Terrestrial Animal Health Standards Commission Report

October 2008

USA Comments

CHAPTER 4.5.

COLLECTION AND PROCESSING OF BOVINE, SMALL RUMINANT AND PORCINE SEMEN

Article 4.5.1.

General considerations

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Article 4.5.2.

Conditions applicable to testing of bulls and teaser animals

Bulls and teaser animals should only enter an *artificial insemination centre* if they fulfil the following requirements.

1. Pre-quarantine

   The *animals* should comply with the following requirements prior to entry into isolation at the *quarantine station*.

   a) …

   e) Bluetongue

      The *animals* should comply with Articles 8.3.5., 8.3.6. or 8.3.7., depending on the bluetongue status of the country of origin of the *animals*.

General Comment: the United States recommends that the Blue tongue testing section be clarified to explain that the testing requirement is for the movement of animals and not a requirement due to disease transmission via semen.

2. Testing in the quarantine station prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, bulls and teaser animals should be kept in a *quarantine station* for at least 28 days. The *animals* should be subjected to diagnostic tests as described below a minimum of 21 days after entering the *quarantine station*, except for *Campylobacter fetus* subsp. *venerealis* and *Trichomonas foetus*, for which testing may commence after 7 days in quarantine. All the results should be negative except in the case of BVD-MD antibody serological testing (see point 2b)i) below).

a) …

c) *Campylobacter fetus* subsp. *venerealis*

i) *Animals* less than 6 months old or kept since that age only in a single sex group prior to quarantine should be tested once on a preputial specimen, with a negative result.

ii) *Animals* aged 6 months or older that could have had contact with females prior to quarantine should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

d) *Trichomonas foetus*

i) *Animals* less than 6 months old or kept since that age only in a single sex group prior to quarantine, should be tested once on a preputial specimen, with a negative result.

ii) *Animals* aged 6 months or older that could have had contact with females prior to quarantine should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

Comment: The testing requirement for *Campylobacter fetus* subsp. *venerealis* and *Trichomonas foetus* of three cultures is insufficient to confirm the animals as negative for these two organisms. US experience has shown that the test sensitivities and specificities are too low to have confidence in this protocol.

Rationale *Campylobacter fetus* subsp. *venerealis*

Campylobacteriosis is a venereal disease of cattle caused by the organism *Campylobacter fetus* subsp *venerealis*. The organism is a nonpyogenic, slow growing, microaerophilic, gram-negative bacterium. Bulls become transient or permanent carriers of the organism after breeding infected females. In the carrier male, the organism is a superficial contaminant of the bull's external genitalia and causes no clinical pathology or changes in semen quality.

The potential for transmission exists as a result of contamination of collected semen with smegma or material from the glans penis and prepuce when infected bulls are used as semen donors. The organism has been shown to survive in untreated or improperly treated semen used for artificial insemination in fresh or frozen states. The carrier state may be eliminated by appropriate treatment with antibiotics available in the USA.

Sample collection consists of aspiration or washing of preputial material from the glans penis and surrounding preputial epithelium. These procedures yield the greatest numbers of organisms from

infected bulls. The collected sample material may be placed in physiological saline (.85%) for short-term transportation or in selective enriched transport media for long-term transportation.

The basic primary testing procedure to confirm a diagnosis of campylobacteriosis is the use of bacteriological examination to include biochemical reactions. Collected samples may be screened for the presence of *Campylobacter fetus* subsp *venerealis* using fluorescent antibody (FA) technique to establish a tentative diagnosis of campylobacteriosis in large populations of bulls. Bulls exhibiting a positive reaction shall undergo direct bacteriological examination. The inherent lack of specificity of the FA test due to cross reactivity with intestinal campylobacterial strains requires the use of culture and biochemical procedures as the final determinant of a bull's status as to the campylobacter carrier state.

Testing Recommendations:

Bulls in an AI center isolation facility shall undergo a series of negative culture tests of preputial material for *Campylobacter fetus* subsp *venerealis*.

As an alternative procedure, the preputial material may be examined using the FA technique as a screening test. Any positive FA test shall be followed by a culture of preputial material, for final determination.

