected in dependence on humidity of culture medium surface (30 minutes is usually enough). Dishes must be identified accurately and any contamination or mistaking must be avoided during handling. The liquid is spread over the plates using a bent sterile plastic / glass stick or the tip of the pipette. The liquid is let dry and the plates are incubated upside down at 37±1°C for 5 - 8 days."

Furthremore, the EU suggests merging sections 1.4.1. and 1.5. and describing each method separately.

LINES 344-381: The EU suggests adding another phenotypic molecular method - MALDI-TOF - in section 1.6.3. These methods are on the second place on the scale of precision (right after PCR).
Test description: "Bacterial colony (a part or whole) is transferred onto target plates as a thin layer, after drying at room temperature it is covered with 1µl MALDI Matrice (Cinamonic Acid) a is let dried again, then MALDI-TOF MS measurement is performed. The device detects specific mass spectra of bacterial ribosomal peptides. After comparison of detected spectra with the database of known spectra the bacterial strain is identified".

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CHAPTER 2.3.3.: Avian infectious laryngotracheitis

General comments

The EU can in general support this revised chapter and has some specific comments.

Specific comments

LINE 6: Please replace the word "pathological reactions" by "observed pathology".

LINE 14: Please insert the word "fowls" between "embryonated" and "eggs".

LINE 64 (Table 1): The EU suggests including histopathology in the table.

LINE 168: Advice regarding the selection of birds for post-mortem is recommended also here, similar to the advice for virus isolation given in **LINES 88-92**.

LINE 186: It would be useful if the time periods (days) for these phases of infection were included.

LINE 194: One of the limitations in diagnosis is that so far there are no molecular tests allowing a differentiation between field and vaccine strains. Therefore, a good history of vaccination in the flock might be necessary to interpret the results of diagnostic tests.

The EU therefore suggests adding the following at the end of the introduction of the molecular tests section: "There are so far no molecular tests available that allow a differentiation between field and vaccine strains. Therefore, a good history of vaccination in the flock might be necessary to interpret the results correctly".

LINE 255: In common with the PCR and RT-PCR sections described above, it would be useful for a recommended method for RFLP to be stated. Other genotyping methods may also be mentioned as well.

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

CHAPTER 2.3.4.: Avian influenza

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 56: The EU suggests replacing the word "nucleocapsid" by the word "nucleoprotein", as the capsid is formed by several nucleoprotein proteins. The same change would be necessary in **LINE 88**.

LINES 74-75: The EU suggests replacing the word "same" by "homologous" and "different" by "heterologous".

LINE 152: The EU suggests clarifying how many swabs taken from dead birds may be pooled.

LINE 153: This recommendation for live birds does not include the possibility of pooling swabs. This seems inconsistent with the recommendation for dead birds (see comment above).

LINE 231: The EU suggests replacing the word "snicking" by "sniffing" or "sneezing".

LINE 619: The EU suggests adding the following sentence: "A recombinant duck enteritis virus in domestic ducks is being tested for potential licensure and use in China (Liu *et al.*, 2011)".

LINE 814: The EU suggests adding the reference for this DNA vaccine: "(Rao *et al.*, 2008)".
Reference: Rao S, Kong WP, Wei CJ, Yang ZY, Nason M, Styles D, DeTolla LJ, Panda A, Sorrell EM, Song H, Wan H, Ramirez-Nieto GC, Perez D, Nabel GJ. "Multivalent HA DNA vaccination protects against highly pathogenic H5N1 avian influenza infection in chickens and mice". PLoS One. 2008;3(6).

DRAFT EU COMMENTS
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CHAPTER 2.3.6.: Avian tuberculosis

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.4.2.: Bovine babesiosis

General comments

The EU can support this revised chapter.

Specific comments

None

<u>DRAFT</u> EU COMMENTS
On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

CHAPTER 2.4.9.: Contagious bovine pleuropneumonia

<u>General comments</u>

The EU can in general support this revised chapter and has specific comments.

In general, the recommended PCR is rather dated and uses a two stage process (i.e. restriction enzyme step) when better tests are available.

Taxonomic changes within the *Mycoplasma mycoides* cluster have designated the causative agent as *M. mycoides* subsp. *mycoides* (the SC is redundant).

There is no mention of latex agglutination test which though lacking some sensitivity can be performed uniquely in the field and help to stop spread of disease.

<u>Specific comments</u>

LINE 7: Please replace by "Diagnosis <u>requires</u> the isolation […]".

