



How to control health status of semen ?

Dr Laurence GUILBERT-JULIEN



**LABORATOIRE NATIONAL DE
CONTRÔLE DES REPRODUCTEURS**

WOAH Collaborating Centre
for Infectious Reproduction
Diseases in Europe



Reference Centre
World Organisation
for Animal Health
Founded in 1967



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How to control health status of semen ?

- The LNCR
- What about the risk of diseases transmission by semen ?
- How is this managed by the European Legislation ?
- What analyses can be performed in semen ?
- Antibiotics and need of control ?
- Conclusion



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Presentation of LNCR

- **LNCR = Laboratoire National de Contrôle des Reproducteurs** = French Veterinary Laboratory for control of breeders in France
- Over 50 years of expertise in livestock reproduction
- Based in Maisons-Alfort, closed to the National Veterinary School and the Animal Health ANSES Laboratory
- 25 staff members, 6 technical units : bacteriology, virology, serology, molecular biology, semen analysis, endocrinology
- 300 000 tests/ year ; 80% serological tests in ruminants & pigs
- Strong Quality assurance system – COFRAC accreditation since 2003. (NF EN ISO/CEI 17025)
 - 90% of tests under COFRAC
- **WOAH Collaborating Centre for Infectious disease in Europe since 2016**
- **High level of performance for analyses on semen**
- **Primary site for the French National Biobank**



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What about the risk of disease transmission by semen

Is there a risk ?

- Answer is clearly YES
- Some virus, bacteria or parasite may be transmitted through semen, for cattle, small ruminants, pigs...
- Cryopreservation of semen allows conservation of the pathogens and risk of transmission by AI
- This result in detection of DNA or RNA of the pathogen in semen by PCR analyse
- ! Detection of DNA or RNA of a pathogen in semen doesn't always imply transmission and infection of female by natural breeding or AI

Animal 2018, 12:51, pp. e165–e171. © The Animal Consortium 2018
doi:10.1017/S1751109818000096



Review: Risks of disease transmission through semen in cattle

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Example of diseases transmitted by semen for cattle

- **FMD** : extremely contagious disease, shedding of virus in all secretion and excretion including semen; FMD free country ...
- **Brucella abortus** : presence in semen
- **BHV1** (Infectious Bovine Rhinotracheitis) : can be transmitted by semen
- **BVD virus** : after infection, replication in genital tract and shedding of virus in semen during a period that can be prolonged
 - In WOA recommendations, seropositive bulls must be controlled by analyse on semen to be sure semen is free of BVD virus
- **Bovine Leukaemia** virus – importance of sanitary status of herd



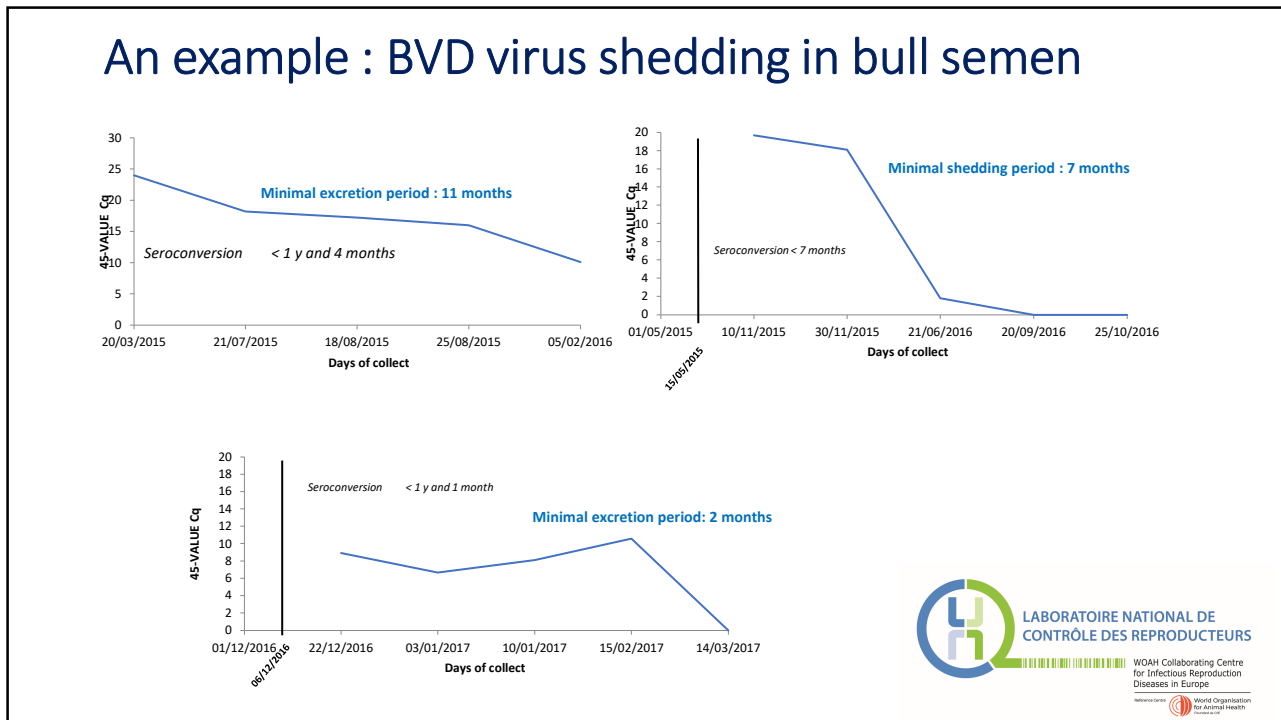
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Example of diseases transmitted by semen for cattle