Bulls may be placed on the following variable testing schedule:

Age of Sire when entering Isolation	Minimum Number of tests at weekly intervals
Under 180 days of age	1
180-364 days of age	3
365 days or older	6

Following successful completion of testing procedures, during the entire period of a bull's residence at an AI center, natural mating shall not occur. Testing frequency shall be negative single tests at 6 month intervals while in the semen production herd.

The recommended diagnostic procedure of choice is a direct culture of material collected from the glans penis and surrounding preputial epithelium. The limited availability of fluorescent antibody technology and the potential for cross reactivity precludes the use of this test as the sole criterion for determining the Campylobacter carrier status of an individual animal. Fluorescent antibody techniques may be used in conjunction with bacterial examination as a means to screen populations of animals with the final status determination based on bacteriological findings.

REFERENCES

Andrews, P.J. and F.S. Frank: Comparison of Four Diagnostic Tests for Detection of Bovine Genital Vibriosis. JAVMA 165(1974):695-697.

Bryner J.H. and J.W. Foley: Diagnosis of Bovine Campylobacteriosis on Bulls by Use of Selective Enrichment Transport Media and FA. From proceedings 2nd International Symposium of Veterinary Laboratory Diagnosticians, Lucerne, Switzerland. Vol II (1980):309-312.

Garcia M.M., G.M. Ruckerbauer, M.D. Eaglesome and W.E. Boisclaire: Detection of Campylobacter fetus in Artificial Insemination Bulls With a Transport Enrichment Medium. Can J Comp Med, 47(1983):336-340.

Hoerlein A.B.: Bovine Genital Vibriosis. In Diseases of Cattle in the Tropics: Economic and Zoonotic Relevance. Martinus Nijhoff Publisher, The Hague (1981):225-235.

Hoffer M.A.: Bovine Campylobacteriosis: A Review. Can Vet J, 22(1981):327-330.

Rationale ***Trichomonas foetus***

Trichomoniasis is a venereal disease of cattle caused by a protozoan flagellate *Trichomonas foetus.* Infected bulls are asymptomatic, harbor the *T.foetus* organism in the prepuce, and unless appropriately treated usually remain permanently infected.

It is recognized that transmission by artificial insemination (AI) may occur when trichomonas infected bulls are the semen donors. Preputial material, or smegma, from a trichomonas infected bull may contaminate the semen. It has been demonstrated that *T.foetus* can survive routine semen processing and cryopreservation procedures.

In *T.foetus* infected bulls, the highest concentration of trichomonads are identified from preputial material collected from the surface of the glans penis and adjacent preputial epithelium. Sample collection consists of aspiration or washing of preputial material from the glans penis and surrounding preputial epithelium.

Following satisfactory collection of preputial material, the specimen may be examined for trichomonads following incubation in culture media, or, by direct microscopic examination. It is generally accepted that the culture of preputial material is more reliable for the diagnosis of trichomoniasis than the direct microscopic examination, primarily because the identification of trichomonads is more repeatable following their multiplication in culture media.

The direct microscopic examination of semen for *T.foetus* is of little value because the millions of motile spermatozoa obscure the *T.foetus* organism.

Testing Recommendations:

Bulls entering an AI center isolation facility shall undergo a series of negative microscopic examinations of cultures of preputial material collected from the fornix.

Bulls may be placed on the following variable testing schedule:

Age of Sire when entering Isolation	Minimum Number of tests at weekly intervals
Under 180 days of age	1
180-364 days of age	3
365 days or older	6

Following successful completion of testing procedures, during the entire period of a bull's residence at an AI center, natural mating shall not occur. Testing frequency shall be negative single tests at 6 month intervals while in the semen production herd.

REFERENCES

Abbitt, B.: Trichomoniasis in Cattle. In Current Therapy in Theriogenology, by Morrow, WB Saunders Co., Philadelphia, PA.: 482-488, 1980.

Bartlett, D.E., K.Moist and F.A. Spurrel: The Trichomonas Foetus - Infected Bull in Artificial Insemination, JAVMA, Vol.122: 366-370, 1953.

Bartlett, D.E., K.G. Teeter, and P.C. Underwood: Artificial Insemination as a means of Transmission of Bovine Venereal Trichomoniasis. JAVMA, Vol.III; 114-115, 1947.

