ANNEX 3

EU COMMENTS
ON THE PROPOSED CHANGES TO THE
WOAH MANUAL OF DIAGNOSTIC TESTS AND VACCINES
FOR TERRESTRIAL ANIMALS
PRESENTED FOR COMMENTS IN OCTOBER 2023[1]

[1] The draft chapters are appended to the BSC Sept. 2023 meeting report available on the WOAH website at: https://www.woah.org/en/document/report-of-the-meeting-of-the-woah-biological-standards-commission-september-2023/

EU COMMENTS on the proposed changes to the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

SUMMARY

1.1.5. Quality management in veterinary testing laboratories

General comment:

The EU supports this chapter.

1.1.9. Tests for sterility and freedom from contamination of biological materials intended for veterinary use

General comment:

The EU can in general support this revised chapter. Specific editorial comments and rationale are provided below.

Specific comments:

LINES 291-292 (Table 1): Please, remove header formatting for first row since it's just a list of agents, not a header.

2.2.4. Measurement uncertainty

General comment:

The EU supports this chapter.

2.2.6. Selection and use of reference samples and panels

General comment:

The EU supports this chapter.

3.1.5. Crimean–Congo haemorrhagic fever

General comment:

The EU supports this chapter.

3.3.6. Avian tuberculosis

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

LINES 18-19: Please add at the end of the sentence, after “pet birds owners” the following: “or caretakers of captive birds”.

LINE 199: Please replace “pet” with “captive”

LINES 212-213: Notation of the gene segment should be corrected and aligned with the rest of the document. Please replace “The isolates of *M. a. avium/M. a. silvaticum* are IS900−, IS901+, IS1245+, the isolates of *M. a. hominissuis* are IS900−, IS901−, IS1245+, and the isolates of *M. a. paratuberculosis* are IS900+, IS901−, IS1245−” with “The isolates of *M. a. avium/M. a. silvaticum* are IS*900*−, IS*901*+, IS*1245*+, the isolates of *M. a. hominissuis* are IS*900*−, IS*901*−, IS*1245*+, and the isolates of *M. a. paratuberculosis* are IS*900*+, IS*901*−, IS*1245*−”.

LINE 253: The common pheasant (*Phasianus colchicus*) is also called ring-necked pheasant. Please add “(*Phasianus colchicus*)” after “common pheasant” to avoid confusion between the two different common names for the same species of bird.

LINES 399-406: In this paragraph on safety regarding testing for living mycobacteria in tuberculin the study design with the use of guinea pigs as target species for evaluation is much less specific with regards to number of animals needed, minimum size of the animal and injection volume per animal. This is contrast to the paragraph before (on PPD) and the paragraph below (on lack of sensitising effect and batch potency), where the number and size of animals needed as well as injection volume is specified. Please specify accordingly.

It should be noted that daily fluid requirement of guinea pigs is approximately 100 ml/kg/day why care should be given when administering large volumes either intraperitonally or subcutaneously (should not exceed 60 ml/kg/day (Ritzman, 2014)).

Reference:

Ritzman, T. K. (2014). Diagnosis and clinical management of gastrointestinal conditions in exotic companion mammals (Rabbits, Guinea Pigs, and Chinchillas). In *Veterinary Clinics of North America - Exotic Animal Practice* (Vol. 17, Issue 2, pp. 179–194). W.B. Saunders. https://doi.org/10.1016/j.cvex.2014.01.003

3.4.1. Bovine anaplasmosis

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

LINE 148: Please replace “parasites” with “bacteria”.

LINES 161 (figure legend): Please replace “initial bodies” with “inclusion bodies” and use the same term throughout the text.,

LINE 169: Please replace “to be able to examine microscopically intact erythrocytes with “to microscopically examine intact erythrocytes”

LINE 188: Please replace “parasites” with “bacteria”

LINE 191: Please replace “nucleic-acid-based” with “nucleic acid-based”

LINE 200: Should “opportunity” be repaced with “risk”?

LINE 241 (TABLE 1): Suggest writing the oligonucleotides as continuous sequences of bases without hyphens or blank spaces. This will simplify searching and copy-paste

LINES 371 and 379: Use “subsection” or “point” – not both

LINES 362-384 (Section 2.2.2): Please be consistent in depicting time and temperature in the subsections. Use the same order e.g. "incubated at XX degrees for XX min"

LINES 399-400: Please replace “reproducibility” with "repeatability” Reproducibility typically refers to inter-laboratory precision. "Precision" would also be acceptable as a broader term.

LINE 412: Please replace “specimens” with “details”.

LINE 441: Missing blank space.

SECTION 2.3.2: Please be consistent in depicting time and temperature in the subsections. Use the same order e.g. "incubated at XX degrees for XX min"

LINE 448: Please replace “100 ul/well” with “100 µl/well”

3.4.7. Bovine viral diarrhoea

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

Page 111, line 64: Now reads "…its epidemiology and pathogenesis." Suggested change: "…**the** epidemiology and pathogenesis."

Page 112, line 113: The previous species *Pestivirus H* is now named *Pestivirus brazilense* according to ICTV. Please change accordingly in the beginning of the sentence on line 113.

Page 114, line 223: Now reads "…maternal antibody to BVDV…". Suggested change: "….maternal antibodies to BVDV…".

Page 114, line 225 AND page 116, line 464: Now reads "…antibodies against BVDV…". Should read: "…antibodies **to** BVDV…"

3.4.12. Lumpy skin disease (vaccine section only)

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

LINES 57: Chordopoxvirinae was replaced by Chordopoxviridae. Please verify if this is correct. It should probably remain Chordopoxvirinae

LINE 76: add comma after capripoxvirus

Line 368: the indicated change from 50 mM to 50 mm does not make any sense. It should read as originally stated

LINE 377-980: the EURL for capripox viruses, who also acts as WOAH ref lab for Lumpy skin disease virus, was responsible for the proposed revision of chapter C: Requirements for vaccines.

LINES 388-415: Make sure that the viruses are spelled correctly throughout the text: sheeppox virus, goatpox virus, capripoxvirus

3.6.9. Equine rhinopneumonitis (infection with equid herpesvirus-1)

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

LINES 15-17: Please replace "Following viraemia EHV1 also causes the more serious complications of abortion, perinatal foal death, or paralytic neurological disease (equine herpesvirus myeloencephalopathy)." with "Following viraemia, EHV1 may also cause the more serious complications of abortion, perinatal foal death, or paralytic neurological disease (equine herpesvirus myeloencephalopathy).".