LINES 7-9: The main problem for control is the lack of extensive vaccine coverage. This should be reflected in the text.

LINE 16: Please replace by "[…] growth of <u>cell-walled</u> bacteria".

LINE 17: It is unclear how the sample type influences isolation. Furthermore, please replace "contamination dose" by "mycoplasma titre".

LINE 18: The sentence should read as follows: "[…] cloudiness <u>which forms swirls</u> when shaken".

LINE 26-28: This part is misleading and is selective use of the literature; the CFT in the right hands has similar sensitivity and specificity as cELISA and detects antibody earlier than cELISA. This is why it remains a prescribed test. Thus, the EU suggests deleting the following sentence "However, […] sensitivity".

LINE 62: Please amend the sentence as follows: "[…] the disease when the causative agent can spread rapidly; in the chronic stage there may be long-term persistence of the agent."

LINE 66: Please amend the sentence as follows: "These silent carriers may be infectious and thus responsible for unnoticed persistence […]". However, it should be noted that it has never been proven that cattle with sequestra are infectious. It is more likely that cattle with unresolved lesions are the carriers. This should be reflected in the text.

LINE 80-84: The wording of the sentences is a bit unclear and should be revised. The EU is of the opinion that test and slaughter with movement

control is the only method to ensure eradication, whereas vaccination on its own even with extensive coverage will not eradicate CBPP unless other intervention step is include like strategic and controlled chemotherapy.

LINE 114: Please delete the words "hard and". Furthermore, the sentence should be amended to read as follows: "[…] non-existent or may be indistinguishable […]".

LINE 116: Please delete the word "suspected".

LINE 119: Please delete "No matter which […] used" and "always preferential and".

LINE 122 (Table 1): Please delete "Biochemical tests" and place "Immunological tests" after "in vitro culture" as simply isolating mycoplasmas is not sufficient; similarly as the chapter recommends isolation for all tests then in vitro culture should precede molecular tests too.

LINE 150: Please change to "[…] proliferation of cell-walled bacteria".

LINE 161: Please change to "needs specialised media to grow".

LINE 173: Please change to "growth of low-passaged *Mycoplasma* spp".

LINE 176: The use of the stomacher is not necessary, as pestle and mortar will do. Therefore, please delete the word "stomacher".

LINE 181: Please replace the word "positive" with "infected".

LINE 185: Please change to "[…] the sample is considered negative".

LINE 186: Please change to "[…] cloudiness which forms swirls when shaken […]".

LINE 187: Please delete "is characterisitic of MmmSC".

LINE 191: As biochemical tests are not able to speciate, the words "biochemical tests" should be deleted.

LINE 216: A sandwich ELISA for Mmm has been described by Ball *et al.* which has advantages over MF dot etc.

LINE 220-227: Please delete this paragraph for the following reasons: It is unclear why the author uses the term "Immunochemical tests". The tests that are mentioned in previous paragraph are described earlier as immunological tests which have replaced the biochemical tests for Mmm in many labs. These tests have been performed well for many years. The disadvantages described in this paragraph are true for many tests including PCR.

LINE 256-266: It is not appropriate to make recommendations in this chapter on PCR *per se* as in the right lab nested PCR is suitable and safe. A single

step PCR by Miles *et al*. is not mentioned and has many advantages over listed PCR (reference: Miles K *et al*., 2006. "Identification and differentiation of European and African/Australian strains of Mycoplasma mycoides subspecies mycoides small colony type using polymerase chain reaction analysis". Journal of Veterinary Diagnostic Investigations 18, 168-171).

LINE 381-382: This statement contradicts **LINES 27-28** which say CFT is limited.

LINE 390: It is unclear what exactly is meant by this statement.

LINE 391: Please change to "[…] epidemiological investigation".

LINE 400: This statement is not correct, as CFT is not laborious in many labs.

LINE 462: Evidence would be needed for this statement which is difficult to understand.

LINES 464-465: Evidence would be needed for this statement which would be true for all serological tests.

LINES 466-469: This would be true also for cELISA, so the statement needs to be amended accordingly.

LINES 582-583: As this general statement would be true for all tests, it should be deleted.

LINE 584: This statement contradicts statement at **LINES 27-28**; here it says CFT and cELISA have equal sensitivity.

LINE 597: Please delete the words "as a consequence".

LINES 602-620: These paragraphs are superfluous. It is not clear why the ELISA format ensures a "proper QMS". These statements are true for all serological tests and not specific to the diagnosis of CBPP.

LINE 651: It should be noted that false positives can also be detect in cELISA.

