Bacterial, fungal, parasitological and pathological analyses of abortions in small ruminants from 2012-2016

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Summary

Abortion in small ruminants presents a clinical and economic problem with legal implications regarding animal health and zoonotic risk by some of the abortive pathogens. Several bacteria, fungi and parasites can cause abortion, but cost-orientated routine diagnostics only cover the most relevant epizootic agents. To cover a broad-range of common as well as underdiagnosed abortifacients, we studied 41 ovine and 36 caprine abortions by Stamp's modification of the Ziehl-Neelsen stain, culture for classical and opportunistic abortive agents, real-time PCR for *C. burnetii*, *C. abortus*, pathogenic *Leptospira* spp., *Toxoplasma gondii* and *Neospora caninum*. When the dam's serum was available detection of antibodies against *B. melitensis*, *C. burnetii*, *C. abortus* and *Leptospira* spp. was performed. In 37 cases sufficient placental tissue was available for pathological and histopathological examination. From the 77 cases 11 (14.3%) were positive by staining whereas real-time PCR detected *C. burnetii* and *C. abortus* in 49.3% and 32.5% of the cases. Antibodies against *C. abortus* and *Leptospira* spp. (33.3 and 26.7%) were detected. In 23.4% a bacterial culturable pathogen was isolated. Fungal abortion was confirmed in 1.3% of cases. A single abortive agent was identified in 44.2% of the cases and in 31.2% multiple possible abortifacients were present. Our study shows that the highest clarification rate can only be achieved by a combination of methods and evidences the role that multi-infections play as cause of abortion.

Keywords: small ruminants, abortion, epizootics, broad-spectrum, molecular diagnostics

Bakteriologische, mykologische, parasitologische und pathologische Untersuchungen von Kleinwiederkäueraborten zwischen 2012–2016

Aborte stellen ein klinisches und wirtschaftliches Problem mit tierseuchenrechtlichen Folgen und Zoonoserisiko durch gewisse Aborterreger dar. Verschiedene Bakterien, Pilze und Parasiten können Aborte verursachen, aber die kostenorientierte Routinediagnostik deckt nur die wichtigsten Tierseuchenerreger ab. Um ein breites Spektrum an gängigen als auch unterdiagnostizierten Aborterregern abzudecken, untersuchten wir 41 Schaf- und 36 Ziegenaborte mittels modifizierter Ziehl-Neelsen Färbung nach Stamp, Kultur auf klassische und opportunistische Aborterreger, real-time PCR auf *C. burnetii*, *C. abortus*, pathogene *Leptospira* spp., *Toxoplasma gondii* und *Neospora caninum*. Wenn Serum des Muttertieres vorhanden war, wurde der Nachweis von Antikörper gegen *B. melitensis*, *C. burnetii*, *C. abortus* and *Leptospira* spp. durchgeführt. In 37 Fällen war genügend Plazentagewebe für pathologische Untersuchungen vorhanden. Von den 77 Fällen waren 11 (14.3%) positiv in der Färbung, wohingegen die Real-time PCR *C. burnetii* und *C. abortus* in 49.3% und 32.5% der Fälle nachwies. Antikörper gegen *C. abortus* und *Leptospira* spp. (33.3 und 26.7%) wurden nachgewiesen. In 23.4% wurde ein bakterieller kulturell anzüchtbarer Pathogen nachgewiesen. Ein Pilzabort wurde in 1.3% der Fälle bestätigt. Ein einzelner Aborterreger wurde in 44.2% der Fälle nachgewiesen und in 31.2% waren multiple mögliche Aborterreger vorhanden. Unsere Studie zeigt auf, dass die höchste Aufklärungsrate nur mit einer Kombination von Methoden erreicht werden kann, und zeigt die mögliche Rolle von Multi-Infektionen als Abortursache auf.