LINES 27-30: Please replace "Viruses can be isolated in ~~equine~~ cell culture from nasal or nasopharyngeal swab extracts taken from horses ~~during the febrile stage of~~ with acute respiratory tract infection, from the placenta, from ~~and~~ liver, lung, spleen, adrenal glands or thymus of aborted fetuses and early foal deaths, and from the leukocyte fraction of the blood of animals ~~with acute~~ during the febrile stage of EHV-1 infection." with "Viruses can be isolated in ~~equine~~ cell culture from nasal or nasopharyngeal swab extracts taken from horses ~~during the febrile stage of~~ with acute respiratory tract infection, from the placenta, from ~~and~~ liver, lung, spleen, adrenal glands or thymus of aborted fetuses and early foal deaths, and from the leukocyte fraction of the blood of animals ~~with acute~~ during the febrile stage ~~of EHV-1 infection~~."

LINES 39-43: Please replace "Most horses possess some level of antibody to EHV-1/4, the demonstration of specific antibody in the serum collected from a single blood sample is therefore not confirmation of a positive diagnosis of recent infection. Paired, acute and convalescent sera from animals suspected of being infected with EHV-1 or EHV-4 should be tested for a four-fold or greater rise in virus-specific antibody titre by either virus neutralisation (VN) or complement fixation (CF) tests." With "As most horses possess some level of antibodies to EHV-1/4, the demonstration of specific antibody in the serum collected from a single blood sample is ~~therefore~~ not confirmation of a positive diagnosis of recent infection. Paired~~,~~ (acute and convalescent) sera from animals suspected of being infected with EHV-1 or EHV-4 should be tested for a four-fold or greater rise in virus-specific antibody titre by either virus neutralisation (VN) or complement fixation (CF) tests."

LINE 64: The current taxonomic names of the viruses are: *Varicellovirus equidalpha1* and *Varicellovirus equidalpha4*.

LINES 76-77: Please replace "In horses under 3 years of age, clinical ER usually takes the form of an acute, febrile respiratory illness that spreads rapidly through the group of animals." with "In horses under three years of age, clinical ER usually takes the form of an acute, febrile respiratory illness that spreads rapidly through the group of animals."

LINES 86-87: Please replace “Like other herpesviruses, EHV-1~~/4~~ causes long-lasting latent infections and latently infected horses represent a potential infection risk for other horses.” with “Like other herpesviruses, EHV-1~~/4~~ causes long lasting latent infections and latently infected horses represent a potential infection risk for other horses.”

LINES 101-102: Please replace “Strain typing has been shown to be not reliable for predicting the clinical outcome of EHV-1 infection but can be useful in epidemiological investigations (Garvey *et al*., 2019; Nugent *et al*., 2006; Sutton *et al*., 2019).” with “Strain typing has been shown to be unreliable for predicting the clinical outcome of EHV-1 infection but can be useful in epidemiological investigations (Garvey *et al*., 2019; Nugent *et al*., 2006; Sutton *et al*., 2019).”

LINES 104-105: Please replace “~~Both~~ EHV-1 ~~and EHV-4~~ is transmitted by the respiratory route and has ~~have~~ the potential to be highly contagious ~~viruses~~ particularly where large numbers of horses are housed in the same air space.” with “~~Both~~ EHV-1 ~~and EHV-4~~ is transmitted by the respiratory route and has ~~have~~ the potential to be highly contagious, ~~viruses~~ particularly where large numbers of horses are housed in the same air space.”

LINES 139-141: Please replace “Virus may be isolated from post-mortem cases of EHV-1 neurological disease by culture of samples of brain and spinal cord but such attempts to isolate virus are often unsuccessful; however, they may be useful for PCR testing and pathological examination.” with “Virus may be isolated from post-mortem cases of EHV-1 neurological disease by culture of samples of brain and spinal cord but such attempts ~~to isolate virus~~ are often unsuccessful; however, they may be useful for PCR testing and pathological examination.”

LINES 312-313: Please replace “EHV-1 and EHV-4 are endemic in most parts of the world and seroprevalence is high; however, serological testing of paired sera can be useful for diagnosis of ER in horses.” with “EHV-1 and EHV-4 are endemic in most parts of the world and seroprevalence is high; notwithstanding, serological testing of paired sera may be useful for diagnosis of ER in horses.”

LINE 396: Should read: “…antibodies to EHV-1”

3.8.1. Border disease

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

LINES 371-372: Please replace “to afford a high level of fetal infection” with “to provide a high level of fetal protection”.

3.8.12. Sheep pox and goat pox

General comment:

The EU can in general support this revised chapter.

Specific comments and rationale are provided below.

Specific comments:

LINES 87: After "…following capripoxvirus infection.." please add "*Surviving animals eventually clear the infection, since there is no evidence of persistently infected animals...".*

LINE 87-88: After 'Capripoxvirus is not infectious to humans' please add the paragraph *'Capripoxvirus is inactivated at 56C for 2 hours or 65C for 30 minutes. The virus survives between pH 6.6-8.6. It is susceptible to highly alkaline or acid pH. The virus is sensitivity to various chemicals: sodium dodecyl sulphate, ether 20%, chloroform, formalin 1%, sodium 2%, iodine compounds, Virkon 2%, quaternary ammonium (0.5%), and phenol 2% for 15 minutes'*

LINE 101: After '..neutralising antibody responses.' add '*In addition to epithelial lesions nasal and buccal swabs can be collected because the virus will be present in nasal and saliva discharges'.*

Line 110: For consistency, 'antigen detection' should be deleted, as in other paragraphs.

Line 115: 'antigen' should be replaced by 'genome'.

Line 205: EDTA-blood and semen are specified as possible sample types for conventional PCR methods, can these matrices be used for Real-time PCR methods as well? - Line 253-257 refers in general to commercial kits for 'blood and tissue'. If blood and semen is valid sample material for nucleic acid recognition methods, it should be included in Line 196.

LINE 246: Please add the Haegeman et al. (2013) assay to the list of pan-capripox virus realtime PCR assays. This test is used by the EURL for capripox viruses and several other national reference laboratories from Europe.

All validation information on this test can be found in following publication:

J Virol Methods. 2013 Nov;193(2):446-51. doi: 10.1016/j.jviromet.2013.07.010. Epub 2013 Jul 11.

Development and validation of three Capripoxvirus real-time PCRs for parallel testing

A Haegeman, K Zro, F Vandenbussche, L Demeestere, W Van Campe, M M Ennaji, K De Clercq

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LINE 285: below point 2. 'Serological tests' add a line '*Blood for antibody detection should be collected in tubes without anti-coagulant. Detectable levels of antibodies develop one week after the animal show clinical signs. The highest antibody levels are detected within one to two months after infection is detected'.*

Line 345: 'different capripoxviruses (LSD or SPP/GTP)' – 'V' should be included in the abbreviations.

3.9.1. African swine fever (vaccine section only)

General comment:

The EU thanks the WOAH Biological Standards Commission for the work being moved forward. The EU can in general support this revised chapter. It appears that there are still several points that need to be clarified and we invite the Commission to review them.