BonDurant, R.H.: Diagnosis, Treatment, and Control of Bovine Trichomoniasis. The Compendium on Continuing Education, Vol 7, No.3:S179-S188, 1985.

Clark, B.L., M.B. White and J.C. Banfield: Diagnosis of Trichomonas Foetus Infection in Bulls. Aust Vet Jour, Vol 47: 181-183, 1971.

Kimsey, P.B., B.J. Darien, J.W. Kendrick and C.E. Franti: Bovine Trichomoniasis: Diagnosis and Treatment. JAVMA, Vol 177, No.7:616-619, 1980.

Parsonson, I.M., B.L.Clark and J. Dufty: The Pathogenesis of Tritrichomonas Foetus Infection in the Bull. Aust. Vet. Jour., Vol 50:421-423, 1974.

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e) IBR-IPV

If the *artificial insemination centre* is to be considered as IBR/IPV free, the *animals* should be subjected, with negative results, to a diagnostic test for IBR/IPV on a blood sample. If any *animal* tests positive, the *animal* should be removed immediately from the *quarantine station* and the other *animals* of the same group should remain in quarantine and be retested, with negative results, not less than 21 days after removal of the positive *animal*.

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f) Bluetongue

The *animals* should comply with Articles 8.3.5., 8.3.6. or 8.3.7., depending on the bluetongue status of the country of origin of the *animals*.

Comment: The testing as described in Articles 8.3.5, 8.3.6 and 8.3.7 is too cumbersome and unnecessary to document the status of animals while in quarantine.

Rationale

Bluetongue viruses (BTV) are arthropod-borne (Culicoides sp.) agents that infect several species of wild and domesticated ruminants. Direct animal-to-animal transmission does not occur. BTV infection of bulls is not persistent. Following exposure, infection in cattle typically lasts less than 60 days[1]. BTV is rarely present in semen, and then only during the period of viremia[2]. In bulls exposed to natural infection and observed for 5 years, there was no evidence of bluetongue virus contamination of semen[3]. Testing for BTV is not included in the "CSS Minimum Requirements for Disease Control of Semen Produced for AI".

Diagnostic tests for BTV include serology (ELISA), which indicates prior exposure. If a bull is serologically positive, diagnostic tests to confirm its current infection status (i.e., the presence of virus in blood) are available. These tests include virus isolation in embryonated chicken eggs, virus isolation on tissue culture or polymerase chain reaction technology (PCR). Because BTV is present in semen only when the bull is viremic (i.e., virus in the blood during the brief interval following initial exposure), blood is the best specimen for virological examination[4]. PCR testing of processed semen is also an acceptable testing methodology.

Recommendation if Bluetongue testing is required

The resident herd was tested every 6 months with negative results to Bluetongue based on agar-gel immunodiffusion or ELISA; **OR**

One negative agar-gel immunodiffusion or ELISA on blood serum from semen donor performed prior to the day of the first semen collection and then between 21 and 60 days after semen for exportation was collected; **OR**

Whole-blood virus isolation test on the semen donor; one negative test at the beginning and conclusion of the collection period, and at least every seven (7) days during the semen collection period; **OR**

Whole blood PCR tests on the semen donor. One negative test at the beginning and conclusion of the collection period, and at least every twenty-eight (28) days during the semen collection period; **OR**

Test sample of frozen semen from each collection code intended for export using PCR.

References:

[1]Summary and Conclusions, 2nd International Symposium, Bluetongue, African Horse Sickness and Related Orbiviruses, June 17-21, 1991. Paris, France. Office International des Epizooties, 1992.

[2]MacLachlan, N.J., S.M. Barratt-Boyes, A.W. Brewer and J.L. Stott. 1991. Bluetongue virus infection of cattle. In; T.W. Walton and B.I. Osburn (eds) Proceedings of the Second International Symposium, Bluetongue, African Horse Sickness and Related Orbiviruses. Paris, France, CRC Press; 725-736.

[3]Gard, G.P., L.F. Melville, J.E. Shorthose. 1989. Investigations of bluetongue and other arboviruses in the blood and semen of naturally infected bulls. Vet Microbiol 20:315-322.