LINE 769: It is not clear what "compatibility with CBPP outbreak detection" means exactly.

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

CHAPTER 2.4.16.: Theileriosis

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.5.8.: Equine piroplasmosis

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.6.1.: Myxomatosis

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.7.6.: Contagious caprine pleuropneumonia

General comments

The EU can in general support this revised chapter and has specific comments.

Specific comments

LINE 20: The EU suggests replacing the words "Diagnosis depends on clinical" by the words "Diagnosis is carried out by clinical".

LINE 33: Please replace the word "saprophytic" by "other".

LINE 36: Please amend as follows: "produces 'comets' when broth cultures are shaken."

LINES 44-46: The EU is of the opinion that CFT does not have significant limitations if properly performed. Therefore, that sentence should be deleted or the words "if not properly performed" added to the end.

LINE 58: Please replace the word "purified" by "semi-purified" as this more accurately describes the situation.

LINE 68: The reference should be as follows: "(Manso-Silvan *et al.*, 2007)".

LINE 70: Please replace "which are usually involved in the cross-reactions with Mccp" by "which may cross-react with Mccp".

LINE 72: Please delete the words "with Mccp".

LINE 75: Please replace "in the Cape" by "to the Cape".

LINE 82: It has also been isolated in Mauritius (Srivastava *et al.*, 2010).
Reference: Srivastava, A. K. Meenowa, D., Barden, G., Churchward, C., Ayling, R.D., Salguero, F. J., Nicholas RAJ. (2010). "Contagious caprine pleuropneumonia in Mauritius". Veterinary Record 167, 304-305.

LINE 95: Also in gazelles in the UAE (Nicholas *et al.*, 2008).
Reference: Nicholas, R., Ayling, R. and McAuliffe, L. (2008). "Mycoplasma diseases in ruminants. Contagious caprine pleuropneumonia. " CABI International, Wallingford, UK. p 123.

LINE 103: The EU does not agree that CFT is a nonspecific test, on the contrary it has very high specificity. Therefore, this statement is completely unacceptable.

LINES 109-110: Please delete the words "; and what has been called "mastitis […] syndrome", as this is not referred to anymore.

<u>DRAFT</u> **EU COMMENTS**
On the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

LINE 114 (Table 1): CFT can be used for “Population freedom from infection" and "Individual animal freedom", so the score should be "++" instead of "-". Furthermore, CFT and cELISA can be used for Confirmation of clinical cases" so the score should be at least "++" instead of "+".

LINES 136-141: It is not clear which PCR these primers refer to.

LINE 144: Some guidance is needed here as to whether a PCR positive without isolation would constitute a confirmed outbreak.

LINE 146: A molecular typing test cannot be used for identification.

LINES 236-237: These two mycoplasmas have been merged into a single subspecies: *M. mycoides* subsp. *capri*.

LINE 260: While sequencing can be useful for molecular typing, it is not essential for identification.

LINE 274: Diagnostic biochemical tests are available (Ozdemir *et al*., 2005).

LINE 310: Sensitivity is not an issue here and the mycoplasma has been grown in broth prior to carrying out the growth inhibition test so is available in sufficient quantity.

LINES 320 and 323: Bovine group 7 has now been designated as *M. leachii*.

LINES 402-404: Please update mycoplasma taxonomy (see comments above).

LINES 438-439: It is unclear why a reference to CBPP would be needed here.

LINES 502-512: The reference to previous ELISA is unnecessary and should be deleted.

LINE 509: Please delete the ";" at end of line.

LINES 657-674: CFT could also be used here.

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

CHAPTER 2.7.10.: Ovine pulmonary adenocarcinoma

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.9.1.: Bunyaviral diseases of animals (excluding RVF)

General comments

The EU can in general support this revised chapter provided the specific comments below are taken into account.

Specific comments

LINES 13-14: For SBV, embryonic losses cannot be excluded for the time being. Therefore, the EU suggests amending the sentence as follows: "[…] ~~embryonic and fetal~~ prenatal losses".

LINE 15: The EU suggests deleting the words "named after the city in Germany where it was first isolated", as this information is irrelevant for the summary of an OIE Manual chapter.

Furthermore, the words "emerged in" should be replaced by "was detected in", as the origin of SBV has not been determined and there is no evidence it originates in the EU. Indeed, it is likely that it arrived in the EU from somewhere else. The efficient and transparent EU surveillance system allowed identifying this virus, and the cutting edge EU laboratories managed to isolate it. However this does not mean SBV is native from the EU.