There is one point in the new section on vaccines that we would like to raise again:

At present, the occurrence of fever is given very high priority. Even if the definition in the later sections is correct and sensible in our opinion (three consecutive days a rise of >2.5°C in an individual pig or group mean 1.5°C above baseline), it sounds to us as if a short fever spike is already an exclusion criterion (see e.g. line 136). Many good vaccines have side effects, including fever, muscle aches etc. To avoid misunderstandings, especially with regulatory bodies, we think the general wording should be worded more carefully.

In addition, we would argue less strictly in the reversion to virulence. It is about relevant increases in virulence and thus impact for the vaccination campaign. Proving complete genetic stability is probably a matter of luck with a live virus and there should be some room for interpretation.

Further, specific comments are provided below.

Specific comments:

LINE 14: consider replacing mortality (referring to the number of deaths in a given population) by lethality (referring to deaths in the sub-population of diseased individuals in a given population). Mortality can be quite low with ASF while the lethality is usually high.

Line 37: It is just in Vietnam that MLV vaccines for ASF have been licenced (?) - see lines 161-162

LINE 39: the distribution expanded… given that e.g. the Dominican Republic and Haiti are affected, the list of countries and continents should be amended.

LINE 77 et seq: consider revision. Suggestion: Animal that have recovered from ASFV infection can stay positive for virus and viral genome for prolonged times and may act as carriers. However, the biological basis for the possible persistence is not well understood and their role in epidemiology remains unclear.

Comment: Should point-of-care tests be mentioned? Some tests of the newer generation could be worthwhile when knowing about their advantages and disadvantages.

LINES 77-80: Please replace “Animals that have recovered from either acute,

subacute or chronic infections may potentially become persistently infected, acting as

virus carriers. The biological basis for the persistence of ASFV is still not well understood, nor it is clear what role persistence plays in the epidemiology of the disease" with "Animal that have recovered from ASFV infection can stay positive for virus and viral genome for some time. However, the biological basis for the possible persistence is not well understood and their role in epidemiology remains unclear.".

LINE 103:
Please replace "and that has been validated with respect to virus identity, sterility, purity, potency, safety, non-transmissibility, stability and immunogenicity.**'**

With 'and that has been validated with respect to virus identity, sterility, purity, potency, stability, safety (including spread), and efficacy (including immunogenicity).**'**

Comment: possible rewording to rationalise wording. According to the minimum standard some virus vaccine transmission might be allowed, so reference to non-transmissibility may send an inaccurate message.

LINE 103-104:
"ASF MLV first generation vaccines – defined as those for which peer-reviewed publications are in the public domain"

Comments: The definition provided is based on the existence of peer reviewed publications in the public domain rather than on the characteristics of the vaccines per se. The number of peer-reviewed publications is not considered an appropriate basis for the definition since publications will inevitably increase over time for different types of vaccines. A definition based on the specific characteristics of such vaccines would seem more appropriate.

LINE 109: Given that problems were seen mainly in breeding animals, their exclusion from the minimum standards may be unwise. I was part of the group discussion on the standards and know how the chapter addition came about. However, I still think that duration of immunity and impact on breeding animals is a must.

LINE 109-110:
Please revise: 'Demonstration of MLV safety and efficacy in breeding-age boars, gilts and pregnant sows, and onset and duration of protective immunity, are also preferred but are not required to meet the minimum standard'

Comments:

Please note that the paragraph above appears to conflict with the minimum standards described in lines 136-146: *'Efficacious: protects against mortality, reduces acute disease (fever accompanied by the appearance of clinical signs caused by ASF) and reduces vertical (boar semen and placental) and horizontal disease transmission;'*

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The text appears to make a distinction between ideal/preferred minimum standards and essential minimum standards which create some confusion. Please consider clarifying.

Considering the potential use of the vaccine (e.g. blanket vaccination), demonstration of safety (at least) of a MLV vaccine in breeding-age boars, gilts and pregnant sows is considered an important aspect.

Onset of immunity: also, if the minimum standards for efficacy described in the efficacy section are followed, the onset of immunity can be set based on the interval of time elapsed between (last vaccination) and challenge, if protection is confirmed. Therefore, the reference made that the onset of immunity is not required for the minimum standards is not understood. Furthermore, onset of immunity is considered a key vaccine attribute and should be defined; it would appear to be very important parameter for the deployment (planning) of vaccine in the field and surveillance purposes. Duration of immunity is also an important vaccine attribute and it is a standard requirement in the EU for obtaining a standard (full) marketing authorisation.

LINE 114: Consider removing “soft-bodied”.

LINE 125: I would not talk about one strain. There is some variation now. I would call it “ASFV p72 genotype II strains”

LINE 136: Is it wise to ask for the absence of fever without giving the definition here (there is a clear definition further down)? A short temperature peak could mean good vaccine action. See above.

LINE 139: Protects against ASF induced mortality

LINE 143-144:

“Quality – potent: the log10 virus titre maintained throughout…” with “Quality – potent: the ~~log10~~ virus titre maintained throughout…”

Comment: this quality attribute is more related to stability i.e. maintain the minimum titre (potency) over the shelf life of the vaccine

LINE 145:

“Identity: based on the capacity to protect against the ASFV B646L (p72) genotype II pandemic strain or other p72 145 genotypes of recognised epidemiologic importance.”

Comment: is this really identity? In section 2.1.2, identity is referred to as ‘Identity of the MSV must be confirmed using appropriate methods (e.g. through the use of vaccine strain-specific whole genome detection methods such as next generation sequencing).’

LINE 145: see above, consider broadening it “strains”

LINE 158 et seq.: See comment on strains. At present, there is no real strains and lineage designation.

LINE 161-162:

Please replace ‘Currently, two gene deleted MLV recombinant vaccines (ASFV-G-ΔI177L and ASFV-G-ΔMGF) have been licenced for field use in Vietnam following supervised field testing to evaluate the safety and effectiveness of several vaccine batches.’
with ‘Currently, two recombinant gene deleted MLV ~~recombinant~~ vaccines (ASFV-G-ΔI177L and ASFV-G-ΔMGF) have been licenced ~~for field~~ in Vietnam for use in domestic pigs ~~following supervised field testing to evaluate the safety and effectiveness of several vaccine batches~~.’

Comment: It is important to include the target species of the vaccines that have been authorised. Some editorial rephrasing is also suggested.

Lines 163-174: With some of the ASFV vaccine candidates that are being developed, e.g. with different single gene deletions (lines 168-169), as licenced in Vietnam, there seems to be the possibility than an animal that inadvertently received two different vaccine strains (with different single gene deletions) could potentially regenerate a fully virulent ASFV by recombination. Should there be consideration of requiring that all MLV ASFV vaccines have at least one attenuating deletion in common so that it is not possible for this to occur?

