Annex 2

Comments on Terrestrial Manual from P.R. China

CHAPTER 2.1.2.

BIOTECHNOLOGY ADVANCES IN THE DIAGNOSIS OF INFECTIOUS DISEASES

Line 29

Text as presented: 1.Nucleic acid extraction

General comments： In this part, more information about high throughput nucleic acid extraction should be mentioned. When treating field isolated samples, the number usually very huge, high throughput technologies are capable to help, it is mostly based on magnetic-beads which has technical maturity. The most advanced one called BOMB (Bio-On-Magnetic-Beads) is a high-throughput method for purification of plasmids, genomic DNA, RNA and total nucleic acid (TNA) from a range of bacterial, animal, plant, environmental and synthetic sources (Oberacker P, 2019). There are many commercial high throughput nucleic acid extraction paltform as well.

Reference: Oberacker P, Stepper P, Bond DM, Höhn S, Focken J, Meyer V, et al. (2019) Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation. PLoS Biol 17(1): e3000107. https://doi.org/10.1371/journal.pbio.3000107

CHAPTER 3.3.3.

AVIAN INFECTIOUS LARYNGOTRACHEITIS

Line 17-19

Text as presented: **Identification of the agent:** Virus isolation may be done by inoculation of suspected material on to the dropped chorioallantoic membrane of embryonated hens' eggs, or into avian embryonic cell cultures. These methods are time-consuming but sensitive.

Proposed alternative text: **dentification of the agent:** Virus isolation may be done by inoculation of suspected material on to the dropped chorioallantoic membrane of embryonated hens' eggs, or into avian embryonic cell cultures. These methods are time-consuming but ~~sensitive~~ remain useful approaches for ILT diagnosis.

Rationale : Virus isolation is not sensitive assay.

CHAPTER 3.3.4.

AVIAN INFLUENZA (INFECTION WITH HIGH PATHOGENICITY AVIAN INFLUENZA VIRUSES)

Line 775

Text as presented: **3.1. Vaccines available and their advantages**

General comment: A series of reverse genetic produced vaccines should be reviewed here. They are the most widely used AI vaccines.

CHAPTER 3.3.5.

AVIAN MYCOPLASMOSIS (*Mycoplasma gallisepticum, M. synoviae*)

Line 128-129

Text as presented: *Ornithobacterium rhinotracheale* and *Enterococcus* joint infections and, in chicken, from infectious tenosynovitis caused by reoviruses.

Proposed alternative text: *Ornithobacterium rhinotracheale* and *Enterococcus* joint

infections and, in chicken, from infectious tenosynovitis caused by ~~reoviruses~~ avian orthoreoviruses.

Rationale: reovirus was renamed as avian orthoreovirus.

CHAPTER 3.3.6.

AVIAN TUBERCULOSIS

Line 98-99

Text as presented: Among domestic animals (mammals), domestic pigs (*Sus scrofa* f. *domesticus*) are the most susceptible to avian tuberculosis.

Proposed alternative text: Among domestic animals (mammals), domestic pigs (*Sus scrofa* f. *domesticus*) are the most susceptible ~~to avian tuberculosis.~~ to be infected by *M. a. avium*

Rationale: It is rational that pigs are the most susceptible to be infected by *M. a. avium*, not avian tuberculosis

CHAPTER 3.4.10.

HAEMORRHAGIC SEPTICAEMIA (*PASTEURELLA MULTOCIDA* SEROTYPES 6:B AND 6:E)

Line 443-444

Text as presented: The test can be applied on direct culture, boiled cell lysate and infected tissues.

1.3.5 Real-time PCR and loop-mediated isothermal amplification (LAMP) assays

Proposed alternative text: The test can be applied on direct culture, boiled cell lysate and infected tissues.