LINE 17: The EU suggests adding the words "and beyond", as also countries outside of Europe reported suspicion of SBV.

LINES 37-38: As isolation from tissues of infected foetuses is difficult and only a limited number of isolations are successful, the EU suggests amending the sentence as follows: "SBV can be isolated from the blood of viraemic adults and occasionally from different tissues of infected foetuses, especially from brain material".

LINE 39: The EU suggests replacing the words "is difficult" by "can be difficult" and "to the cell culture" by "to cell culture".

LINE 40: Please replace "is necessary for in-vitro growth of SBV" by "may occur in-vitro".

LINES 40-44: The EU suggests amending the sentence as follows, in order to be less prescriptive of a specific test:
"Real-time RT-PCRs have been established (e.g. Bilk *et al.*, 2012) and commercial PCR kits are available allowing highly sensitive and specific virus detection in blood of acutely infected ruminants as well as in organs of infected fetuses, such as brain, as well as placenta, amniotic fluid, and meconium. Nevertheless, detection of SBV-genome is possible only in a part of the infected and malformed fetuses and not equally well in all tissue due to virus clearance by the fetal immune response (De Regge *et al.*, 2013)."

LINE 44: The EU suggests adding the following at the end of the paragraph: ", probably due to clearance of the virus during gestation".

LINE 74: The EU suggests adding the following sentence after "[…] mainly sheep": "Experimental infection of ovine foetuses has confirmed the role of CVV in causing malformation (Rodrigues Hoffman et al., 2012).".

LINES 108-110: The EU suggests amending the wording of the sentence as follows: "Other potential pathogens in the Simbu serogroup include Aino, Peaton, Schmallenberg, Shamonda and Tinaroo viruses".

LINES 131-132: The EU suggests amending the sentence as follows: "Generally disease can be observed in the fetus of naïve ewes or cows following infection between 30 and 70 days […]".

LINES 156-157: This statement should be deleted or revised, as it is still a matter of scientific debate (see Tohru Yanase *et al.* "Genetic reassortment between Sathuperi and Shamonda viruses of the genus Orthobunyavirus in nature: implications for their genetic relationship to Schmallenberg virus". Archives of Virology (2012) 157:1611-1616.).

LINE 161: The EU suggests amending the sentence as follows: "[…] has been confirmed by real-time reverse-transcriptase […]".

LINES 172-176: The EU suggests moving this paragraph to the beginning of the section on SBV (i.e. before current **LINE 154**).

LINE 176: The EU suggests changing the last sentence at the end of this paragraph:
"Since then, SBV has been detected in many European countries as well as in Kazakhstan. Suspicions of past infections have also been reported in Turkey and South Africa".
(References:
- Leask *et al.* "Schmallenberg virus – Is it present in South Africa?". Journal of the South African Veterinary Association; Vol 84, No 1 (2013), 4 pages; and
- Azkur *et al.* "Antibodies to Schmallenberg virus in domestic livestock in Turkey". Trop Anim Health Prod. 2013 May 4.
- Report of the 2013 OIE General Session, page 63 indicates that SBV has been detected in Russia, Kazakhstan and Ukraine).

LINES 177-178: As this reference to WAHID is very general and would be valid for any disease, the EU suggests deleting that paragraph entirely. Furthermore, as SBV it is not an OIE listed disease and there is no obligation for surveillance for this virus anywhere in the world, WAHID cannot be expected to contain reliable information on the distribution of SBV.

LINE 247: The EU suggests adding the following sentence at the end of this paragraph:
"A recent duplex real-time RT-PCR for the California serogroup and Cache Valley virus has been published (Wang *et al.*, 2009) although this has not been validated for use with veterinary samples."
Reference: WANG H., NATTANMAI S., KRAMER L.D., BERNARD K.A. AND TAVAKOLI, N.P. (2009). "A duplex real-time reverse transcriptase polymerase

chain reaction assay for the detection of California serogroup and Cache Valley viruses". Diagn. Microbiol. Infect. Dis. 65, 150-157.

LINE 270: The EU suggests adding the following sentence at the end of this paragraph:
"An isothermal alternative to RT-PCR detection of AKAV has been reported using reverse transcription loop-mediated isothermal amplification (RT-LAMP) capable of detecting as little as 5 tissue culture infectious dose 50 (TCID50) / mL virus (Qiao *et al.*, 2013)."
Reference: QIAO J., WANG J., MENG Q., WANG G., LIU Y., HE Z., YANG H., ZHANG Z., CAI X. & CHEN C. (2013). "Rapid detection of Akabane virus by a novel reverse transcription loop-mediated isothermal amplification assay (RT-LAMP)". Virol. J. 10, 288.