LINE 166: wild boar

LINE 168 et seq: consider to combine these viruses… all were designed by homologous recombination (see lines 196 et seq) and are deletion mutants with a different number of genes deleted.

LINE 179 et seq.: Would state it stricter… there is no inactivated vaccine with any level of protection that could be acceptable.

LINE 193:
Please replace “Characteristics of the seed” with “Characteristics of the seed virus”.

LINE 194:
Please replace “Biological characteristics of the master seed” with “Biological characteristics of the master seed virus”

LINE 195-196:
Please replace “MLVs are produced from ASFV field strains derived from naturally attenuated field isolates or using 195 DNA homologous (genetically targeted) recombination techniques in cell cultures to delete one or 196 more ASFV genes or gene families.” **with** “ASF MLVs are generally produced from ASFV field strains derived from naturally attenuated field isolates or using 195 DNA homologous

(genetically targeted) recombination techniques in cell cultures to delete one or 196 more ASFV genes or gene families."

Comment: some of the vaccines vaccines in the pipeline are based on naturally-attenuated MLVs which then are subject to modification to delete certain genes. At the moment, the definition give would not encompass these as it is an 'or' (naturally attenuated or subject to gene deletion) but not both. Perhaps adding generally, any approach would be covered.

LINE 214-217:
The following sentence can be deleted: '~~Live vaccines must be shown not to cause disease or other adverse effects in target animals in accordance with chapter 1.1.8, Section 7.1 Safety tests (for live attenuated MSVs), that includes target animal safety tests, increase in virulence tests, assessing the risk to the environment) 216 and if possible, no transmission to other animals.'~~

Comment: The paragraph refers to safety requirements but it is placed under the heading "Quality criteria (sterility, purity, freedom from extraneous agents)", so it does not appear to be the appropriate place. The safety requirements are explained elsewhere.

LINE 239:
Please replace "ASF vaccine should be presented in a suitable pharmaceutical form (e.g. lyophilisate or liquid form)." **With** "ASF vaccines should be presented in a suitable pharmaceutical form (e.g. lyophilisate or liquid form)."

Comment: editorial.

LINE 242: MLV contains already "virus", remove the additional "virus"

LINE 246-248:
Please replace 'Thus, preferably a master cell bank based established, continuous cell line shown to support genetically stable ASFV replication and acceptable titres over several passages should be used.
With
'Thus, preferably a master cell bank based on an established, continuous cell line shown to support genetically stable ASFV replication and acceptable titres over several passages should be used.

Comment: editorial.

LINE 280-281
Please replace: 'The test should be carried out consistent with VICH44 GL26 (Biologicals: Testing of Residual Moisture, 200345). Required for MLV vaccines presented as lyophilisates for suspension for injection.'

With: ‘‘The test should be carried out consistent with VICH44 GL26 (Biologicals: Testing of Residual Moisture, 200345). Required for MLV vaccines presented as lyophilisates ~~for suspension for injection~~.’

Comment: The test will be required for any lyophilised or freeze-dried vaccine regardless of the route of administration (e.g. it will be required for a lyophilised vaccine for nasal or oral administration) and the final reconstituted dose form (suspension, emulsion, solution etc.)

LINE 295-296 Additional demonstration of MLV safety in breeding age gilts and pregnant sows is preferred but not required as a minimum standard.

Comment: Please note that if a vaccine is intended for use in breeding animals, the evaluation of impact of the vaccine in reproductive performance is a standard safety requirement in the EU (for full marketing authorisation). As stated, in a different comment, given the potential use of ASF vaccines (blanket vaccination), the evaluation of impact on reproductive parameters would appear to be an important safety parameter.

LINE 298-299 Unless the most sensitive category for safety testing is considered to be pigs of 6-10 weeks of age, a more flexible wording would be preferable e.g. wording used in line 421 which is aligned with VICH GL44.
Please replace: ‘Carry out the test by each recommended route of administration using, in each case, piglets a minimum of 6-weeks old and not older than 10-weeks old.’

With: ‘Carry out the test by each recommended route of administration using, in each case, piglets of a suitable age (e.g. between 6 and 10 weeks of age) ~~a minimum of 6-weeks old and not older than 10-weeks old.~~’

Comment: In line 295, it is stated that the safety of the vaccine should be tested in pigs of the ‘target age’ whilst in line 299, lines 334-335, lines 381-382, a specific age is pre-defined (i.e. minimum of 6-weeks old and not older than 10-weeks old). VICH GL 44 (Target animal safety for veterinary live and inactivated vaccines) states that ‘animals should be appropriate for the purpose of the test with regard to species, age, and class for which the product will be used’. It also states that ‘generally the most sensitive class, age and sex proposed on the label should be used.’

LINE 307
Please replace: ‘To obtain individual and group mean baseline temperatures, the body temperature of each vaccinated piglet is measured…’

With: ‘To obtain individual and group mean baseline temperatures, the body temperature of each ~~vaccinated~~ piglet is measured…’

Comment: editorial. The test refers to temperature before vaccination.

LINES 309-315

To confirm the presence or absence of fever accompanied by acute and chronic disease, observe the piglets 4 hours after vaccination and then at least once daily for at least 45 days, preferably 60 days post-vaccination. Carry out the daily observations for signs of acute and chronic disease using a quantitative clinical scoring system adding the values for multiple clinical signs (e.g. Gallardo et al., 2015a). These clinical signs should include fever, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling and necrotic lesions around the joints, respiratory distress and digestive findings).

Comment: The monitoring period proposed is far longer than that proposed in the VICH GL44 for target animal safety for veterinary live and inactivated vaccines.

When injection site adverse reactions are present at the end of the 14 days observation, the observation period should be extended until clinically acceptable resolution of the lesion has occurred or, if appropriate, until the animal is euthanized and histopathological examination is performed.

LINES 321-322
Please replace: 'No piglet shows abnormal (local or systemic) reactions, reaches the pre-determined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;"

With: 'No piglet shows abnormal (local or systemic) reactions, or notable signs of disease, or reaches the pre-determined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;"

Comment: The current wording could be interpreted as if vaccinated piglets showing notable signs of disease but not reaching the pre-determined humane endpoint would comply with the test. For this reason, some re-wording is suggested.

Line 328-329: In contrast to the text, there have been experimental studies looking at the transmission of a genotype II ASFV from pregnant sows to the foetuses, see Lohse et al., (2022). In these studies, it was shown that low levels of ASFV DNA were detected in tonsils, spleen and lymph nodes of some of the foetuses. However, the deaths of the sows may have limited the time available for virus replication in these foetuses.

Reference is: Lohse L, Nielsen J, Uttenthal Å, Olesen AS, Strandbygaard B, Rasmussen TB, Belsham GJ, Bøtner A. Experimental Infections of Pigs with African Swine Fever Virus (Genotype II); Studies in Young Animals and Pregnant Sows. Viruses. 2022 Jun 25;14(7):1387. doi: 10.3390/v14071387).