Schlüsselwörter: Kleinwiederkäuer, Abort, Tierseuchen, Breitspektrum, Molekulardiagnostik

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Introduction

Abortion is defined as the termination of gestation with expulsion of a nonviable fetus before completion of gestation [Swiss Ordinance on epizootic diseases (TSV) SR.916.401; Article (Art.) 6]. Even though an abortion rate up to 2% is considered normal for sheep (Scott, 2015), abortion is a common, important clinical problem that can cause considerable economic loss (von Tavel *et al.*, 2005; Williams and O'Donovan, 2009). Abortion in small ruminants can have numerous infectious and non-infectious causes (Givens and Marley, 2008; Williams and O'Donovan, 2009; van den Brom *et al.*, 2012; Borel *et al.*, 2014) including bacterial agents with zoonotic potential (Pospischil *et al.*, 2002; Longbottom and Coulter, 2003; Roest *et al.*, 2013). According to Swiss Federal Law all abortions in small ruminants are notifiable (TSV SR.916.401; Art. 129). Any infectious agent that is able to reach the junction between maternal and fetal placenta could cause abortion (Kirkbride, 1993) and studies conducted among cattle in Switzerland suggested that some protozoans (Häsler *et al.*, 2006; Reitt *et al.*, 2007) and some neglected opportunistic pathogens play an important role in abortion (Vidal *et al.*, 2016). However, due to cost efficiency or depending on the legislation, only the most likely etiologies and those with epizootic potential like *Brucella* (*B.*) spp., *Coxiella* (*C.*) *burnetii* and *Chlamydia* (*C.*) *abortus*, are in focus of routine diagnostics (Borel *et al.*, 2014). These analyses include microscopy for those three bacterial diseases and serological testing for brucellosis; furthermore, in cases of suspicion, serological analysis of coxiellosis and chlamydiosis is generally included (TSV SR.916.401; Art. 129b and 190-195). For a complete, broad-spectrum investigation of an abortion, the sample material should include placenta (cotyledons especially any with lesions), fetal stomach content and organs (for sheep and goats it is convenient to submit the entire fetus) and dam's serum. Furthermore, it should be submitted for analysis as clean and fresh as possible and preventing any leakage to avoid cross-contamination (Kirkbride, 1990). An abortive agent needs to fulfill the following criteria to be considered etiologic (Borel *et al.*, 2014): a) presumptive abortive agent present in large number and/or pure culture in fetal abomasal content and/or liver and lung, b) associated inflammation in placenta, c) other common abortive agents were excluded. Unfortunately, routine diagnostics often fail to clarify the cause of abortion because the before mentioned requirements are rarely met.

Table 1: Overview of targets, primer and probe sequences, corresponding references and reagents of the real-time and end-point PCRs.

Pathogen	Target	Primer (5′ – 3′)	Probe (5′ – 3′)
T. gondii	B1	F - GGAGGACTGGCAACCTGGTGTCG	CGGAAATAGAAAGCCATGAGGCACTCC
		R - TTGTTTCACCCGGACCGTTTAGCAG	ACGGGCGAGTAGCACCTGAGGAGAT
N. caninum	Nc5	F - CCCAGTGCGTCCAATCCTGTAAC	CACGTATCCCACCTCTCACCGCTACCA
		R - CTCGCCAGTCAACCTACGTCTTCT	TCCCTCGGTTCACCCGTTCACACAC
C. burnetii	IS*1111*	F - AATTTCATCGTTCCCGGCAG	TGTCGGCGTTTATTGG
		R - GCCGCGTTTACTAATCCCCA	
Chlamydiales	16S rRNA	F - CCGCCAACACTGGGACT	CTACGGGAGGCTGCAGTCGAGAATC
		R - GGAGTTAGCCGGTGCTTCTTTAC	
C. abortus	*ompA*	F - GCAACTGACACTAAGTCGGCTACA	TAAATACCACGAATGGCAAGTTGGTTTAGCG
		R - ACAAGCATGTTCAATCGATAAGAGA	
pathogenic *Leptospira* spp.	*lipL32*	F - AGAGGTCTTTACAGAATTTCTTTCACTACCT	AAGTGAAAGGATCTTTCGTTGC
		R - TGGGAAAAGCAGACCAACAGA	
C. burnetii	repetitive transposon	F - TATGTATCCACCGTAGCCAGTC	-
		R - CCCAACAACACCTCCTTATTC	
Chlamydia sp.	23S rRNA	F - GGGCTAGACACGTGAAACCTA	-
		R - CCATGCTTCAACCTGGTCATAA	
C. abortus	23S rRNA	F - AATCATCTATCATTGTACGC	-
		R - AGACTAGGTTTCACGTGTCTAG	
C. fetus subsp. *fetus*	*nahE*	F - GGTTATTTTTTATAACTGTAGGAATGCAGAT	-
		R - GATCGCTTAAATCTTGTACTTTTAGCTTTT	-

[a] References: [1] Costa *et al.*, 2000; [2] Scheidegger *et al.*, 2005; [3] Müller *et al.*, 2002; [4] Howe *et al.*, 2009; [5] Croxatto *et al.*, 2011; [6] Pantchev *et al.*, 2009; [7] Villumsen *et al.*, 2012; [8] Willems *et al.*, 1994; [9] Nordentoft *et al.*, 2011; [10] Abril *et al.*, 2007

[b] Roche Diagnostics (Basel, Switzerland). Light Cycler® FastStart DNA Master HybProbe Kit (Roche) was used.

