**USA COMMENTS IN RED FONT**

# CHAPTER 12.7.**~~EQUINE PIROPLASMOSIS~~ *INFECTION* with *Theileria equi* AND *Babesia caballi*(Equine piroplasmosis)**

Article 12.7.1.

**General provisions**

The *infection* with use of the term equine piroplasmosis indicates clinical diseases caused by the transmission of *Theileria equi (T. equi)* or *Babesia caballi (B. caballi)* established after transmission of these pathogenic agents through competent ticks or iatrogenic practices may be asymptomatic or may cause a clinical disease known as equine piroplasmosis. Vertical transmission from mares to foals has also been reported. This chapter deals not only with the occurrence of clinical disease signs caused by infection with T. equi or B. caballi, but also with asymptomatic infections the presence of infection with T. equi or B. caballi in the absence of clinical signs.

Susceptible animals for *infection* with *T*. *equi* or *B*. *caballi* are primarily domestic and *wild* equids. Although old-world camelids are susceptible to *infection* and are potential reservoirs, they are not found to play a significant role in the epidemiology of the disease.

Equids infected with *T. equi* or *B. caballi* may remain carriers of these blood parasites for long periods, sometimes lifelong and act as sources of *infection* for competent Ixodid tick *vectors* ~~of~~ including the genera *Dermacentor, Rhipicephalus,* *Hyalomma* and *Amblyomma*.

**RATIONALE:** There are other tick genera that have been demonstrated to be competent vectors (Onyiche et al, 2019). It is misleading to limit the list to only these tick genera when there are other documented competent Ixodid vectors. Previous feedback from Code Commission noted that the Terrestrial Manual only had these vectors listed so did not want to conflict. Suggest that until the Terrestrial Manual can be updated, to avoid conflicting with the Terrestrial Manual, generalize as edited here.

For the purposes of the *Terrestrial Code*, the following defines *infection* with *T. equi* or *B. caballi*:

1) identification of the parasite by microscopic examination of a sample from an equid which may be showing clinical or pathological signs consistent with *infection* with *T. equi* or *B. caballi* or epidemiologically linked to a confirmed or suspected *case* of *infection* with *T. equi* or *B. caballi*; or

2) antigen or genetic material specific for *T. equi* or *B. caballi* has been identified in a sample from an equid which may be showing clinical or pathological signs consistent with *infection* with *T. equi* or *B. caballi* or epidemiologically linked to a confirmed or suspected *case* of *infection* with *T. equi* or *B. caballi*; or

3) antibodies specific to *T. equi* or *B. caballi* have been identified in a sample from an equid which may be showing clinical or pathological signs consistent with *infection* with *T. equi* or *B. caballi* or epidemiologically linked to a confirmed or suspected *case* of *infection* with *T. equi* or *B. caballi.*

For the purposes of the *Terrestrial Code*, the *incubation period* of *infection* with *T. equi* or *B. caballi* in equids shall be 30 days and the *infective period* shall be lifelong.

For the purposes of this chapter, a temporary importation refers to the introduction of ~~equids~~horses into a country or *zone*, for a defined period of time, not exceeding 90 days, during which the *risk* of transmission of the *infection* is mitigated through specific measures under the supervision of the *Veterinary Authority.* Temporarily imported horses are re-exported ~~or slaughtered~~ at the end of this period. The duration of the temporary importation period and the destination after this period, as well as the conditions required to leave the country or *zone,* should be defined in advance.

When authorising import or transit of the *commodities* listed in this chapter, with the exception of those listed in Article 12.7.2. *Veterinary Authorities* should require the conditions prescribed in this chapter relevant to the status of *infection* with *T. equi* and *B. caballi* of the *exporting* *country* or *zone.*

Standards for diagnostic tests ~~and vaccines~~ are described in the *Terrestrial Manual*.

Article 12.7.2.

**Safe commodities**

When authorising importation or transit of the following *commodities*, *Veterinary Authorities* should not require any ~~conditions related with~~ *infection* with *T. equi* or *B. caballi-*related conditions, regardless of the *~~infection~~* health status of the *animal population* of the *exporting country* or *zone*:

1) *milk* and *milk products*;

2) *meat* and *meat products*;

3) hides and skins;

4) hooves;

5) gelatine and collagen;

6) semen collected;

7) sterile filtered horse serum;

8) embryos collected, processed and stored in accordance with Chapters 4.9. and 4.10.