- **Blue Tongue Virus (BTV)** : presence of live virulent virus in semen clearly demonstrated – WOA recommendations imply regular PCR on blood or serological analyses to be sure of no risk for semen
- **Schmallenberg virus** : detection of virus in semen; commercial issue for exportation in some countries
- **Other :**
 - *Tritrichomonas foetus* : Sexually transmitted
 - *Campylobacter fetus venerealis* : idem
 - *Leptospira*
 - *Coxiella burnetii*
 - *Mycobacterium bovis*
 - *Mycobacterium avium paratuberculosis*
 - *Mycoplasma bovis*
 - *Histophilus somni*
 - *Ureaplasma*
 - And the **Epizootic Haemorrhagic Disease...NEW** in France



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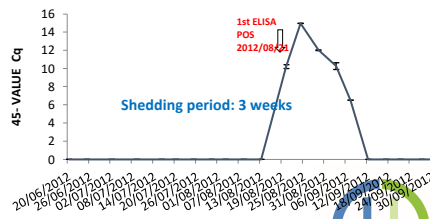
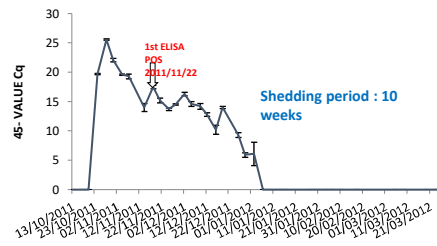
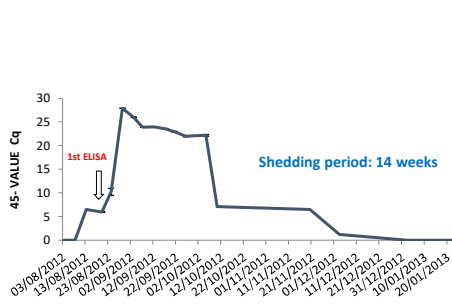
BVD virus in bull semen

- Shedding in semen is always existing for some BVD seropositive bulls, despite program of monitoring of this virus in France...

| | | | |
|---|-------------------------|------------------------------|----------------------------|
| Dossier N°22/76441 | Enregistrement N°AJ9302 | 2ème Contrôle en Quarantaine | Reçu le 14 Juin 2022 |
| Commentaire du dossier : | | | |
| Site : | | | Prélevé le 13 Juin 2022 |
| Nature du prélèvement : Sperme Pur | | | |
| © #Recherche du génome viral de la DVB/MM (RT-PCR sur semence - Extraction automatisée - Amplification VetMAX BVDV Screening (Thermo Fisher)) : *** Détecé (Ct = 29.81) *** | | | |
| Dossier N°22/77072 | Enregistrement N°AK3197 | Contrôle en Quarantaine | Reçu le 12 Juillet 2022 |
| Commentaire du dossier : | | | Prélevé le 11 Juillet 2022 |
| Nature du prélèvement : Sperme Pur | | | |
| © #Recherche du génome viral de la DVB/MM (RT-PCR sur semence - Extraction automatisée - Amplification VetMAX BVDV Screening (Thermo Fisher)) : *** Détecé (Ct = 34) *** | | | |
| Dossier N°22/77784 | Enregistrement N°AK6565 | 2ème Contrôle en Quarantaine | Reçu le 17 Août 2022 |
| Commentaire du dossier : | | | Prélevé le 16 Août 2022 |
| Nature du prélèvement : Sperme Pur | | | |
| © #Recherche du génome viral de la DVB/MM (RT-PCR sur semence - Extraction automatisée - Amplification VetMAX BVDV Screening (Thermo Fisher)) : *** Détecé (Ct = 34) *** | | | |
| Dossier N°22/79275 | Enregistrement N°AL3795 | Contrôle en CIA | Reçu le 17 Octobre 2022 |
| Commentaire du dossier : | | | Prélevé le 13 Octobre 2022 |
| Nature du prélèvement : Sperme Pur | | | |
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An example : Schmallenberg virus shedding in bull semen