[c] Applied Biosystems (Zug, Switzerland); TaqMan® Fast Advanced Master Mix (Applied Biosystems, Foster City, California, USA) was used.

[d] Biometra GmbH (Goettingen, Germany); for end-point PCRs Firepol DNA polymerase (Solis BioDyne, Tartu, Estonia) was used.

In this study we analyzed abortion material from sheep and goats submitted over a four-year period by broad-spectrum culture, end-point PCR and real-time PCR, serology and histopathology to gain an overview of the diagnostic value of the current approach to abortion investigations. Bacterial, fungal and parasitological agents involved in abortion in small ruminants were addressed to determine the most reliable method or combination of methods for their detection.

Animals, Material and Methods

From 2012 to 2016 we studied cases of abortion in 77 small ruminants submitted for prescribed routine abortion diagnostics. The samples originated from sheep (n= 41) and goat (n= 36) and included a total of 182 tissue samples (placenta, n=59; fetal abomasal content, n=42; fetal liver, n=43; fetal lung, n=38). The cases occurred in 11 different cantons in Switzerland [Bern (n=55), Ticino (n=6), Fribourg (n=5), Jura (n=3), Neuchâtel (n=2), Vaud (n=1), St. Gallen (n=1), Schaffhausen (n=1), Basel-Stadt (n=1), Schwyz (n=1) and Zurich (n=1)]. In 40 cases the sera of the dams were also available. The abortions were analyzed with an extended spectrum for bacteria and fungi, and were moreover examined by parasitological and/or pathological analyses if requested by the sender.

Staining and microscopy

All tissue samples were analyzed with the Stamp's modification of the Ziehl-Neelsen (mod-ZN) staining (Alton *et al.*, 1988), a modified acid-fast staining for the presumptive evidence of *Brucella* organisms that stain red against blue background. However, this method does not allow clear differentiation of *C. abortus* and *C. burnetii* from *Brucella* organisms (OIE, 2016).

Broad-spectrum culture

For the identification of culturable bacteria a piece of approximately 2 cm diameter from a cotyledon of the placenta and/or 1 ml of fetal abomasal content was homogenized in 5 ml of 0.85% NaCl using an Ultra-Turrax® Tube Drive Workstation (IKA®-Werke GmbH, Staufen, Germany). Thereof 100 µl and approximately 20 mg of fetal liver and/or lung were plated onto trypticase soy agar with 5% sheep blood (Becton Dickinson, New Jersey, USA), phenylethyl alcohol agar (Becton Dickinson, Le Pont de Claix, France) and modified Brucella selective agar (Abril *et al.*, 2011) and incubated at 37 °C in an atmosphere containing 5% CO_2 for 48h and up to 7d for the Brucella selective medium.

The same amounts were plated onto MacConkey agar, Palcam Listeria agar, Sabouraud chloramphenicol agar (Oxoid, Hampshire, UK) and incubated aerobically at 37 °C for 48h. Furthermore, 500 µl of the homogenized material was enriched in Müller-Kauffmann Tetrathionate-Novobiocin broth for detection of *Salmonella* spp. After 24 hours at 37 °C, 100 µl were plated onto Brilliant Green Agar and Salmonella Chromagar (Oxoid) and incubated at 37 °C for 24h. 500 µl of the homogenized material was enriched in Thomann Transport and Enrichment medium (Harwood *et al.* 2009) and after 48 hours at 37 °C, 100 µl were plated onto Skirrow agar (Oxoid) for detection of *Campylobacter* spp. after incubation at 37 °C in a microaerophilic atmosphere for 5d. The microbial isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS, Bruker).