LINE 274: The recommendations for sample storage should be generalised for this chapter, as they do not only relate specifically to SBV.

LINES 276-279: It should be clarified that only the viral genome could be detected in the tissues mentioned (except the brain), i.e. it is not proven that amniotic fluid, placenta and meconium contain infectious virus. This is not sufficiently reflected in the last sentence in **LINES 278-279**.

LINE 352: The EU suggests adding the following sentence at the end of this paragraph:
"A real-time RT-PCR has been developed to detect NSD virus (Bin Tarif *et al.*, 2012) but has not been fully validated for field-derived clinical samples. "
Reference: BIN TARIF A, LASECKA L., HOLZER B. & BARON M.D. (2012). "Ganjam virus / Nairobi sheep disease virus induces a pro-inflammatory response in infected sheep". Vet. Res. 43, 71.

LINES 431-434: This section B.2.3. could be expanded and devised as sections B.2.1 and B.2.2. The neutralisation assay could be explained in more details.

LINE 534: The EU suggests adding the following sentence at the end of this paragraph:
"An inactivated experimental trivalent vaccine (Aino, Akabane and Chuzan viruses) has been developed and successfully tested in cattle (Kin *et al.*, 2011)."
Reference: KIN Y.H., KWEON C.H., TARK D.S., LIM S.I., YANG D.K., HYUN B.H., SONG J.Y., HUR W. & PARK S.C. (2011). "Development of inactivated trivalent vaccine for the teratogenic Aino, Akabane and Chuzan viruses". Biologicals 39, 152-157.

LINES 537-539: The last 2 sentences should be deleted as they are not relevant for this section on requirements for vaccines. Furthermore, control of potential vectors is difficult and not feasible in many countries, thus that sentence would be of no real added value.

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

CHAPTER 2.9.2.: Camelpox

General comments

The EU can in general support this revised chapter and has some specific comments, some of which are purely linguistic.

Specific comments

LINE 13: The EU suggests adding the words "and pregnant females" after "in young animals".

LINE 122: Please delete this sentence (" Field reports […]"), as it is redundant.

LINE 124: Please add the words "in humans".

LINE 125: Please remove the words "at the time of writing", as this is uncommon in an OIE standard.

LINE 126: The wording is a bit odd ("However, they and mild human infections seem […]") and should be reviewed.

LINE 148: Please replace "fetal" by "foetal" (typographical error).

LINE 225: Please insert the following sentence after the one ending with "[…] orthopoxviral DNA":
"Several gel-based PCR methods have been described for the detection of camelpox viral DNA (Balamurugan *et al*., 2009; Meyer *et al*., 1994; Meyer *et al*., 1997; Ropp *et al*., 1995)."
(References: BALAMURUGAN V., BHANUPRAKASH V., HOSAMANI M., JAYAPPA K.D., VENKATESAN G., CAUHAN B., SINGH R.K. (2009). "A polymerase chain reaction strategy for the diagnosis of camelpox". J. Vet. Diagn. Invest. 21, 231-237; MEYER H., ROPP S.L., & ESPOSITO J.J., (1997). "Gene for A-type inclusion body protein is useful for a polymerase chain reaction assay to differentiate orthopoxviruses". J. Virol. Meth. 64, 217-221; ROPP SL., JIN Q. & KNIGHT J.C., MASSUNG R.F., & ESPOSITO J.J. (1995). "PCR strategy for identification and differentiation of smallpox and other orthopoxviruses". J. Clin. Microbiol. 33, 2069-2076.)

LINE 231: Please add the words "for gel-based PCR" to the title of the section.

LINE 314: Please add the following sentence at the end of this paragraph:
"In case Vero cells or other fast growing cells are used the concentration of the cells should be decreased to 240.000 cells /ml".

LINE 386: Please amend the sentence as follows: "Currently live attenuated and inactivated vaccines are commercially available."

LINES 399-400: Please amend the text as follows:

"Samples (such as crusty material from the nose, skin lesions or scabs) are collected from a camel calf showing generalised camel pox lesions. The samples are crushed in MEM with antibiotics, centrifuged […]".

LINE 405: Please replace the word "proven" by "confirmed".

LINE 406: Please delete the words "The isolate was designated as 298/89" and "of isolate 298/89", as this information is irrelevant.

LINES 419-420: Please amend the sentence to read as follows: "When the cell monolayer is confluent, the cells are infected with the vaccine virus".