1.3.5 Pasteurella multocida multiplex LPS PCR typing system

P. multocida strains can be classified into 16 somatic or lipopolysaccharide (LPS)

serovars (serotypes) using the Heddleston gel diffusion precipitin test. The genes required for LPS assembly has been identified. Multiplex PCR method was developed for LPS molecular typing based on LPS outer core structure genes. (Harper et al., 2015)

Primers:

BAP6119: 5'- ACA-TTC-CAG-ATA-ATA-CA-CCCG-3'

BAP6120: 5'- ATT-GGA-GCA-CCT-AGT-AAC-CC-3'

BAP6121: 5'- CTT-AAA-GTA-ACA-CTC-GCT-ATT-GC-3'

BAP6122: 5'- TTT-GAT-TTC-CCT-TGG-GAT-AGC-3'

BAP7213: 5'-TGC-AGG-CGA-GAG-TTG-ATA-AAC-CAT-C-3'

BAP7214: 5'- CAA-AGA-TTG-GTT-CCA-AAT-CTG-AAT-GGA-3'

BAP6126: 5'- CTT-TAT-TTG-GTC-TTT-ATA-TAT-ACC-3'

BAP6125: 5'- TTT-CCA-TAG-ATT-AGC-AAT-GCC-G-3'

BAP6129: 5'- AGA-TTG-CAT-GGC-GAA-ATG-GC-3'

BAP6130: 5'- CAA-TCC-TCG-TAA-GAC-CCC-C-3'

BAP7292: 5'- TCT-TTA-TAA-TTA-TAC-TCT-CCC-AAG-G-3'

BAP7293: 5'- AAT-GA-AGG-TTT-AAA-AGA-GAT-AGC-TGG-AG-3'

BAP6127: 5'- CCT-ATA-TTT-ATA-TCT-CCT-CCC-C-3'

BAP6128: 5'- CTA-ATA-TAT-AAA-CCA-TCC-AAC-GC-3'

BAP6133: 5'- GAG-AGT-TAC-AAA-AAT-GAT-CGG-C-3'

BAP6134: 5'- TCC-TGG-TTC-ATA-TAT-AGG-TAG-G-3'

Size of resulting fragments:

L1 (Heddleston Serovar 1 and14)	BAP6119/ BAP6120	1307bp
L2 (Heddleston Serovar 2/5)	BAP6121/ BAP6122	810bp
L3 (Heddleston Serovar 3 and 4)	BAP7213/ BAP7214	474bp
L4 (Heddleston Serovar 6 and 7)	BAP6126/ BAP6125	550bp
L5 (Heddleston Serovar 9)	BAP6129/ BAP6130	1175bp
L6 (Heddleston Serovar 10, 11, 15, and 12)	BAP7292/ BAP7293	1668bp
L7 (Heddleston Serovar 8 and 3)	BAP6127/ BAP6128	931bp

L8 (Heddleston Serovar 16) BAP6133/ BAP6134 255bp

PCR conditions:

Each of the final LPS-mPCRs (50- µ l final volume) was performed in 1×Taq polymerase buffer (10 mM Tris-HCl, 1.5 mM MgCl, 2, 50 mM KCl) containing 0.4 M each primer (Table 2), 0.2 mM deoxynucleoside triphosphates (dNTPs), and 1.7 U Taq polymerase. For each colony PCR, material from 2 to 3 well-isolated P. multocida colonies was collected using a sterile tip on a 20 µl micropipette (volume set at 20 µl) inserted into the middle of each colony. The collected material was then added to a 50-µl PCR mixture and mixed thoroughly by pipetting. For PCR using genomic DNA, approximately 50 ng of column-purified DNA was added to each PCR mixture. All reaction mixtures were mixed briefly then centrifuged (10 s, 13,000 ×g). For colony PCR, the cycling conditions were 96°C for 10 min, followed by 30 cycles of 96°C for 30 s, 52°C for 30 s, and 72°C for 2.5 min, with a final extension at 72°C for 5 min. For PCR using genomic DNA as the template, the cycling conditions were identical to those in the colony PCR, except that the initial denaturation step at 96°C was reduced to 5 min.

1.3.~~5~~ 6 Real-time PCR and loop-mediated isothermal amplification (LAMP) assays

Rationale： A multiplex PCR method has been established to replace the Heddleston serotyping (somatic typing). Thus, it is suggested to introduce this method.

Reference： Harper, M., John, M., Turni, C., Edmunds, M., St, M.F., Adler, B., Blackall, P.J., Cox, A.D., Boyce, J.D. (2015). Development of a rapid multiplex PCR assay to genotype Pasteurella multocida strains by use of the lipopolysaccharide outer core biosynthesis locus. *J CLIN MICROBIOL*, 53, 477-485.