Molecular detection of bacteria and parasites

Table 1 shows an overview of all primer and probe sequences as well as targets and reagent specifications for the molecular analyses. For detection of bacterial agents (*C. burnetii*, *C. abortus* and pathogenic *Leptospira* spp.) total DNA was extracted from placenta (n=59) and fetal abomasal content (n=42) with Ultra Clean® Tissue&-Cells DNA Isolation Kit (MoBio) according to the manufacturer's guidelines. DNA concentrations of all ex-

	Probe label	Ref.[a]	Cycler
	3′ fluorescein	[1; 2]	Light Cycler[b]
	5′ LC-Red 640 3′ phosphorylated		
	3′ fluorescein	[3]	
	5′ LC-Red 640		
	5′ FAM 3′ MGB	[4]	7500 FAST real-time PCR system[c]
	5′ FAM 3′ BHQ1	[5]	
	5′ FAM 3′ TAMRA	[6]	
	5′ FAM 3′ MGBNFQ	[7]	
	-	[8]	Biometra thermal cycler[d]
	-	[9]	
	-	[9]	
	-	[10]	
	-		

tractions were determined by fluorometry (QuantiFluor®, Promega, Dübendorf, Switzerland). In case of uninterpretable culture of *Campylobacter* spp. due to contamination, direct PCR of the enrichment medium was carried out for *C. fetus* subsp. *fetus* (Abril *et al*., 2007). In case of a positive reaction to the *Chlamydiales* screening the result was confirmed as *C. abortus* by real-time PCR according to Pantchev *et al*. (2009). For detection of parasites [*Neospora* (*N.*) *caninum* and *Toxoplasma* (*T.*) *gondii*] total DNA was extracted from 30 samples as follows: 50 µg of homogenized brain or placental tissue was used for DNA extraction with the Qiagen tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted in a final volume of 100 µl. Of these, 0.5 and 2 µl, respectively, were added to a PCR mix of 8 µl. To check for inhibitory effects control reactions were carried out in parallel for each sample for all real-time PCRs.

Serology

Upon availability (sample volume) sera from the dams were screened for antibodies against *B. melitensis* (n=40), *C. burnetii* (n=37), *C. abortus* (n=36) and pathogenic *Leptospira* spp. (n=30). We used Enzyme Linked Immunosorbent Assays (ELISA) (Idexx Brucellose Serum X2, Idexx Q fever, Idexx Chlamydiosis Total Ab Antibody Test Kits, IDEXX Laboratories Inc., Liebefeld-Bern, Switzerland) according to manufacturer's instructions and the microscopic agglutination test (MAT) according to the World Organisation for Animal Health (OIE) standards (OIE, 2012). The following strains were used to detect antibodies against 9 pathogenic leptospiral serovars [*L. kirschneri* serovar Grippotyphosa Moskva V; *L. borgpetersenii* serovars Ballum Mus127, Sejroe M84 and Tarassovi Perepelitsin; *L. interrogans* serovars Australis Ballico, Pomona Pomono, Canicola Hond Utrecht IV, Icterohaemorrhagiae RGA and Hardjo Hardjoprajitno]. Sera were initially screened for agglutination at a dilution of 1:100. Reactive sera were titrated in a serial two-fold dilution to determine the end-point titer defined as the highest serum dilution at which at least 50% agglutination occurs. The sole detection of antibodies without confirmation of the presence of the agent in the abortion material was not considered relevant.

Pathology

From the 77 cases 37 placentae were available for pathological analysis. For the triage of suitable samples for histopathology macroscopic changes and the degree of autolysis were recorded and subsequently histopathology was carried out on 18 placentae. The samples were fixed in 10% buffered formalin and routinely processed for histology. Histological sections were stained with hematoxylin and eosin. Periodic-Acid-Schiff (PAS)-staining was carried out in case of lesions suspicious for fungal placentitis.

Statistical analysis

Calculation of sensitivity and specificity of a technique requires the comparison with a gold standard which in bacteriology is usually the isolation of a pathogen in culture. The three abortive bacterial agents, *Coxiella burnetii*, *Chlamydiales* and *Leptospira* spp., belong to the fastidious bacteria for which the gold standard regarding abortion diagnostics is the detection of the pathogens in placentae and/or fetal organs together with histopathological lesions. Especially in abortion diagnostics this is often not possible due to either the lack of the placenta or autolysis that entails loss of tissue architecture and cellular detail. We calculated Cohen's kappa (κ) coefficient to assess the level of agreement between the serological and molecular analysis for *Coxiella burnetii*, *Chlamydiales* and *Leptospira* spp. Percentages and κ coefficients with 95% confidence intervals (CIs) were calculated using NCSS 11 Statistical Software (2016) (NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.). Standard cutoffs were used to define poor ($\kappa < 0.4$), fair ($\kappa = 0.41$-0.6), good ($\kappa = 0.61$-0.80) and very good agreement ($\kappa \geq 0.80$). Suspicious serological results were excluded from the comparison.