Article 12.7.3.

**Country or zone free from infection with *T. equi* and *B. caballi***

1) Historical freedom as described in Chapter 1.4. does not apply to *infection* with *T. equi* and *B. caballi.*

2) A country or a *zone* may be considered free from *infection* with *T. equi* and *B. caballi* when:

a) *infection* with *T. equi* and infection with *B. caballi* have been notifiable diseases in the entire country for at least the past 10 years and, in the country or *zone*:

~~EITHER:~~

i) there has been no *case* of *infection* with *T. equi* and no *case* of *infection* with *B. caballi* during the past six years; and

ii) a *surveillance* programme performed in accordance with Article 12.7.9. has demonstrated no evidence of *infection* with *T. equi* and no evidence of *infection* with *B. caballi* in the past six years and has considered the presence or absence of competent *vectors* in the epidemiological situation;

~~OR~~

~~iii)~~ ~~an ongoing~~ *~~surveillance~~* ~~programme performed in accordance with Article 12.7.9. has found no competent tick~~ *~~vectors~~* ~~for at least six years;~~

b) imports of equids into the country or *zone* are carried out in accordance with this chapter. A country or *zone* free from *infection* with *T. equi* and *B. caballi* in which an epidemiological investigation has been conducted with favourable results ~~ongoing~~ *~~vector surveillance~~*~~, performed in accordance with Article 12.7.9., has found no competent tick~~ *~~vector~~* will not lose its free status through the introduction of seropositive or infective equids imported temporarily in accordance with Article 12.7.6.;

c) a country or *zone* free from *infection* with *T. equi* and *B. caballi* adjacent to an infected country or *zone* should include a high-risk area in which ~~continuous serological, agent and~~ *~~vector~~ surveillance* is conducted in accordance with Article 12.7.9.

Article 12.7.4.

**Recovery of a free status**

When *infection* with *T. equi* or *B. caballi* is detected in a previously free country or *zone*, Article 12.7.3. applies.

Article 12.7.~~2~~5.

**Recommendations for the importation of ~~equines~~ equids**

*Veterinary Authorities* ~~of~~*~~importing countries~~* should require the presentation of an *international veterinary certificate* attesting that ~~the animals~~:

1) the animals showed no clinical signs ~~equine piroplasmosis~~ of *infection* with *T. equi* or *B. caballi* on the day of shipment, and

2) EITHER:

a) the animals were kept in a country or *zone* free from *infection* with *T. equi* and *B. caballi* since birth;

OR

~~2)~~ ~~were subjected to diagnostic tests for equine piroplasmosis (Theileriaequi and Babesia caballi) with negative results during the 30 days prior to shipment;~~

b) i) were subjected to a serological ~~or and agent identification~~ test validated to international standards ~~with molecular techniques~~ for the detection of *T. equi* and *B. caballi* with negative results carried out on a blood sample taken within the 14 days prior to shipment; and

**RATIONALE:** The Terrestrial Manual Chapter 3.6.8. Equine Piroplasmosis states: “in carrier animals, low parasitaemias make it extremely difficult to detect parasites, especially in the case of B. caballi infections, although they may sometimes be demonstrated by using a thick blood smear technique,” and that molecular techniques “have been developed and continue to expand.” The chapter goes on to classify PCR as the recommended method (+++) for detection of the agent for all purposes, but in a footnote recommends the use of a combination of agent identification methods on clinical samples, presumably due to known sampling and sensitivity issues. For detection of immune response, only two assays are presented in the evaluation chart: the IFA and cELISA; the US and other countries have demonstrated that the complement fixation (CF) assay to be instrumental in detecting early infections that would have otherwise been classified negative if performing cELISA or PCR only. The IFA, although reportedly a sensitive test, is a highly subjective, is a low throughput procedure, therefore it is only performed by the USDA APHIS National Veterinary Services Laboratory (NVSL) for anticomplementary samples or as needed to meet export requirements. The chapter continues:

Negative results in agent identification or serological tests do not necessarily mean that the animals are free from infections. In persistently infected carrier animals, the parasites may be sequestered in organs…while the parasites and their genetic materials are undetectable…Similarly, during the early stage of infections, horses may be seronegative…while such animals may be positive by PCR assays (Abedi et al., 2014; Posada-Guzman et al., 2015).