Line 477-484

Text as presented:

Multilocus sequence typing (MLST), a sequence-based typing system based on seven housekeeping genes has been used to identify strain diversity of bovine isolates of *P. multocida* (Davies *et al.*, 2004). MLST of 55 isolates of *P. multocida* associated with

HS found that the majority include sequence type (ST) 122 (*n* = 50), and rarely ST63, ST147 and ST162 in comparison with other members of the species isolated from different lesion types and hosts (Petersen *et al.*, 2014). However, these techniques are largely used for research purposes and require specialised equipment. Moreover these profiles are not unique to country of origin or host species.

Proposed alternative text: Multilocus sequence typing (MLST), a sequence-based typing system based on seven housekeeping genes has been used to identify strain diversity of bovine isolates of *P. multocida* (Davies *et al.*, 2004). There were two MLST schemes (Rural Industries Research and Development Corporation (RIRDC) MLST and Multi-host MLST) for P. multocida MLST (Davies et al., 2004, Subaaharan et al., 2010). MLST database of P. multocida (https://pubmlst.org/) has been develop for for global and long-term epidemiology studies. RIRDIC. MLST of 55 isolates of *P. multocida* associated with HS found that the majority include sequence type (ST) 122 (*n* = 50), and rarely ST63, ST147 and ST162 in comparison with other members of the species isolated from different lesion types and hosts (Petersen *et al.*, 2014). RIRDC Multilocus sequence typing of a global collection of P. multocida isolates from cattle andother host species demonstrates Clonal Complex 13 was associated with bovine respiratory (Hotchkiss et al., 2011). ~~However, these techniques are largely used for research purposes and require specialised equipment. Moreover these profiles are not unique to country of origin or host species.~~

Rationale: Molecular typing based on gene sequences provides definitive and objective results. Especially, MLST provides higher discriminatory power, and can be used to analyze the relationship of worldwide clinical isolates. MLST is considered to be the most appropriate technology for global and long-term epidemiological research, and is being used by more and more laboratories. Therefore, it is suggested that this part be stated separately. Two MLST schemes have been developed (RIRDC MLST and Multi-host MLST) for P. multocida, which both have been successfully used in clinical practice. Therefore, both methods should be mentioned.

Reference: 1. Subaaharan, S., Blackall, L.L., Blackall, P.J. （2010）, Development of a multi-locus sequence typing scheme for avian isolates of Pasteurella multocida. *VET Microbiology* 141, 354-361.

2. Hotchkiss, E.J., Hodgson, J.C., Lainson, F.A., Zadoks, R.N. (2011), Multilocus sequence typing of a global collection of Pasteurella multocida isolates from cattle and other host species demonstrates niche association. *BMC Microbiology*, 11, 115.

CHAPTER 3.6.2.

RABBIT HAEMORRHAGIC DISEASE

Line 46

Text as presented: Although the capsid RHDVs has been expressed as recombinant VPLs,

Proposed alternative text: Although the capsid RHDVs has been expressed as recombinant ~~VPLs,~~ VLPs,

Rationale: There is a spelling error here. The VLPs is the abbreviation of virus-like particles，as showed in line 41 in the manuscript.

Line 301-311

Text as presented: ~~HA is less evident or non-existent when RBCs of other species are used. Washed RBCs are suspended at 0.75% in PBS. A twofold dilution of the clarified supernatant of a 10% tissue homogenate of liver or spleen is incubated with an equal volume of washed RBCs in a sealed round-bottom microtitre plate at, preferably, 4°C. After 1 hour (range from 20 minutes to 2 hours) of incubation, agglutination at an end-point dilution of >1/160 is considered to be positive. Lower titres should be regarded as suspicious and should be checked using other methods. Around 10% of samples found to be positive by ELISA or EM give negative results in HA (HA false-negative). Some RHD isolates may exhibit temperature-dependent differences in haemagglutinating characteristics and could show HA activity only~~

~~when the test is performed at 4°C. Nevertheless, the HA false negativity is mainly detected in organs of rabbits showing a subacute/chronic form of the disease and it depends on the characteristics of the VLPs.~~