Results

Staining and microscopy

Out of 77 cases analyzed by Stamp's modification of the Ziehl-Neelsen staining 17 tissue samples corresponding to 11 cases (14.3%, 95% CI [7.4, 24.1]) were positive showing red intracellular organisms suspicious for coccobacilli against blue background and hence hinting at the presence of *Brucella* spp., *C. abortus* and/or *C. burnetii*.

Broad-spectrum culture

From the 182 tissue samples corresponding to 77 cases of abortion we could not assign a possible bacterial abortifacient, based on the criterion that it occurred in large number and/or pure culture in fetal abomasal content and/or liver and lung, by broad-spectrum culture in 59 cases. In 18 cases (23.4%, 95% CI [14.5, 34.4]) (Table 3) a bacterial abortive agent could be identified: *Escherichia* (*E.*) *coli* (n=10), *Salmonella* (*S.*) *enterica* subsp. *diarizonae* (n=3), *Streptococcus* (*S.*) *uberis* (n=1; mixed culture with *E. coli*), *S. lutetiensis* (n=1), *Histophilus* (*H.*) *somni* (n=1), *Listeria monocytogenes* (n=1), *Enterococcus* (*E.*) *casseliflavus* (n=1) and (*S.*) *enterica* subsp. *enterica* serovar Abortusovis (n=1). No *Campylobacter* spp. was found; in 30 cases of overgrowth of the culture by contaminant bacteria, the PCR was carried out to confirm the absence of *C. fetus* subsp. *fetus*.

In 16 cases (20.8%, 95% CI [12.4, 31.5]) fungi were cultured belonging to the filamentous fungi (n=10) [not identifiable (n=6), *Lichtheimia corymbifera* (n=3), *Mucor*

circinelloides (n=1)] and to the yeasts (n=6) [not identifiable (n=2), *Candida* sp. (n=1), *Cryptococcus* sp. (n=1), *Debaromyces* sp. (n=1), *Wickerhamomyces* sp. (n=1)]. Only in the case where *Wickerhamomyces* sp. was isolated from placenta and fetal abomasal content) the histopathology could furthermore demonstrate intralesional fungal structures and thereby confirm causality (1.3%, 95% CI [0.0, 7.0]) (Table 3, Table 4).

Molecular detection

From the 101 samples of placenta and fetal abomasal content samples corresponding to the 77 cases of abortion analyzed by end-point PCR 11 cases (14.3%, 95% CI [7.4, 24.1]) and two cases (2.6%, 95% CI [0.3, 9.1]) were positive for *C. abortus* and *C. burnetii*, respectively. Real-time PCR revealed the presence of *C. abortus* in 25 cases (32.5%, 95% CI [22.2, 61.0]) and of *C. burnetii* in 38 cases (49.4%, 95% CI [31.7, 61.0]) (Table 2, Table 3). Only 3 samples [placenta (n=2); fetal abomasal content (n=1)] from 3 cases of ovine abortion were positive for pathogenic *Leptospira* species, (3.9%, 95% CI [0.8, 11.0]). Out of 30 cases tested for T. gondii, three were positive by PCR (10%, 95% CI [2.1, 26.5]). One out of 13 tested cases (7.7%, 95% CI [0.2, 36.0]) was positive for *Neospora* (*N.*) *caninum*. In total, real-time PCR could identify a possible cause of abortion in 50 cases (64.9%, 95% CI [0.532, 0.755]).

Serology

Serology was negative for antibodies against *B. melitensis* and *C. burnetii*. Antibodies against *C. abortus* were detected in 12 sera of the dams (33.3%, 95% CI [18.6, 51.0]), in one case the serum sample was suspicious (2.8%, 95% [0.1, 14.5]); in 9 corresponding abortions the agent was also detected by real-time PCR. Out of the 30 sera tested by MAT 8 sera (26.7%, 95% CI [12.3, 45.9]) were positive, with 7 sera (23.3%, 95% CI [9.9, 42.3]) [sheep (n=5); goat (n=2)] being seropositive for one serovar [*L. interrogans* serovars Australis (n=1), Pomona (n=2), Hardjo (n=2), *L. borgpetersenii* serovar Ballum (n=2)] and one serum (3.3%, 95% CI [0.1, 17.2]) [sheep (n=1)] being positive for two serovars [*L. borgpetersenii* serovar Sejroe and *L. interrogans* serovar Hardjo, with the latter showing the higher titer.