3 Positive bulls

Parham et al. Veterinary Research 2014, 45:37
http://www.veterinaryresearch.org/content/45/1/37

VR VETERINARY RESEARCH

SHORT REPORT Open Access

Evidence of excretion of Schmallenberg virus in bull semen

Clair Ponsant¹, Nathalie Pozo¹, Emmanuel Biard², Virginie Catroc², Guillaume Vard², Corinne Salléau², Cyril Vianouge², Julie Gouzé², Martin Beer², Stepan Zentgraf² and Damien Vitouz²

Abstract

Schmallenberg virus (SBV) is a novel orthobornavirus, discovered in Germany in late 2011. It mainly infects cattle, sheep and goats and could lead to congenital infection, causing abortion and fetal abnormalities. SBV is transmitted by biting midges from the Culicoides genus and there is no evidence that natural infection occurs directly between ruminants. Here, we could detect SBV RNA in infected bull semen using qRT-PCR three bulls out of seven tested positive; 29 positive semen batches out of 136. We also found that highly positive semen batches from SBV infected bulls can provoke an acute infection in IFNAR^{-/-} mice, suggesting the potential presence of infectious virus in the semen of SBV infected bulls.

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What about small ruminants?

- Similarity with cattle
 - FMD
 - BTV
 - *Brucella abortus*
 - *Coxiella burnetii*
 - *Chlamydia...*
- Some specificities :
 - *Brucella ovis*
 - *Border Disease Virus...*

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What about swine ?

- Many virus and bacteria can be detected in semen and transmitted by AI :

- Aujeszky disease,
- FMD
- CSF
- ASF
- PRRS
- Parvovirus
- PED
- Swine Influenza
- Swine Vesicular Disease
- Leptospira...

DIAGNOSTIC NOTES

Detecting PRRSV in boar semen

Jane Christopher-Hennings, DVM, MS; Eric A. Nelson, PhD; and David A. Benfield, PhD



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An example : PEDV virus shedding in boar semen

RESEARCH ARTICLE

Open Access



Evidence of porcine epidemic diarrhea virus (PEDV) shedding in semen from infected specific pathogen-free boars

Sarah Gallien^{1,2,3*}, Angélique Moro^{1,2}, Gérald Lediguerher^{1,2}, Virginie Catinot⁴, Frédéric Paboeuf^{1,2}, Lionel Bigault^{1,2}, Mustapha Berri³, Phillip C. Gauger³, Nathalie Pozzi⁴, Edith Authié⁴, Nicolas Rose^{1,2} and Béatrice Grasland^{1,2}

Genome load (log (PEDV-genome copies/mL) by mL of sperm rich and seminal fractions of semen and gelatin plug during the trial

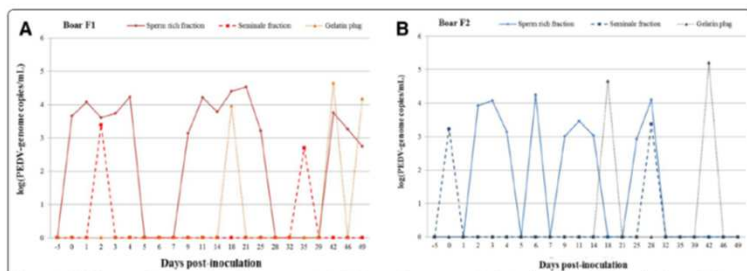


Figure 2 PEDV genome loads (number of genome copies/mL) detected in semen and gelatin plugs from SPF boars before and after inoculation (A Boar F1; B Boar F2). Individual virological data: PEDV viral genome load in sperm rich fraction, in seminal fraction and in gelatin plug [log(number of genome copies/mL)] in the two inoculated SPF boars (F1 and F2).



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What is mandatory for germinal products in AHL - delegated regulation 2020/686 ?