LINES 432-433: Please amend the sentence to read as follows: "The procedure for testing for sterility and freedom from contamination of biological materials is described in chapter 1.1.7.".

LINE 436: Please change to "[…] using ten times the recommended dose per animal".

LINES 439-440: Please change to "The amount of virus present in the live attenuated vaccine is titrated on cell culture and the end titre is calculated".

LINES 452-457: Please change to "Efficacy in susceptible animals is demonstrated in naive dromedaries. The experimental animals should be vaccinated twice with the live attenuated vaccine and three weeks after the last vaccination the camels should be challenged with a virulent camelpox field strain. The virulence of the challenge virus should be demonstrated by inoculation of the virus into unvaccinated control animals. The vaccinated animals should not show any clinical signs whereas the unvaccinated group should develop characteristic clinical signs of camelpox. The long-term immunity provided by the live attenuated vaccine can also be confirmed by challenging the vaccinated animals 6 years later. The efficacy of the live attenuated vaccine should be further evaluated by measuring the antibody levels against camelpoxvirus 21-30 days after vaccination using ELISA and virus neutralisation test".

LINE 473: Please add the following sentence at the end of this paragraph: "The production of commercially available inactivated camelpox vaccine is described below".

LINE 517: Please change to "[…] using two times the recommended field dose per animal".

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

CHAPTER 2.9.4.: Cryptosporidiosis

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.9.5.: Cysticercosis

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.9.7.: Listeria monocytogenes

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 37: The EU suggests deleting the words "DNA restriction enzyme digestion pattern (conventional and".

LINE 59: The EU suggests inserting the words "and other animal species" after the word "ruminants".

LINE 112: After the word "literature", the EU suggests inserting the following sentence: "Although outbreaks have been reported from several countries, the majority of human cases are sporadic".

LINE 809: The EU suggests adding the following sentence: "In Europe, the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) are building databases with PFGE profiles of *L. monocytogenes* isolated from human cases and food and veterinary sources, respectively, with the aim of being able to solve transnational outbreaks."

LINE 894: The EU suggests inserting the words ", cold smoked fish" after the words "soft cheeses".

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

CHAPTER 1.1.3.: Standard for managing biorisk in veterinary laboratory and animal facilities

General comments

The EU has a few important general comments on this revised chapter, in addition to specific comments.

The EU appreciates that the OIE is adapting this chapter to motivate member countries to carry out their own risk assessments which is in accordance with the intention of current and previous versions of this chapter, and with the ideas promoted in the CWA 15793 Laboratory biorisk management standard (2008).

However, the proposed change of the chapter from a standard on how to classify veterinary pathogens and suggesting specific controls for work at four defined containment levels to a general standard on how to carry out a risk assessment for veterinary pathogens without a common framework for the handling of various biorisk levels may have serious consequences.

Firstly, although some member countries have national laws and formalised systems for classifying the work in their veterinary laboratories, others rely on referring to the four containment levels defined in this OIE Manual chapter when deciding on controls necessary for handling various veterinary pathogens, e.g. when a new pathogen emerges or a new facility is being constructed.

Member countries without a national legislative framework are already risk-evaluating work with veterinary pathogens in their laboratories, but most likely in a more informal manner using as guidance the OIE Manual, expert opinions and scientific publications, and have thus already established which OIE containment level is required for this type of work according to their individual situation.

The new version of this chapter removes the framework on which these decisions have been based on, and forces the member countries to formalise their systems for risk assessment and possibly develop national legislation to regulate the work with veterinary pathogens, which will be resource-demanding and time-consuming processes.

Indeed, in order to be able to implement this task, a lot of resources (time and money) would have to be put on education and information about the content of this standard and also to update or revise risk assessments already in place according to the suggested form. Furthermore, abandoning the "risk group and biocontainment level"-concept would require time and communication especially concerning the zoonotic agents.

Moreover, national and EU legislation in some instances make reference to the containment levels in the OIE Manual to define the containment

level necessary for work with particular pathogens, and these will be affected by the proposed change.

Secondly, the current and previous versions of this chapter support collaboration between member countries by promoting a common framework of understanding of biocontainment and biorisk in relation to working with veterinary pathogens.

This makes sharing of samples and reagents between laboratories more straightforward, and is also very helpful in the training of students and employees in new laboratory environments. Thus it is important for maintaining the necessary levels of biosafety measures and controls in a facility.