Pathology

A total of 37 placentae were analyzed macroscopically. In seven cases autolysis impeded further analysis, three cases presented malformations and in eight cases no macroscopic changes were detected; for the remaining 19 placentae a histopathological examination was carried out (Table 4). The most frequent pattern in the morphological diagnosis was an acute necrotizing placentitis (n=16). In seven cases bacteria were visible histopathologically. Six placentae presented with addi-

Table 2: Comparison of the results of the molecular detection of *Coxiella burnetii* and *Chlamydia abortus* by real-time and end-point PCR in the 77 cases from sheep (41 cases) and goat (36 cases).

PCR	Sheep						Goat					
	C. burnetii			*C. abortus*			*C. burnetii*			*C. abortus*		
	pos.	%	95% CI	pos.	%	95% CI	pos.	%	95% CI	pos.	%	95% CI
real-time	19	46.3	30.7, 62.6	17	41.5	26.3, 57.9	19	52.8	35.5, 69.6	8	22.2	10.1, 39.2
end-point	1	2.4	0.1, 12.9	8	19.5	8.8, 34.9	1	2.7	0.1, 14.5	3	8.3	1.8, 22.5

Table 3: Summary of the results with the different diagnostic methods indicating percentages of positive cases (+) with 95% confidence intervals (CI).

Analysis		Sheep		Goat		Total	
		+ / total cases	Percentage (95% CI)	+ / total cases	Percentage (95% CI)	+ / total cases	Percentage (95% CI)
Staining		6/41	14.6 (5.6, 29.2)	5/36	13.9 (4.7, 29.5)	11/77	14.3 (7.4, 24.1)
Cultured possible abortive bacteria		10/41	24.4 (12.4, 40.3)	8/36	22.2 (10.1, 39.2)	18/77	23.4 (14.5, 34.4)
Cultured fungi*		12/41	29.3 (16.1, 45.5)	4/36	11.1 (3.1, 26.1)	16/77	20.8 (12.4, 31.5)
Real-time PCR	*C. burnetii*	19/41	46.3 (30.7, 62.6)	19/36	52.8 (35.5, 69.6)	38/77	49.4 (37.8, 61.0)
	C. abortus	17/41	41.5 (26.3, 57.9)	8/36	22.2 (10.1, 39.2)	26/77	33.8 (23.4, 45.4)
	Leptospira spp.	3/41	7.3 (1.5, 19.9)	0/36	0	3/77	3.9 (0.8, 11.0)
	T. gondii	3/17	17.6 (3.8, 43.4)	0/13	0	3/30	10 (2.1, 26.5)
	N. caninum	0/7	0	1/6	16.7 (0.4, 64.1)	1/13	7.7 (0.2, 36.0)
Serology	*B. melitensis*	0/23	0	0/17	0	0/40	0
	C. burnetii	0/22	0	0/15	0	0/37	0
	C. abortus	8/21	38.1 (18.1, 61.6)	4/15	26.7 (7.8, 55.1)	12/36	33.3 (18.6, 51.0)
	Leptospira spp.	6/19	31.6 (12.6, 56.6)	2/11	18.2 (2.3, 51.8)	8/30	26.7 (12.3, 45.9)
Multiple abortifacients		16/41	39.0 (24.2, 55.5)	8/36	22.2 (10.1, 0.32)	24/77	31.2 (21.1, 42.7)

* Only in one case confirmed as abortive agent [sheep: 1/41, 2.4% (0.1, 12.9); total: 1/77, 1.3% (0.0, 7.0)].

tional vasculitis and in two cases fungal structures were found. Tachyzoites from *T. gondii* were observed in one case. Table 4 relates the pathological findings for 27 placentae (eight without macroscopical changes and 19 with histopathological results) to the bacteriological, mycological and parasitological results and point out the most likely etiology.

Overall, in 19 cases (24.7%, 95% CI [15.6, 35.8]) of the total of 77 no bacterial, fungal or parasitic cause of abortion could be identified. In 34 cases (44.2%, 95% CI [32.8, 55.9]) a single abortive agent was identified and in 24 cases (31.2%, 95% CI [21.1, 42.7]) multiple possible infectious abortifacients were found. A summary of the results by animal species and overall is provided in Table 3.