- **Bulls**, controls include diagnostic of :
 - Bovine leukaemia
 - BHV1
 - BVD
 - Brucella abortus
 - Tuberculosis
 - *Campylobacter fetus*
 - *Trichostrongylus axei*
 - BTV
- **Ovine and caprine**
 - Tuberculosis
 - Brucella abortus
 - Brucella ovis for rams
 - BTV
- **Boars** :
 - Brucella
 - Aujeszky disease
 - CSF
 - PRRS including serological analyse and PCR



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What can be detected by PCR on semen – available tools for cattle, small ruminants and swine

- 2 different situations
 - For some diseases, kits and methods have been validated for semen
 - For the other, the validation has not yet been made
- PCR on semen is different from PCR on other samples...



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PCR on semen

RUMINANTS

- BVD
- Border Disease
- BHV1
- BTV
- SBV
- Brucella
- Leptospira
- Coxiella burnetii
- Campylobacter fetus venerealis
- Tritrichomonas fetus
- Mycobacterium bovis
- Mycobacterium paratuberculosis

- Neospora caninum
- Listeria
- Mycoplasma bovis

- And the new EHD...

SWINE

- PRRS
- Aujeszky
- CSF
- ASF
- Brucella
- Parvovirus
- PCV2
- PED
- Leptospira
- Influenza
- Bordetella
- Haemophilus parasuis
- Salmonella
- Actinobacillus
- Mycoplasma hyopneumoniae
- Pasteurella Multocida T
- Coronavirus (GET/CVRP)
- Delta Coronavirus...



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Is there a need to control bacteria if antibiotics are used on semen ?

- Add antibiotics to semen is necessary to avoid transmission of some diseases :
 - Leptospira, Mycoplasma, and Campylobacter for bovine semen (directive 88/407/ECC)
 - Leptospira for porcine semen (Directive 90/429/EEC)

- Limit also multiplication of bacterial flora that can have a negative impact on semen quality
- Can also limit transmission of other bacteria by semen
- The antibiotics association used in germinal products need to have bacterial activity at least equivalent to Penicillin Streptomycin Lincomycin Spectinomycin (PSLS) or Gentamycin Tylosin Lincomycin Spectinomycin (GTLS) combination

- If the information about addition of antibiotics and nature of the combination are known before prior to enter the GR in biobank, it could allow not to test the presence of some pathogen bacteria.

NB : use of antibiotics was mandatory but in the Delegated Regulation(EU) 2023/647 of 13th of January 2023 amending Delegated Regulation (EU) 2020/686 supplementing Regulation (EU) 2016/429, it is now indicated : « Where necessary, antibiotics or mixtures of antibiotics may be added to semen or contained in semen diluents”

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Limits of antibiotics

- Antibiotics in semen could be considered as insufficient to prevent transmission of some pathogens like *Mycoplasma bovis*
- NZ authorities make it mandatory to detect *Mycoplasma bovis* on bull semen before importation with a very precise protocol, (or to increase concentration of antibiotics).



Semen as a source of *Mycoplasma bovis* mastitis in dairy herds

Vera Haapala^{a,*}, Tarja Pohjanvirta^b, Nella Vähänikkilä^b, Jani Halkilahti^c, Henri Simonen^d, Sinikka Pelkonen^b, Timo Soveri^a, Heli Simojoki^a, Tiina Autio^b

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What could be the right protocol ?

- Having data about the herd status of animals,
- Establish a minimum list of pathogens, cf AHL requirements :

▪ CATTLE

- BVD
- BHV1
- BTV
- SBV
- *Campylobacter fetus venerealis*
- *Tritrichomonas fetus*
- *Mycobacterium bovis*
- *Mycobacterium paratuberculosis*
- *Brucella*
- And the new EHD...

▪ OVINE and CAPRINE

- Border Disease
- BTV
- *Brucella*
- *Brucella ovis*
- *Mycobacterium bovis*
- *Mycobacterium paratuberculosis*

▪ SWINE

- PRRS
- Aujeszky
- CSF
- ASF
- *Brucella*



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Conclusion

- Many pathogens could be detected by PCR on semen, despite it is not a common matrix for this type of analyse,
- Some tests can be performed on routine, some are more complicated to do or not yet adapted to semen
- You will find only what you are looking for, and the list could be long...
- Define a minimum list of pathogens to control, taking in account the herd sanitary status,
- ! PCR is always more expensive than serological analyses
- And it is genetic resources consuming.



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