Proposed alternative text: HA is less evident or non-existent when RBCs of other species are used. Washed RBCs are suspended at 0.75% in PBS. A twofold dilution of the clarified supernatant of a 10% tissue homogenate of liver or spleen is incubated with an equal volume of washed RBCs in a sealed round-bottom microtitre plate at, preferably, 4°C. After 1 hour (range from 20 minutes to 2 hours) of incubation, agglutination at an end-point dilution of >1/160 is considered to be positive. Lower titres should be regarded as suspicious and should be checked using other methods. Around 10% of samples found to be positive by ELISA or EM give negative results in HA (HA false-negative). Some RHD isolates may exhibit temperature-dependent differences in haemagglutinating characteristics and could show HA activity only when the test is performed at 4°C. Nevertheless, the HA false negativity is mainly detected in organs of rabbits showing a subacute/chronic form of the disease and it depends on the characteristics of the VLPs.

Rationale: HA test is a rapid and convenient method for clinical diagnosis. Although considering the risk from working with human red cells, this method has been replaced by ELISA in Europe, the HA test is still the widely used method in developing countries. Safe and regular human red cells can be obtained by purchase. In addition, the HA test has also been described in part C 2.2.3 and 2.2.4 (page 15 line 714 and page 16 line 741) in the manuscript, so we suggest to keep the HA test here.

Line 431

Text as presented: ~~2.1. Haemagglutination inhibition~~

Proposed alternative text: 2.1. Haemagglutination inhibition

Rationale: HI test is a rapid, convenient and valuable method for the serological diagnosis of RHDV. Considering the HI titer of serum is associated with the immune effects of RHDV vaccine, it is recommend to keep the HI test here.

Line 908-910

Text as presented: This type of recombinant vaccine, i.e. a modified *Myxoma* virus expressing the main RHDV protein, has been developed and registered, and is commercially available in several countries for administration by the parenteral route.

Proposed alternative text: This type of recombinant vaccine, i.e. a modified Myxoma virus expressing the main RHDV protein, has been developed and registered, and is commercially available in several countries for administration by the parenteral route. Importantly, a recombinant baculovirus expressing VP60 has been registered and is commercially available in China for administration by the subcutaneous route.

Rationale: The RHD recombinant subunit vaccine has been registered and commercially used in China. It is recommended that this commercially vaccine of RHD should be known with global rabbit industry colleagues.

CHAPTER 3.7.8.

OVINE PULMONARY ADENOCARCINOMA

(adenomatosis)

General comments: Overall, CHAPTER 3.7.8. is much improved by the revision, which has introduced the updated knowledges about the diagnostic techniques for diagnosis of OPA, in particular PCR and immunohistochemistry. However, the revised chapter failed to mention use of RT-PCR for detection of viral RNA. JSRV is a beta retrovirus which belongs to the RNA virus family *Retroviridae*. These viruses encode a reverse transcriptase enzyme that converts the viral RNA genome into viral DNA during the retroviral life cycle, which then becomes integrated into the host genome. Therefore, both viral DNA or RNA can be detected in tumour, draining lymph nodes, lung fluid and peripheral blood mononuclear cells from OPA-affected sheep. A previous study demonstrated that lung fluid from different OPA cases contained between 10^7 and 1010 copies of JSRV RNA per ml (Christina Cousens et al 2009). A quantitative RT-PCR (qRT-PCR) (Cousens C et al 2009, Lee AM et al 2017) and

RT-PCR based on primers derived from the env of the JSRV (Zhang K et al 2014) have been developed, which demonstrated that RT-PCR detect viral RNA in several tissues. It would be, therefore, very useful to include this additional information about RT-PCR for diagnosis of OPA in the Chapter.

Reference ： 1. Christina Cousens et al 2009. Jaagsiekte sheep retrovirus is present at high concentration in lung fluid produced by ovine pulmonary adenocarcinoma-affected sheep and can survive for several weeks at ambient temperatures. Research in Veterinary Science 87: 154–156.

2. Keshan Zhang et al 2014 Diagnosis and phylogenetic analysis of ovine pulmonary adenocarcinoma in China. Virus Genes 48:64–73 DOI 10.1007/s11262-013-0988-x.

3. Lee AM et al 2017 First confirmation by PCR of Jaagsiekte sheep retrovirus in Ireland and prevalence of ovine pulmonary adenocarcinoma in adult sheep at slaughter. Ir Vet J. 2017 Dec 19;70:33. doi: 10.1186/s13620-017-0111-z. eCollection 2017.

