



Use of novel molecular techniques for improvement of abortion diagnostics in ruminants

Sabrina Rodriguez Campos¹, Vincent Perreten¹, Egija Zaura², Horst Posthaus³

¹Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, CH-3012 Bern, ²Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam, University of Amsterdam, NL-1081 Amsterdam, ³Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, CH-3012 Bern

Key words

Abortion in ruminants, opportunistic pathogens, bacteria, fungi, molecular diagnostics, next generation sequencing, microbiota, microarray

Aim of the study

The study was performed to: 1) evaluate the current situation of routine abortion diagnostics, 2) perform broad-spectrum analyses of abortions from ruminants including serology, microscopy, broad-spectrum culture and conventional as well as real-time PCR for detection of difficult to grow abortifacients, 3) detect potential novel abortifacients by next generation sequencing (amplicon sequencing technique), 4) improve the diagnostic workflow providing a cost-efficient molecular tool.

Material and methods

A total of 493 cases of abortions received between 2012-2016 for routine diagnostics were included in the study. A complete microbiological abortion analysis should comprise placenta, fetal organs (abomasal content, liver, lung) and blood serum of the dam. However, the complete sample material is often not available, thus, different studies within the project were conducted on varying sample numbers.

- a) Microscopy: Tissue smears were examined microscopically after Stamp's modification of the Ziehl-Neelsen (mod ZN) staining.
- b) Serology: Maternal sera were tested for antibodies against *B. abortus*, *C. burnetii* and *C. abortus* using the prescribed, approved ELISA test kits. The serological detection of antibodies against *Leptospira* spp. was performed by microscopic agglutination test (MAT).
- c) Broad-spectrum culture was carried out for culturable bacteria and fungi according to Schnydrig et al. (2017).
- d) Molecular detection of the difficult to grow bacteria *Coxiella burnetii*, pathogenic *Leptospira* spp., *Chlamydiae* and *C. abortus* was performed in cattle and small ruminants according to Vidal et al. (2017b) and Schnydrig and colleagues (2017), respectively, using classical end-point PCR and/or real-time PCR. In case of uninterpretable culture of *Campylobacter* spp. due to overgrowth with contaminants, direct PCR of the enrichment medium was additionally carried out.
- e) Histopathological analyses and immunohistochemistry were performed according to Vidal et al. (2017b and c)
- f) Next generation sequencing (NGS) to disclose the bacterial and fungal microbiota was performed according to Vidal et al. (2017a and c).
- g) The suitability of microarrays was evaluated with the ChlamType-23S AS-4 Kit (Alere Technologies GmbH).

Results and significance

The project outcome highlights the importance of a multi-methodological approach to abortion diagnostics in order to achieve the highest possible clarification rate. This approach requires complete submission of suitable sample material. Our studies revealed a wide range of opportunistic bacteria, including zoonotic agents, which

play a role in abortion in ruminants and often occur as multi-infections. The currently prescribed staining method (mod ZN) lacks sensitivity for the detection of *C. burnetii* and *C. abortus*, which play a major role in abortion in ruminants. The microarray as a single tool was discarded during this study due to lower sensitivity than the real-time PCR technique. The NGS approach, while not suitable as diagnostic tool, is useful to screen for emerging and/or novel agents. As a further outcome of this project a working group was initiated with pathologists, parasitologists, virologists and bacteriologists of both Vetsuisse Faculties as well as veterinary clinicians from the Vetsuisse Bern that is currently elaborating recommendations for an improved diagnostic workflow.

Publications, posters and presentations

Peer-reviewed journal articles

- Vidal, S.; Kegler, K.; Posthaus, H.; Perreten, V.; Rodriguez-Campos, S.; (2017) a. Amplicon sequencing of bacterial microbiota in abortion material from cattle. *Veterinary Research* 48, 64.
- Vidal, S.; Kegler, K.; Greub, G.; Aeby, S.; Borel, N.; Dagleish, M.P.; Posthaus, H.; Perreten, V.; Rodriguez-Campos, S. (2017) b. Neglected zoonotic agents in cattle abortion: tackling the difficult to grow bacteria. *BMC Veterinary Research*, 13(1):373.
- Schnydrig, P.; Vidal, S.; Brodard, I.; Frey, C.; Posthaus, H.; Perreten, V.; Rodriguez-Campos, S. (2017) Bacterial, fungal, parasitological and pathological analyses of abortions in small ruminants from 2012-2016. *Schweizer Archiv für Tierheilkunde*, 159(12):647-656.
- Vidal, S.; Brandt, B.W.; Dettwiler, M.; Abril, C.; Bressan, J.; Greub, G.; Frey, C.F.; Perreten V.; Rodriguez-Campos, S. (2017) c. A broad-spectrum approach to bovine infectious abortion: limited added value of fungal ITS amplicon sequencing. Under review in *Theriogenology*.

Posters

- Vidal, S.; Syring, C.; Rodriguez-Campos, S. (2015) Implications of *Leptospira* spp. in bovine abortion. 2nd ELS (Euro-pean Leptospirosis Society) meeting. Amsterdam (The Netherlands), 16th-18th April 2015.
- Vidal, S.; Perreten, V.; Rodriguez-Campos, S. (2015) Analysis of samples from bovine abortion by 16S ribosomal DNA amplicon sequencing. 73rd Annual Assembly of the Swiss Society for Microbiology. Lugano (Switzerland), 28th-29th May 2015.
- Vidal, S.; Perreten, V.; Rodriguez-Campos, S. (2016) Analysis of the mycobiota present in abortion material from cattle by ITS amplicon sequencing. 74th Annual Meeting SSM, Bern (Switzerland), 13.-15.06.2016
- Schnydrig, P.; Vidal, S.; Perreten, V.; Rodriguez-Campos, S. (2016) Leptospirosis as possible cause of abortion in small ruminants. 74th Annual Meeting SSM, Bern (Switzerland), 13.-15.06.2016
- Schnydrig, P.; Brodard, I.; Overesch, G.; Rodriguez-Campos, S. (2016) Typing of *Salmonella enterica* subspecies diari-zonae serovar 61:(k):1,5,(7) in sheep in Switzerland by pulsed-field gel electrophoresis. 4th EAVLD Con-gress of the European Association of Veterinary Laboratory Diagnosticians, Prague (Czech Republic), 6.-09.11.2016
- Vidal, S.; Overesch, G.; Thomann, A.; Rodriguez-Campos, S. (2016) Implication of *Campylobacter* spp. in bovine abortion in Switzerland. 4th EAVLD Congress of the European Association of Veterinary Laboratory Diagnosticians, Prague (Czech Republic), 6.-09.11.2016

Presentations

- Vidal, S.; Brodard, I.; Posthaus, H.; Perreten, V.; Rodriguez-Campos, S. (2015) Comprehensive analysis of bovine abortion combining classical and next generation sequencing approaches, XVII International Symposium of the World Association of Veterinary Laboratory Diagnosticians, Saskatoon (Canada), 15th-18th June 2015.
- Vidal, S.; Greub, G.; Aeby, S.; Perreten, V.; Rodriguez-Campos, S. (2016) Neglected zoonotic agents in cattle abortion: molecular and serological screening of difficult to grow bacteria. 7th World Congress on Microbiology, Valencia (Spain), 28th–29th November 2016.

Project 1.14.07

Project duration November 2014 – January 2018