LINE 333: Consider not to comingle directly. If oral vaccines are considered, environmental contamination with vaccine virus could lead to "vaccination" of naïve contacts.

LINES 336-338

Please replace: 'All piglets are housed together from day 0 and the number of vaccinated animals is the same as the number of naïve, contact animals. Co-mingle equal numbers of vaccinated and naïve, contact piglets in the same pen or room.

Comment: The information provided in the first and the second sentence is the same. The text should be rationalised.

LINES 367-368
Please replace: 'No piglet shows abnormal (local or systemic) reactions, reaches the pre-determined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;"

With: 'No piglet shows abnormal (local or systemic) reactions, or notable signs of disease, or reaches the pre-determined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;"

Comment: The current wording could be interpreted as if vaccinated piglets showing notable signs of disease but not reaching the pre-determined humane endpoint would comply with the test. For this reason, some re-wording is suggested.

LINES 428-429
LINES 457-458
LINES 465-466
LINES 470-471

"Observe inoculated animals daily for the appearance of at least two and preferably at least three clinical signs and record daily body temperatures."

Comment: The reference to 'at least two and preferably at least three clinical signs" is not understood. The observation should include all relevant parameters typical for the disease that could indicate increase in virulence.

LINES 438-439
Please replace: 'Based on results from at least one completed vaccine virus blood and tissue distribution dissemination study, Section C.2.3.2.iv above) euthanise piglets'

With: 'Based on results from at least one completed vaccine virus blood and tissue ~~distribution~~ dissemination study~~,~~ (Section C.2.3.2.iv above), euthanise piglets'

Comment: editorial.

LINES 482-484

"Absence of chronic and acute clinical signs and gross pathology over the entire test period or minimal chronic clinical signs (defined as mild swollen joints with a low clinical score that resolve within 1 week)."

Comment: the reference to chronic clinical signs which are then defined as resolving within 1 week appears to be inconsistent.

LINES 485-488
Please replace: 'Based on results from at least one completed vaccine virus blood and tissue distribution dissemination study, Section C.2.3.2.iv above) euthanise piglets'

With: 'Based on results from at least one completed vaccine virus blood and tissue ~~distribution~~ dissemination study~~,~~ (Section C.2.3.2.iv above), euthanise piglets'

Comment: editorial.

LINES 492-496 "In addition, the vaccines in their commercial presentation before being authorised for general use should be tested for safety in the field (see chapter 1.1.8 Section 7.2.3). Additional field safety evaluation studies may include but are not limited to: environmental persistence (e.g. determination of virus recovery from bedding or other surfaces), assessment of immunosuppression, and negative impacts on performance."

Suggested rewording:

In addition, for regulatory approval, ASF MLV vaccines should be tested for safety under field conditions (see chapter 1.1.8 Section 7.2.3). Safety field studies generally include measurement of body temperatures, observation of local or systemic reactions and, where appropriate, performance measurements".

Comments: Suggested rewording. Assessment of immunosuppression (i.e. impact on immunological functions of vaccines) is usually addressed in the pre-clinical studies; environmental persistence is usually addressed in the preclinical safety studies (spread).

LINES 496 Additional comment:

Please note that in the EU, if the recommended vaccination schedule consists of more than one administration (including the number of administration of the primary vaccination schedule and the first revaccination, if revaccination is recommended) the safety of the repeated administration of one dose is a standard requirement.

LINES 513-514
Please replace: 'Twenty-eight days (±2 days) after the single injection of vaccine (or if using two injections of the 513 vaccine then 28 days [±2 days] following the second injection)'

With: 'Twenty-eight days (±2 days) after the single ~~injection~~dose of vaccine (or if using two doses~~injections~~ of the vaccine then 28 days [±2 days] following the second dose ~~injection~~)…'

Comment: Preferable to use a more neutral wording. Injection would restrict the scope to vaccines administered parenterally (e.g. would exclude vaccines administered nasally, described in literature).

LINES 551-552
Please replace:
'No vaccinated challenged piglet shows abnormal (local or systemic) reactions, reaches the humane endpoint or dies from causes attributable to ASF'

With: 'No vaccinated challenged piglet dies or ~~shows abnormal (local or systemic) reactions,~~ reaches the humane endpoint ~~or dies~~ from causes attributable to ASF'

Comment: Suggested rephrasing. Abnormal (local or systemic) reactions usually refer to safety aspects. The criteria of acceptability for clinical signs of ASF is given in the third bullet point. For clarity and to avoid misinterpretation, it would be preferable to omit that reference here. The change does not impact in the described efficacy criteria.

LINES 575-576
Please replace: 'Twenty-eight days (±2 days) after the single injection of vaccine (or if using two injections of the vaccine then 28 days [±2 days] following the second injection)'

With: 'Twenty-eight days (±2 days) after the single ~~injection~~dose of vaccine (or if using two doses~~injections~~ of the vaccine then 28 days [±2 days] following the second dose ~~injection~~)…'

Comment: Preferable to use a more neutral wording. Injection would restrict the scope to vaccines administered parenterally (e.g. would exclude vaccines administered nasally, described in literature).

LINES 584-586
Please replace: 'Twenty-eight days (±2 days) after the single injection of vaccine (or if using two injections of the vaccine then 28 days [±2 days] following the second injection)'

With: 'Twenty-eight days (±2 days) after the single ~~injection~~dose of vaccine (or if using two doses~~injections~~ of the vaccine then 28 days [±2 days] following the second dose ~~injection~~)…'

LINES 587-588
'Approximately 18-24 hours later, re-introduce naïve piglets to vaccinated, challenged piglets and allow for direct nose to nose contact exposure with vaccinated, challenged piglets. Allow for continuous contact exposure by co-mingling both groups through the end of the study.

Comment: Please note that in the case of the test for the assessment for horizontal transmission, there is no description on how the piglets are housed, and then in line the text states 're-introduce naïve piglets' (line 587) and 'allow for continuous contact exposure by co-mingling…' (line 589). A brief explanation in the initial description of the test animals, housing will be useful (e.g. similar to the explanation included at the start of the section 2.3.2.iii) Horizontal transmission).

LINES 625-626
Please replace: 'No naïve, contact exposed piglet shows abnormal (local or systemic) reactions, reaches the defined humane endpoint or dies from causes attributable to ASF'

With: 'No naïve, contact exposed piglet ~~shows abnormal (local or systemic) reactions~~, reaches the defined humane endpoint or dies from causes attributable to ASF'

Comment: Suggested rephrasing. Abnormal (local or systemic) reactions usually refer to safety aspects. The criteria of acceptability for clinical signs of ASF is given in the second bullet point. For clarity and to avoid misinterpretation, it would be preferable to omit that reference here. The change does not impact in the described efficacy criteria. Alternatively, the following text would be suggested 'No naïve, contact exposed piglet shows notable signs of disease, reaches the defined humane endpoint or dies from causes attributable to ASF' but it is somehow included in the second bullet point.