Abedi et al (2014) evaluated only IFA and multiplex conventional PCR. The authors found these assays to be “in moderate agreement without significant differences in results.” They propose that samples they determined to be PCR negative, but IFA positive, may have been drawn during early infection, attributing this conclusion to Farkas et al (2013). Abedi states that the Farkas’ study “showed that blood samples drawn from horses at the onset of infection, before antibodies developed, were responsible for seronegative IFA results;” however, this statement is, itself, speculation introduced by Farkas, et. al., in their discussion. The actual statement in Farkas, et. al., (2013) is as follows, “DNA of T. equi was detected in five seronegative horses which most likely have become recently infected, i.e., blood samples were taken before specific antibodies developed in the animals.” The sampling strategy, experimental design, and analyses of these studies were not formulated to address these claims.

Posada-Guzman, et. al., (2015) evaluated cELISA (T. equi and B. caballi), blood smear, and PCR; and the sampling strategy prioritized horses that were clinically ill. Out of the 130 horses analyzed by cELISA, 124 (95.4%) were positive, and only 1 and 7 samples were cELISA negative/PCR positive for B. caballi and T. equi, respectively. The authors propose that “horses with positive PCR and negative ELISA results represent animals with recent infections.”

 USDA APHIS’S experience with diagnostic testing and research, direct molecular detection of the causative agents of equine piroplasmosis in positive horses sometimes lags behind serological detection during early stages of infection, and PCR is the first diagnostic method to lose signal in chronic illness or during early stages of treatment. This has been observed across positive horses identified on importation, under USDA APHIS quarantine, and experimentally infected for reagent production. The seronegative/PCR positive horses in the aforementioned studies were likely horses that would have been classified as equine piroplasmosis reactors on complement fixation testing.

USDA APHIS began investigating the utility of PCR for equine import testing in 2020 and has noted PCR to often be discordant with serological testing. To highlight the potential for false negative results if using only cELISA and/or PCR, we have determined that out of 13 CF positive/ELISA negative horses, 1 horse was PCR positive and yielded piroplasmosis organisms on in vitro parasite culture; and 4 horses were PCR negative, 2 of which yielded organisms on culture. There is no PCR that has been validated to meet the WOAH international standards for the detection of T. equi or B. caballi for the purpose of clearing individual equines for movement, and USDA APHIS communications with the Yokoyama laboratory group (WOAH reference laboratory for equine piroplasmosis) have confirmed this interpretation. The omission of complement fixation testing from the chart summary in Chapter 3.6.8 and the brief description of the method in section 2.3 are not supported by USDA APHIS experience and research. The chapter presents the false dichotomy of complement fixation testing versus cELISA, despite the evident solution of parallel testing on both assays, which has provided excellent coverage of both acute and chronic cases of equine piroplasmosis in the USA. The added reasoning that antigen production for complement fixation testing necessitates experimental infection of horses seems beside the point of whether the test performs well for the purpose for which it was validated, especially as many other WOAH recommended tests and reagents require similar animal work.

In conclusion, USDA APHIS does not support the performance of unvalidated PCR for clearance of animals for exportation or movement clearance, and we do not support the replacement of the complement fixation test in the list of recommended tests with PCR or IFA.

References:

[Abedi, Vali, et al. "Molecular and serological detection of *Theileria equi* and *Babesia caballi* infection in horses and ixodid ticks in Iran." Ticks and tick-borne diseases 5.3 (2014): 239-244.](https://www.sciencedirect.com/science/article/abs/pii/S1877959X14000223?via%3Dihub)

[Farkas, R., et al. "Serological and molecular detection of *Theileria equi* infection in horses in Hungary." Veterinary parasitology 192.1-3 (2013): 143-148.](https://www.sciencedirect.com/science/article/abs/pii/S0304401712005316)

[Posada-Guzmán, María Fernanda, et al. "Detection of *Babesia caballi* and *Theileria equi* in blood from equines from four indigenous communities in Costa Rica." Veterinary Medicine International 2015 (2015).](https://www.hindawi.com/journals/vmi/2015/236278/)

~~3)~~ ~~were maintained free from ticks, by preventive treatment when necessary, during the 30 days prior to shipment.~~

ii) were maintained free from competent ticks in accordance with Article 12.7.7. and not subjected to any practice that may present a risk of iatrogenic transmission of *infection* with *T. equi* or *B. caballi* during the 30 days prior to sampling and after sampling until shipment ~~and throughout the transport to the destination country or~~ *~~zone~~*.