Thirdly, for zoonotic pathogens, the specific part of the current and previous versions of this chapter advances a more comparable level of risk of exposure for the worker between the member countries.

The approach in the new version will likely lead to a more varied evaluation of risk for humans between member countries, which in turn will lead to less uniform containment facilities and to more variety of the protection offered to the workers by veterinary laboratories/national authorities in the member countries.

Finally, the EU would also like to stress that even if a pathogen has been isolated in a given member country, this does not mean that it is endemic in that country. Likewise, the fact that a certain pathogen is endemic in a member country does not mean that introduction or spread of a new or less common strain will not happen – possibly with detrimental results.

Thus, even in endemic – or not-free – member countries, a too relaxed level of containment of work with veterinary pathogens may result in spread of new strains or pathogens with the laboratory playing an unfortunate role and *de facto* worsening the situation.

In conclusion, though the EU does support the promotion of more formalised processes of risk assessment by the OIE and the CWA, the EU would like to suggest that the specific part of the chapter defining the four OIE containment levels be maintained.

Lastly, the EU would kindly invite the OIE to answer the following two questions, which would be crucial in order to decide on whether the new approach proposed with this revised chapter could be supported by the EU in the near future:

1) Are there plans e.g. in the WHO laboratory biosafety manual also to leave the "risk group and biocontainment level"-concept? It would be important for the veterinary and human biorisk community to go in the same direction and work towards a harmonisation in this field.

2) How are different laboratories going to compare different facilities when e.g. sending samples to each other? Probably the risk assessments will be written in the countries own language and not always translated into English, which would further complicate collaboration.

Specific comments

LINES 236 to 248: The EU notes that this paragraph is repeated in **LINES 336 to 348**, thus one should be deleted.

LINES 9-10: As literally speaking it is not the laboratory and the facilities that are responsible for having a certain management system, the EU suggests the following alternative wording (in line with the wording in lines 119-122): "The management of laboratory and animal facilities is responsible for […]".

LINES 12-13: The EU does not agree with the deletion of the words "commitment to biosafety and laboratory biosecurity". Indeed, it would be important to clearly indicate from the beginning that biorisk is the combination of biosafety and biosecurity (in line with lines 181-182).

LINE 30: The EU suggests clarifying that these specific hazards (radiation exposure etc.), for which biorisk issues may have to be taken into account due to the laboratory setting in which biological agents are present, belong to prevention management and are not dealt with specifically in this chapter.

LINES 108-109: The EU suggests modifying the sentence as follows: "It is the responsibility of the management of the laboratory to ensure […]" (see also comment on **LINES 9-10**). A similar change would also be necessary in **LINE 218**.

LINE 181: Please delete reference to Table A as it is proposed for deletion.

LINE 189 (Flowchart 1): Under Biohazard Identification, the EU suggests splitting the first box in two separate boxes, one for "biological agent" and one for "Laboratory processes". This may stress the complementarity between "the risk class of a biological agent" and "the activity / manipulations / practices / laboratory processes" to identify a biohazard.

LINES 264-265: This sentence is difficult to understand. It should be clarified how an inventory management requirement links to the access of people, animals and vehicles.

LINES 304-330: The EU suggests making reference to bio-containment levels in this section, as many of the specific recommendation would depend on the bio-containment level.

LINE 311: The EU suggests replacing the words "as appropriate" by "as determined by risk assessment", as this would be more appropriate here.

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

LINES 336-348: The EU suggests deleting the whope paragraph, as it is the same as in **LINES 236-248**.

LINE 472: Please replace "B.3" by "B.2", as it relates to management rather than analysis.

APPENDIX 1.1.3.2. (p. 16): In the table, column 1, under "Biological Material", "Routes of transmission", the EU suggests adding "iatrogenic".

APPENDIX 1.1.3.2. (p. 17): In the table, column 1, under "Laboratory processes/activities", the EU suggests adding the words "- bites or scratches" as a further bullet point after "- shedding potential", as this would be most relevant factors concerning use of animals.

APPENDIX 1.1.3.2. (p. 18): In the table, column 2, under "Risk path controls", the EU suggests deleting the "low integrity controls" header. Indeed, the concept of integrity controls would need to be introduced or explained earlier in the document. It is unclear what this means in the context of the column heading "Considerations in assessment of the biorisk pathway".

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

CHAPTER 2.1.14: Rift Valley Fever

General comments

The EU can support this revised chapter.

Specific comments

None

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

CHAPTER 2.8.3.: Classical Swine Fever

General comments

The EU can in general support this revised chapter and has a specific comment.