LINES 641-644 'In addition, the vaccines in their commercial presentation before being authorised for general use should be tested for efficacy in the field (see chapter 1.1.8 Section 7.2.3). Additional field efficacy evaluation studies may include but are not limited to: onset of immunity, duration of immunity, and impact on disease transmission.'

Suggested rewording:

In general, for regulatory approval, ASF MLV vaccines should be tested for efficacy under field conditions (see chapter 1.1.8 Section 7.2.3). Field efficacy studies generally include measurement of relevant efficacy parameters including but limited to mortality, clinical signs, impact on disease transmission, performance parameters".

Comments: Suggested rewording. Unless a validate correlate or surrogate of protection exists, establishment of onset of immunity and duration of immunity will require a challenge study under laboratory conditions. Also, batches of vaccine used in field trials does not generally contain the minimum virus content (minimum protective dose); routine production batches (i.e. not minimum virus content) are generally used. For this reason, more neutral wording could be appropriate.

LINES 646-648 'Although not included in the guidance for ASF MLV first generation vaccines, manufacturers are encouraged as part of the authorisation procedure, to

demonstrate the duration of immunity of a given vaccine by evaluation of potency at the end of the claimed period of protection.'

Suggested re-wording

'Although not included in the guidance for ASF MLV first generation vaccines, manufacturers ~~are encouraged~~ may be required, as part of the authorisation procedure, to demonstrate the duration of immunity of a given vaccine by evaluation of efficacy (potency) at the end of the claimed period of protection.'

Comments: Please note that in the EU, demonstration of duration of immunity is a requirement for standard marketing authorisation (i.e. it is not a strict requirement for vaccines authorised in the EU under exceptional circumstances). Some re-wording is suggested to cover the point whilst keeping flexibility, as requirements may vary across jurisdictions.

3.10.4. Infection with *Campylobacter jejuni* and *C. coli*

General comment:

The EU can in general support this revised chapter. However, in a future revision, the wider significance of *Campylobacter* spp. as a zoonotic agent could be taken into account, and the information about other *Campylobacter* species of zoonotic relevance (e.g. *C. lari*, *C. upsaliensis*, *C. hyointestinalis*, *C. helveticus*, *C. hepaticus* and *C. bilis*) could be extended in the chapter, or added to a new chapter in the manual.

Specific comments and rationale are provided below.

Specific comments:

LINES 30-33:

MALDI-TOF accounts as a phenotypic method. There is an inconsistency in the chapter regarding tests for detection, confirmation and identification.

Please replace “Phenotypic identification is based on reactions under different growth conditions. Biochemical and molecular tests, including PCR and MALDI-TOF (matrix assisted laser desorption ionisation–time of flight) mass spectrometry can be used to identify Campylobacter strains at species level. PCR assays can also be used for the direct detection of C. jejuni and C. coli.” with “Phenotypic confirmation and identification of C. jejuni and C. coli can be achieved through biochemical tests or MALDI-TOF (matrix assisted laser desorption ionisation–time of flight) mass spectrometry, and PCR can be used for detection of C. jejuni and C. coli directly from the sample or for confirmation and species identification from isolated bacteria.”

LINES 42-43:

The majority of cases of abortion due to Campylobacter is diagnosed without sequencing, and it would therefore be preferred to specify the statement, for example as followed:

Please replace “One specific C. jejuni clone has been associated with abortion in sheep (Tang et al., 2017)” with ”One specific C. jejuni clone has been predominantly associated with abortion in sheep in the United States”

LINES 43-45:

The information about spotty liver disease has been removed from the chapter. If not in this chapter, a new chapter should be written on C. hepaticus and C. bilis, which cause spotty liver disease in laying hens. The disease has appeared in several countries, in so far reported at least from United Kingdom, France, Australia, United States and the Netherlands.

LINES 46-48:

The latter part of the sentence is only true for high-income countries, and recent studies have shown that a large proportion of the campylobacteriosis cases in low- and middle-income countries (LMIC), mostly in children, may be caused by non-C. coli/jejuni strains. Please rephrase to make this limitation clear.

For example, please replace “Campylobacter is the main cause of human bacterial intestinal disease identified in many industrialised countries (CDC, 2022; EFSA, 2021), and C. jejuni and C. coli together account for more than 90% of all human campylobacteriosis cases.” with “In many industrialised countries, Campylobacter is the main cause of human bacterial intestinal disease identified, and C. jejuni and C. coli together account for more than 90% of all human campylobacteriosis cases (CDC, 2022; EFSA, 2021).”

Also, consider adding a note on campylobacteriosis in LMIC.

Reference:

François R, Yori PP, Rouhani S, Siguas Salas M, Paredes Olortegui M, Rengifo Trigoso D, Pisanic N, Burga R, Meza R, Meza Sanchez G, Gregory MJ, Houpt ER, Platts-Mills JA, Kosek MN. The other Campylobacters: Not innocent bystanders in endemic diarrhea and dysentery in children in low-income settings. PLoS Negl Trop Dis. 2018 Feb 7;12(2):e0006200. doi: 10.1371/journal.pntd.0006200.

LINES 46-48:

We know very little about the ratio of C. jejuni and C. coli in human cases in countries outside the EU and the US, and far from all reported cases include speciation, please rephrase.

LINE 65: Please update the number of strains to “43 *Campylobacter* species recognised (July 2023)”.

LINE 71: Please replace “Some species, particularly *C. jejuni*, *C. coli* and *C. lari*, are thermophilic,” with “Some species, particularly *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis,* are thermophilic,”.

Lines 74–76:

Since *C. jejuni* subsp. *doylei* is not thermotolerant and does not normally grow at 42 °C, the statement (“no added value”) in this sentence is somewhat confusing and not in alignment with lines 71–72.

Please consider to keep at least the information of differing growth temperature spans between the two subspecies.

Table 1

Isolation is not an identification method, but a preliminary step in the identification process where biochemical tests, MALDI-TOF or PCR is used. Please clarify the table.

LINE 81:

As PCR is still in the table, please keep the full explanation for the abbreviation of PCR.

LINE 82:

Please replace “(a)Regarding the control of the agent: Campylobacter jejuni and C. coli are endemic globally and very rarely cause disease” with “(a)Regarding the control of the agent: Campylobacter jejuni and C. coli are endemic globally and very rarely cause disease in animals”.

LINE 85:

Please replace “and prevalence of infection surveillance are filled in” with “and ”prevalence of infection – surveillance” are filled in”

LINES 89-95:

Is seems relevant to keep the information about ISO 17995 since this chapter includes information about life stock and sampling at farms, where well water or agricultural water samples are often analysed.