Article 12.7.~~3~~6.

**Recommendations for the temporary importation of ~~equids~~ horses ~~of competition horses on a temporary basis~~**

~~Veterinary Authorities of importing countries should consider the possibility of importing competition horses on a temporary basis and which are positive to the testing procedure referred to in point 2) of Article 12.7.2. under the following safeguards:~~

If the importation of ~~equids~~horses on a temporary basis does not comply with the recommendations in Article 12.7.5., *Veterinary Authorities* of *importing countries* should:

~~1.~~

1) require ~~that~~:

a) ~~the~~ ~~horses are~~ the ~~animals~~horses be accompanied by a passport in accordance with the model contained in Chapter 5.12. or be individually identified as belonging to a high health status *subpopulation* as defined in Chapter 4.17.;

~~2.~~b) ~~the Veterinary Authorities of importing countries require~~ the presentation of *an international veterinary certificate* attesting that the ~~animals~~horses:

~~a.~~i) showed no clinical sign of ~~equine piroplasmosis~~ *infection* with *T. equi* or *B. caballi* on the day of shipment;

~~b)~~ ~~were treated against ticks within the seven days prior to shipment;~~

ii) were maintained free from ticks in accordance with Article 12.7.7. during the 30 days prior to shipment and during transport;

c) the duration of the temporary importation period and the destination after this period, as well as the conditions required to leave the country or *zone,* be defined;

~~3)~~ ~~the horses are kept in an area where necessary precautions are taken to control ticks and that is under the direct supervision of the Veterinary Authority;~~

~~4)~~ ~~the horses are regularly examined for the presence of ticks under the direct supervision of the Veterinary Authority.~~

2) ensure that during their stay in the country or *zone*:

a) the ~~animals~~horses are protected from ticks in accordance with Article 12.7.7.;

b) ~~equids~~horses are examined daily for the presence of competent Ixodid tick vectors ~~of~~ including the genera *Dermacentor*, *Rhipicephalus*, *Hyalomma* and *Amblyomma* with particular attention to the ears, false nostrils, inter-mandibular space, mane, lower body areas, including the axillae, and inguinal region, and the perineum and tail, with negative results;

**RATIONALE**: There are other tick genera that have been demonstrated to be competent vectors (Onyiche et al, 2019). It is misleading to limit the list to only these tick genera when there are other documented competent Ixodid vectors. Previous feedback from Code Commission noted that the Terrestrial Manual only had these vectors listed so did not want to conflict. Suggest that until the Terrestrial Manual can be updated, to avoid conflicting with the Terrestrial Manual, generalize as edited here.

c) the ~~animals~~horses are not subjected to any practice that may represent a risk of iatrogenic transmission of *infection* with *T. equi* or *B. caballi*.

Article 12.7.7.

**Protecting equids from ticks**

Under the direct supervision of the *Veterinary Authority*:

1) equids are kept in tick-protected facilities and transported in protected ~~vehicles~~ *vehicles/vessels* according to Article 12.7.8.;

2) equids have been preventively treated according to the manufacturer's recommendations with an acaricide effective against the competent ticks.

Article 12.7.8.

**Protecting facilities and transports from ticks**

The *establishment* or facility should be approved by the *Veterinary Authority* and the means of protection should at least comprise the following:

1) measures to limit or eliminate habitats for competent tick *vectors* should be implemented for an appropriate time and over an appropriate distance in the vicinity of the area where equids are kept;

2) the facility and immediate surroundings of the stables and exercise or competition areas should be treated with an effective acaricide before the arrival of equids;

3) when transporting ~~animals~~ equids through infected countries or *zones*:

a) the vehicle should be treated with an effective acaricide before transporting the animals;

b) preventive treatment of the equids with an acaricide with an extended residual effect that lasts at least for the duration of any stopover during the trip should be conducted.