Specific comments

LINE 307: RT-PCR methods have proven to be very useful tools for CSF nucleic acid detection and are actually used for 1st rapid and reliable diagnostic in case of suspicion. Some commercial kits are available, and are fully validated and easy to handle for routine diagnostic laboratories. The EU therefore proposes the addition of the following sentence and one reference:

"Moreover several commercial RT-PCR kits have been fully validated and are easy to handle for routine diagnostic (Le Dimna et al, 2008)".

Reference : Le Dimna M, Vrancken R., Koenen F.,Bougeard S, Mesplède A, Hutet E, Kuntz-Simon G and Le Potier M.-F. (2008). "Validation of two commercial real-time RT-PCR kits for rapid and specific diagnosis of classical swine fever virus". Journal of Virological Methods 147, 136–142.

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

GUIDELINE 3.5.: Biorisk Analysis: Biological agent-specific risk assessments

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments (Note: no line numbering available)

p. 7, Example 2 FMD, Pathogen and disease: The EU suggests adding the following sentences:

"Pathogen and disease: FMD is a highly contagious viral disease of cattle and swine. It also affects sheep, goats, deer, and other cloven-hooved ruminants. A variety of wild animals are also susceptible and may contribute to the natural epidemiology of FMDV. The disease is caused by […]".

"The transmission of FMDV occurs either by direct contact between infected and susceptible animals by deposition of infectious aerosols in the respiratory tract, by direct contact of susceptible animals with contaminated material or objects (hands, clothing etc. contaminated with excretions and secretions (saliva, urine, milk, semen, feces)), mechanical transfer of FMDV through virus entry via cuts or abrasions in the skin or mucosae or by consumption of untreated contaminated meat products (primarily by pigs). […]."

"[…] Morbidity may approach 100%. Mortality in general is low in adult animals (1–5% (occasionally high mortality rates (30-50%) have been recorded)) but higher in young calves, lambs and piglets (20% (up to 50%) or higher). […]".

p. 8, column 1 "Risk assessment", Health risk: The EU suggests adding the following sentence:
"[…] is documented (references on file). Humans can be considered as potential vectors for passive transport of FMDV".

p. 9, column 2 "Risk managament", point 2: The EU suggests amending the point as follows:
"Laboratory policy requires that staff agree to not come in direct contact with susceptible species for ~~96~~ 72 hours after working in a laboratory where ~~with~~ FMDV diagnostic cases and assays are performed."

p. 9, column 2 "Risk managament", point 7: The EU suggests amending the point as follows:
"7. Waste management policy addresses carcasses, tissues, fluids, and all laboratory wastes ~~containing potentially infectious material~~. All ~~potentially infectious~~ laboratory wastes are autoclaved or incinerated prior to removal from the site."

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

VALIDATION GUIDELINE 3.6.1.: Development and optimisation of antibody detection assays

General comments

The EU can in general support this revised chapter and has a few specific comments.

Specific comments

LINE 82 (Table 1): According to our understanding of follow-up on vaccination campaigns and the text re. purpose 6 on page 4, PPV should be given the score "+++" instead of "+", and NPV the score "+" instead of "+++".

LINES 134-142: A remark on confirmatory testing of samples positive in screening programs could be useful here.

LINE 172: The EU suggests inserting the words "egg yolk" after the words "meat juice".

LINE 374-381: The EU suggests that steric hindrance, as it appears in certain very targeted blocking assays, be mentioned as a phenomenon, which may give rise to specificity problems.

LINE 536: The EU suggests including the term "predictive values" in the header.

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

VALIDATION GUIDELINE 3.6.2.: Development and optimisation of antigen detection assays

General comments

The EU can support this revised chapter.

Specific comments

None

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

VALIDATION GUIDELINE 3.6.3.: Development and optimisation of nucleic acid detection assays

General comments

The EU can support this revised chapter.

Specific comments

None

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

VALIDATION GUIDELINE 3.6.4.: Measurement uncertainty

General comments

The EU can support this revised chapter.

Specific comments

None

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

VALIDATION GUIDELINE 3.6.5.: Statistical approaches to validation

General comments

The EU can support this revised chapter.

Specific comments

None

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

VALIDATION GUIDELINE 3.6.6.: Selection and use of reference samples and panels

General comments

The EU can support this revised chapter.

Specific comments

None

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

VALIDATION GUIDELINE 3.6.7.: Principles and methods for the validation of diagnostic tests for infectious diseases applicable to wildlife

General comments

The EU can support this revised chapter.

Specific comments

None