Please replace “Two ISO (International Organization for Standardization) procedures for detection of Campylobacter exist. ISO 10272 describes a horizontal method for detection and enumeration of thermotolerant Campylobacter spp. in food and animal feeding stuffs with 2 parts: part 1 detection method (ISO 10272-1:2017) and part 2 colony count technique (ISO 10272-2:2017) with “There are two ISO (International Organization for Standardization) standards for detection of Campylobacter: ISO 10272 and ISO 17995. ISO 10272:2017 describes a horizontal method for detection (part 1) and enumeration (part 2) of thermotolerant Campylobacter spp. in samples of the food chain. ISO 10272:2017 was amended in 2023 (ref included last among the comments). ISO 17995:2019 describes a method for detection and enumeration of thermotolerant Campylobacter spp. in water samples.”

LINES 90-91: Suggest replacing ISO version with most current ones: ISO 10272-1:2017+A1:2023 and ISO 10272-2:2017+A1:2023**.**

LINES 98-99:

It is wrong to generalise that poultry is less often colonised with C. coli than with C. jejuni. Due to several countries claiming C. coli to be more common in poultry than C. jejuni, the reporting of antimicrobial resistance in the EU has been updated so it is no longer pre-defined which of the two species is the most prevalent, and the

harmonised method has been changed to better promote the finding of both species. Recently, C. coli was found to be the predominant species in retail chicken meat in the United Arab Emirates, see ref below:

Ihab Habib et al. Genomic characterization of molecular markers associated with antimicrobial resistance and virulence of the prevalent Campylobacter coli isolated from retail chicken meat in the United Arab Emirates. Curr Res Food Sci, 2023. doi: 10.1016/j.crfs.2023.100434

Andritsos ND, Tzimotoudis N, Mataragas M. Prevalence and distribution of thermotolerant *Campylobacter* species in poultry: A comprehensive review with a focus on the factors affecting the detection and enumeration of *Campylobacter jejuni* and *Campylobacter coli* in chicken meat. Applied Sciences. 2023; 13(14):8079. https://doi.org/10.3390/app13148079

Please replace “Poultry is frequently colonised with C. jejuni (65–95%), less often with C. coli and rarely with other Campylobacter species (Newell & Wagenaar, 2000 Wagenaar et al., 2023)” with “Poultry is frequently colonised with C. jejuni and/or C. coli and less common with other Campylobacter species”

Lines 107–108 and lines 118–119
In section *1.1.1. Poultry at the farm* the word “must” is used in “**Such samples must be prevented from drying out before culturing.**”, wheras in section *1.1.2. Cattle, sheep and pigs at the farm* the word “should” is used: “**they should be prevented from drying out.**” Is this intentionally?

Suggestion: be consistent and use the same word in these two situations.

LINES 122-124: Should neck skin sampling be mentioned alongside caeca?
For example: ‘To monitor levels of carcase contamination, samples of neck skin can also be collected from poultry carcases. These samples are usually pools of neck skin samples from at least three carcases and are taken using a sterile scalpel or scissors post-chilling. They can be submitted to the laboratory in a suitable container.’

LINES 127–128:
Please replace “utmost attention should be given to make sure that campylobacters do not die.” with “utmost attention should be given to maintain the viability of the campylobacters.”

LINES 134-136:

Propose to rewrite since the word ”accepted” relates to the specific harmonised protocol for isolation of Campylobacter for monitoring of antimicrobial resistance in the EU Member States and you may want to rephrase to give recommendations rather than strict directions.

Please replace “Transport to the laboratory and subsequent processing should therefore be as rapid as possible preferably the same day, but It is recommended to process the samples within 72 hours, but if not possible, storage of samples is accepted up to 96 hours (Tast Lahti et al., 2022) within at least 3 days. With “Transport to the laboratory and subsequent processing should therefore be as rapid as possible, preferably the same day but at least within 4 days after sampling (Tast Lahti et al., 2022).

LINES 139-140:

Suggest a broader interval to align with the EFSA harmonised protocol for isolation of Campylobacter from caecal samples for monitoring of antimicrobial resistance: 2-8 °C.

LINES 155-157:

Similarly to the previous comment. Please replace “It is recommended to process the samples within 72 hours, but if not possible, storage of samples is accepted up to 96 hours, whereby C. coli is more sensitive for long storage times than C. jejuni” with “If direct processing is not possible, it is recommended to analyse samples as soon as possible and at least within 96 hours after sampling.”

LINES 166-168 (and also line 201):

Enrichment can also be considered for environmental samples such as swabs and sock samples.

Please replace “Enrichment can be considered to enhance the culture sensitivity of potentially environmentally stressed organisms or in the case of low levels of organisms in faeces for example from cattle, sheep or pigs.” With “Enrichment can be considered to enhance the culture sensitivity in the case of low levels of organisms in faeces for example from cattle, sheep or pigs or in environmental samples such as swab and sock samples.”

LINEs 173–174:
According to ISO 10272:2017, the use of mCCDA is mandatory. Other media may be used in addition.

Please replace “is the most commonly recommended medium and is prescribed in the ISO standard, although alternative media may be used (ISO, 2017)” with “is the most commonly recommended medium and is prescribed in the ISO standard (ISO, 2017), although alternative media may be used”.

LINES 200-202: It's particularly important to update the ISO (10272-Part1) reference for this paragraph, as the amended version includes the addition of a growth supplement for Preston Broth that is not in the previous 2017 version.

LINES 228–229:
According to ISO 10272-1:2017, which includes detection of *Campylobacter* spp. in primary production samples, e.g. chicken caeca, in its scope, the incubation temperature is 41.5 C. Please consider to mention this and give the reference to the current standard in the text.

LINES 238–239:
Are there references for the description of the appearance on different agars? According to our experience, greyish, flat colonies with a tendency to spread may very well be the appearance of campylobacters not only on charcoal-based but also on blood-containing agars.

As an example, the colony morphology on Skirrow agar is described as follows in the short communication *Skirrow Campylobacter selective agar* available in *Handbook of Culture Media for Food Microbiology*, Volume 37, 2003. doi: 10.1016/S0079-6352(03)80092-9:

"Typical colonies are flat, glossy and effuse, with a tendency to form a spreading film if the agar is moist. Mature colonies are low convex, often tan coloured.**"**

LINE 252:

Missing the expected result. Please add: "Examine the plate for absence of growth of colonies"

After LINE 255:

Please add a 1.4.6 'PCR'

PCR can be used to confirm Campylobacter, e.g. a validated real-time PCR targeting the 16S rRNA gene of C. jejuni, C. coli and C. lari (ref ISO 10272:2017/Amd.1:2023, Ferrari et al. 2023)

LINE 260:

Also C. avium is hippurate positive.