Line 79-81

Text as presented: These can detect JSRV in several tissues, including peripheral blood mononuclear cells ~~of unaffected in-contact sheep from flocks with OPA~~, from OPA-affected sheep,

Proposed alternative text: These can detect JSRV in several tissues, including peripheral blood mononuclear cells and lung fluid ~~of unaffected in-contact sheep from flocks with OPA~~, from OPA-affected sheep,

Rationale: In many OPA cases the tumours secrete large amounts of a sero-mucous fluid (lung fluid) which runs from the nostrils when the sheep's head is lowered. Lung fluid contains infectious JSRV which is an important source for virus transmission within sheep flocks because lung fluid from different OPA cases contained between 107 and 1010 copies of JSRV RNA per ml (Cousens et al 2009). Therefore, examination of molecular assays on lung fluid samples is a useful tool in diagnosis of OPA (Cousens et al 2009; K.Voig et al 2007).

CHAPTER 3.7.9.

PESTE DES PETITS RUMINANTS (INFECTION WITH PESTE DES PETITS RUMINANTS VIRUS)

Line 361-363

Text as presented: The seed should either have passed an innocuity test in animals (rodents, sheep and goats) or have been shown to be free of harmful contaminants by other validated means (e.g. deep sequencing);

Proposed alternative text: The seed should either have passed an innocuity test in animals (rodents, sheep and goats) or have been shown to be free of harmful contaminants by other validated means ~~(e.g. deep sequencing)~~;

Rationale: Deep sequencing technology, also referred as high-throughput sequencing, is not a validated assay to detect harmful contaminants in vaccine seed. Furthermore, it is very complicated and expensive, so it is also not practical to use such technology to screen any potential contaminating pathogen.

Line 427

Text as presented: g) Read the plate after 7–10 days of incubation.

Proposed alternative text: Change medium in plate with maintenance medium containing 2% (v/v) fetal calf serum every 2-3 days and Read the plate after 7–10 days of incubation.

Rationale: PPRV grows slowly in the cells invitro. Normally clear CPE can be observed only after 5 days of incubation at least. So, in order to keep these inoculated cells alive before appearance of CPE, cells medium should be replaced with fresh maintenance medium at a regular interval of 2-3 days.

CHAPTER 3.8.1.

AFRICAN SWINE FEVER (INFECTION WITH AFRICAN SWINE FEVER VIRUS)

Line 17-18

Text as presented: Animals that have recovered from either acute or chronic infections may potentially become persistently infected, acting as virus carriers.

Proposed alternative text: Animals that have recovered from either acute or subacute or chronic infections may potentially become persistently infected, acting as virus carriers.

Rationale: The ASF clinical syndromes vary from peracute, acute, subacute to chronic, depending on the virulence of the virus and any pig which has recovered from infections could potentially become persistently infected no matter it is acute, subacute or chronic

CHAPTER 3.9.5.

CYSTICERCOSIS (including infection with *Taenia solium*)

Line 93

Text as presented: Recently, seizures have been observed in pigs naturally infected with *T. solium* (Trevisan *et al.*, 2016).

Proposed alternative text: Recently, central nervous system syndromes, including seizures, depressed spirit have been observed in pigs naturally infected with *T. solium* (Trevisan*et al.*, 2016; Dzikowiec et al., 2017).

Rationale： Epilepsy is only one of the neurological symptoms, central nervous system diseases have been reported occasionally.

Reference: Dzikowiec M, Góralska K, Błaszkowska J. Neuroinvasions caused by parasites. Ann Parasitol. 2017;63(4):243–253.

Line 468-470

Text as presented: The site of *T. multiceps* cysts in sheep brain might be identified by clinical signs presented and, possibly, softening of the skull overlying the coenurus.

Proposed alternative text: The site of *T. multiceps* cysts in sheep brain might be identified by clinical signs presented and, possibly, softening of the skull overlying the coenurus. Sometimes, the coenurus could be observed at the superficial surfaces of affected muscles, especially in the neck region.

Rationale: In addition to the brain, there are also clinical manifestations on the surface of muscles and other locations.