[3]Melville L.F. and G.P. Gard. 1991. Investigations of the effects of natural infection with orbiviruses on reproduction in cattle. In: T.W. Walton and B.I. Osburn (eds) Proceedings of the Second International Symposium, Bluetongue, African Horse Sickness and Related Orbiviruses. Paris, France, CRC Press; 744-750.

[4]Bowen, R.A., T.H. Howard, K.W. Entwistle, and B.W. Pickett. 1983 Seminal shedding of bluetongue virus in experimentally infected mature bulls. Am J Vet Res 43:1907-1911.

[4]Howard, T.H., R.A. Bowen, and B.W. Pickett. 1985. Isolation of bluetongue virus from bull semen In: T.L. Barber and M.M. Jochim (eds), Bluetongue and related Orbiviruses. Liss. New Your, 127-134.

Additional References:

Parsonson, I.M., L.H. Thompson, T.E. Walton, 1994. Experimentally induced infection with bluetongue virus serotype 11 in cows. Am J Vet Res, 55:1529-1534.

Parsonson, I.M..1993. Bluetongue virus infection of cattle. Proceedings of the ninety-seventh annual meeting of the United States Animal Health Association, October 23-29, 1993. Las Vegas, Nevada.

Parsonson, I.M., A.J. Della-Porta, D.A. McPhee. 1981. Isolation of bluetongue virus serotype 20 from the semen of an experimentally-infected bull. Aus Vet J. 57:252-253.

Pearson, J.E., G.A. Gustafson, A.L. Shafer, and A.D. Alstad. 1991 Distribution of bluetongue in the United States. In; T.W. Walton and B.I. Osburn (eds) Proceedings of the Second International Symposium, Bluetongue, African Horse Sickness and Related Orbiviruses. Paris, France, CRC Press; 128-139.

Walton, T. and B. Osburn, 1991. In; T.W. Walton and B.I. Osburn (eds) Proceedings of the Second International Symposium, Bluetongue, African Horse Sickness and Related Orbiviruses. Paris, France, CRC Press.

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3. Testing for BVD-MD prior to the initial dispatch of semen from each serologically positive bull

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5. Testing programme for bulls and teasers resident in the semen collection facilities

   …

   e) Bluetongue

The *animals* should comply with the provisions referred to in Articles 8.3.5., 8.3.6. or 8.3.7., depending on the bluetongue status of the country of origin of the *animals*.

Comment: The United States requests that the science be provided to justify the blue tongue testing regime described in this article. Such testing is not supported by current literature – see rationale above.

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Article 4.5.6.

Conditions applicable to the collection of semen

1. The floor of the mounting area should be easy to clean and to disinfect. A dusty floor should be avoided.

Comment: There are many impervious floors used both in the United States and Europe that have limestone screenings piled on them. Flexibility needs to be allowed for these types of floorings.

2. …

4. The artificial vagina should be cleaned completely after each collection where relevant. It should be dismantled, its various parts washed, rinsed and dried, and kept protected from dust. The inside of the body of the device and the cone should be disinfected before re-assembly using approved *disinfection* techniques such as those involving the use of alcohol, ethylene oxide or steam. Once re-assembled, it should be kept in a cupboard which is regularly cleaned and disinfected.

Comment: The word "dismantled" needs to be clarified. In practice, the 'mini-tub disinfectant system leaves the artificial vagina (less the cone) together for disinfection.

5. …

Article 4.5.7.

Conditions applicable to the handling of semen and preparation of semen samples in the laboratory

1. Diluents

   a) …

   d) Whenever milk, egg yolk or any other animal protein is used in preparing the semen diluent, the product must be free of pathogens or sterilised; milk heat-treated at 92°C for 3-5 minutes, eggs from SPF flocks when available. When egg yolk is used, it should be separated from eggs using aseptic techniques. Alternatively, commercial egg yolk prepared for human consumption or egg yolk treated by, for example, pasteurisation or irradiation to reduce bacterial contamination, may be used. Other additives must also be sterilized before use.

Comment: fresh eggs do not need to be of SPS status to be biologically safe for extender production.

   e) …