Article 12.7.9.

**Surveillance strategies**

1. General principles of surveillance

A Member Country should justify the *surveillance* strategy chosen as being adequate to detect the presence of *infection* with *T. equi* and the presence of *infection* with *B. caballi*, ~~even in the absence of clinical signs,~~ given the prevailing epidemiological situation in accordance with Chapter 1.4. and Chapter 1.5. and under the responsibility of the *Veterinary Authority*.

An active programme of *surveillance* of equids to detect evidence of *infection* with *T. equi* and evidence of *infection* with *B. caballi* by serological or agent identification molecular testing is required to establish the status of a country or *zone* considering that asymptomatic carriers play an important role in the maintenance and transmission of the *infection*.

The *Veterinary Services* should implement programmes to raise awareness among *veterinarians*, horse owners, riders and workers who have day-to-day contact with equids, as well as *veterinary paraprofessionals* and diagnosticians, who should report promptly any suspicion of *infection* with *T. equi* and any suspicion of *infection* with *B. caballi* to the *Veterinary Authority.*

Under the responsibility of the *Veterinary Authority*, Member Countries should have in place:

‒ a formal and ongoing system for detecting and investigating cases;

‒ a procedure for the rapid collection and transport of samples from suspected cases of *infection* with *T. equi* or *B. caballi* to a laboratory for diagnosis;

‒ a system for recording, managing and analysing diagnostic and surveillance data.

2. Clinical surveillance

Clinical *surveillance* aims at detecting clinical signs by close physical examination of equids.

3. Serological and agent surveillance

An active programme of *surveillance* of equids to detect evidence of *infection* with *T. equi* and evidence of *infection* with *B. caballi* by serological or agent identification test with molecular techniques is required to establish the status of a country or *zone* considering that asymptomatic carriers play an important role in the maintenance and transmission of the *infection*.

The study population used for a serological survey should be representative of the population at risk in the country or *zone*.

4. Surveillance in high-risk areas

Disease-specific enhanced *surveillance* in a free country or *zone* should be carried out over an appropriate distance from the border with an *infected* country or *zone*, based upon geography, climate, history of *infection* and other relevant factors. The *surveillance* should be carried out particularly over the border with that country or *zone* unless there are relevant ecological or geographical features likely to limit the spatial distribution and thereby prevent the *infestation* of equids from competent ticks and interrupt the transmission of *infection* with *T. equi* or *B. caballi*.

5. Vector surveillance

*Infection* with *T. equi* or *B. caballi* is transmitted between equine hosts by species of competent Ixodid ticks including the genera *Dermacentor*, *Rhipicephalus*, *Hyalomma,* and *Amblyomma.*

**RATIONALE:** There are other tick genera that have been demonstrated to be competent vectors (Onyiche et al, 2019). It is misleading to limit the list to only these tick genera when there are other documented competent Ixodid vectors. Previous feedback from Code Commission noted that the Terrestrial Manual only had these vectors listed so did not want to conflict. Suggest that until the Terrestrial Manual can be updated, to avoid conflicting with the Terrestrial Manual, generalize as edited here.

*Vector surveillance* is aimed at demonstrating the absence of tick *vectors* or defining high, medium and low-risk areas and local details of seasonality by determining the various species present in an area, their respective seasonal occurrence, and abundance. *Vector surveillance* has particular relevance to potential areas of spread. Long term *surveillance* can also be used to assess *vector* abatement measures or to confirm the continued absence of *vectors*.

*Vector surveillance* sampling should be scientifically based. The choice of surveillance methods ~~the number and types of traps~~ to be used in *vector surveillance* and the frequency of their use should consider the size and ecological characteristics of the area to be surveyed as well as the biology and behavioural characteristics of the local *vector* species of Ixodid ticks.

**RATIONALE:** To allow for surveillance methods other than traps.

The use of a *vector surveillance* system to detect the presence of circulating *T. equi* or *B. caballi* is not recommended as a routine procedure. Animal-based *surveillance* strategies are preferred to detect *T. equi* or *B. caballi* transmission than entomological *surveillance*.

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