Please replace "Generally, C. jejuni can be differentiated from other Campylobacter species on the basis of the hydrolysis
of hippurate as this is the only hippurate-positive species isolated from veterinary or food samples." With "Generally, C. jejuni can be differentiated from other

Campylobacter species on the basis of the hydrolysis of hippurate as this is the only commonly encountered hippurate-positive species isolated from veterinary or food samples."

LINE 261:

Table 2 was updated in the Amendment 1 of each part of ISO 10272:2017. The edits do not affect the table in this document, but you still may want to refer to the latest version. Please also align the table with the ISO standard and include the catalase test.

LINE 267: *C. coli* is negative for hydrolysis of Hippurate, the position of the mins in Table 2 should be corrected
Table 2 needs to be corrected as the content is correct but is not formatted the same throughout the table.

Lines 268-269 and Table 3

This text should be moved to after 1.4.4.

LINES 280-282

This text was updated in Amd1 to ISO 10272:2017 after conducting an ILS where it was discovered that the text was interpreted in several different ways.

Please replace "If indoxyl acetate is hydrolysed a colour change to dark blue occurs within 5–10 minutes. No colour change indicates hydrolysis has not taken place". with

"If the indoxyl acetate is hydrolysed, a colour change to blue occurs within 5 min to 10 min. If there is an unclear result after 10 min, a better result can be obtained after waiting for another 20 min. No colour change indicates hydrolysis has not taken place."

LINE 295 - 312: An extension of this chapter including genotyping of *Campylobacter* is suggested as whole genome sequencing techniques with subsequent bioinformatic data analysis are already established in many countries and are a powerful diagnostic tool:
Suggested new title: **"**1.6. Molecular detection, identification and genotyping of *Campylobacter"*
Suggested additional paragraph for this chapter:
"Whole genome sequencing of *Campylobacter jejuni* with subsequent bioinformatic data analysis is already established in many countries for *C. jejuni* genotyping, outbreak analyses and for the detection of genetic markers for certain phenotypes (antibiotic resistance and virulence) (El-Adawy et al., 2023). For sequencing and data analysis, the current version of the ISO 23418: 2022 guideline should be observed. DNA isolation and creation of sequencing libraries should be carried out according to the manufacturer's instructions. The data analysis begins with a quality control and the reads are assembled to predict contigs. The assembled genomes should have a

size of 1.4 to 1.7 MB and an N50 value of 15 kB. Based on the assembled genomes, genetic factors for resistance to antibiotics, virulence and mobile genetic elements can be detected by comparison with corresponding databases (e.g. AMRFinderPlus, VFDB, Plasmidfinder). For rough typing, the classic Multi Locus Sequence Typing (MLST, 7 genes) is possible. High-resolution typing can be carried out on the one hand by determining single nucleotide polymorphisms (SNPs). On the other hand, methods based on core-genome Multi Locus Sequence Typing (cgMLST) have become established."

Reference to be added, in case of acceptance:

- El-Adawy H, Hotzel H, García-Soto S, Tomaso H, Hafez HM, Schwarz S, Neubauer H, Linde J. (2023). Genomic insight into *Campylobacter jejuni* isolated from commercial turkey flocks in Germany using whole-genome sequencing analysis. Front Vet Sci. 10:1092179. doi: 10.3389/fvets.2023.1092179
- ISO 23418:2022, Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria General requirements and guidance, 1st Edition, P. 1-45

LINES 310-311

Complement with reference: ISO 10272:2017/Amd.1:2023.

LINE 323: A chapter on antimicrobial susceptibility testing of *Campylobacter* is missing, therefore we suggest to add the following paragraph:

"The antimicrobial susceptibility testing of *Campylobacter* to antibacterial agents can be carried out using various nationally or internationally recognized methods such as disc diffusion, agar diffusion, broth micro-dilution or the Epsilometer (E) test. The methods and interpretation criteria are based on the standard for antimicrobial susceptibility testing for bacteria of the Clinical and Laboratory Standards Institute (CLSI, 2015; CLSI; 2018)."

Reference to be added, in case of acceptance:

- CLSI. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI guideline M45 (ISBN 1-56238-917-3 [Print]; ISBN 1-56238-918-1 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2015.
- CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 4th ed. CLSI supplement VET08. Wayne, PA:, USA 2018.

LINES 373-376

Please replace "Microbiology of food and animal feeding stuffs" with "Microbiology of the food chain"

And, either updating the ISO 10272 reference to the latest version or add the amendments as separate references:

ISO 10272-1:2017/Amd.1:2023 and ISO 10272-2:2017/Amd.1:2023

3.10.8. Toxoplasmosis

General comment:

The EU can in general support this revised chapter. Specific comments and rationale are provided below.

Specific comments:

LINE 30: the term “morphologically” is appropriate in this context and should be maintained.

New suggested amendments:

LINES 84-85: Please replace “When a susceptible animal ingests sporulated oocysts, the sporozoites are released to penetrate the intestinal lining, become tachyzoites, and establish an infection.”

with

“When a susceptible animal ingests sporulated oocysts, the sporozoites are released to penetrate the intestinal lining. Once reached the underlying lamina propria, they differentiate into tachyzoites, which will spread the infection systemically”.

Justification: Sporozoite migration from invaded enterocytes to the cells of the lamina propria is crucial to allow tachyzoite development and replication in a suitable site for parasite dissemination”.

LINE 95 delete “main”

LINE 105 delete “and” before 29, replace “with” with “and”

LINE 110 Table first and second column replace “freedom” with “free” or rewrite the sentences using the term “Uninfected” instead of free

LINE 130 add “banana shaped” after “ovoid”

LINE 212 delete “validated”

LINE 292 add “gene” after B1, add “genomic region” after 529RE

LINE 309 replace “a” with “the”

LINES 341-343: Please replace **“***Toxoplasma gondii* can be grown and maintained in tissue culture by twice-weekly passage in African green monkey kidney (Vero) cells. Other cell lines (e.g. MARC145 cells) are also suitable. Cell lines are available from repositories (e.g. ATCC).”

with

“*Toxoplasma gondii* can be grown and maintained in tissue culture by twice-weekly passage in African green monkey kidney (Vero) cells. Other cell lines, e.g. human foreskin fibroblasts (HFF) and MARC145 cells are also suitable. Cell lines are available from repositories (e.g. ATCC).”

EU COMMENTS on the proposed changes to the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

Justification: human foreskin fibroblasts (HFF) are widely used and should be mentioned as they offer several advantages over Vero cells (no need to be frequently passaged because HFF do not overgrow; much easier observation of the intracellular tachyzoites).

LINE 410: Please replace “It is important to treat sera with 0.2 2-mercaptoethanol to avoid…”
with
“It is important to treat sera with 0.2 M 2-mercaptoethanol to avoid…”

LINE 459: Please replace ” In addition, severam recombinant antigens…”
with
”In addition, severaml recombinant antigens…”