Table 4: Pathological findings in relation to the bacteriological, mycological and parasitological results from 27 placentae with the most likely etiology.

Case	Histopathology		Bacteriological and mycological culture	Molecular detection		Serology[a,c]	Most likely etiology
	Placentitis	Vasculitis		Bacteria[a]	Parasites[b]		
12Ue1433			*Escherichia coli*	all neg	Tg neg		*E. coli* septicemia
12Ue1448	ND	ND		Ca / Cb / L	Tg neg		Suspicion of intoxication
12Ue1466	ND	ND	*Escherichia coli*	all neg	Tg neg		*E. coli* septicemia
12Ue1509	X	X		Ch	Tg neg		Not further identifiable *Chlamydiales*
13Ue0067	ND	ND		Ch / Cb	Tg neg		Inconclusive, no pathological lesions
13Ue0201	ND	ND		all neg	-		Undetermined, probably non-infectious
13Ue0273	ND	ND	*Salmonella enterica* subsp. *diarizonae* 61:(k):1,5,(7)	Ch	-		Inconclusive, no pathological lesions
13Ue0428	ND	ND		Ch	-		Inconclusive, no pathological lesions
13Ue0485	ND	ND		Ch	Tg / Nc neg	Ca	Inconclusive, no pathological lesions
13Ue1427	ND	ND		all neg	Tg neg		Undetermined, probably non-infectious
15A0007	X	X		Ca	-	Ballum	*C. abortus*
15A0013	X			Cb	-		*C. burnetii*
15A0039	X			Cb	-		*C. burnetii*
15A0055	X			Cb	-		*C. burnetii*
15A0056	X			all neg	-	Hardjo	Inconclusive, no *Leptospira* sp. DNA detected
15A0062	X	X		Ca	-	Hardjo	*C. abortus*
15A0064	X		*Enterococcus casseliflavus*	Cb	-		*C. burnetii* with *E. casseliflavus* septicemia
15A0072				Ch / Cb	-		Inconclusive, no pathological lesions
15A0073	X			Cb	-		*C. burnetii*
15A0189	X	X		Ca / Cb	-		*C. abortus, C. burnetii*
16A0011	X	X		Ca / Cb	-		*C. abortus, C. burnetii*
16A0016				Ca / Cb	-		Inconclusive, no pathological lesions
16A0021	X		*Escherichia coli*	Ca / Cb	Tg		*T. gondii* (presence of tachyzoites) with *E. coli* septicemia
16A0028	X		*Salmonella enterica* subsp. *diarizonae* 61:(k):1,5,(7)	Cb	Tg		*T. gondii*
16A0061	X		*Wickerhamomyces* sp.	Ch / Cb	-	Pomona	Fungal abortion (intralesional fungal structures)
16A0066	X	X		Ca / Cb	Nc / Tg neg	Ca	*C. abortus*
16A0074	X		*Salmonella enterica* subsp. *enterica* Abortusovis	Ca / Cb	Tg neg		*S.* Abortusovis

[a] Ca = *C. abortus*, Cb = *C. burnetii*, Ch = *Chlamydiales*, L = pathogenic *Leptospira* spp., neg = negative; [b] Tg = *Toxoplasma gondii*, Nc = *Neospora caninum*; [e] leptospiral serovar

Statistical analysis

The level of agreement, calculated as Cohen's kappa coefficient, between serological analyses and real-time PCR for *C. burnetii*, *C. abortus* and pathogenic *Leptospira* spp. is shown in Table 5. For *C. abortus* the two methods showed a moderate level of agreement ($\kappa = 0.464$), while for *C. burnetii* and *Leptospira* spp. the level of agreement was poor with $\kappa = 0.000$ and $\kappa = 0.108$, respectively.

Discussion

Requirements for a comprehensive broad-spectrum abortion investigation (Kirkbride, 1993; Borel *et al.*, 2014) are demanding as outlined in the introduction. Incomplete submission of abortion material and a reduced diagnostic spectrum due to economic reasons hamper in most cases a final diagnosis. This entails that our understanding of abortions remains to date limited in some regards. For example, the role of *S. enterica* subsp. *diarizonae* 61:(k):1,5,(7), detected in three cases of ovine abortion, remains unclear in two cases since the agent could only be demonstrated in placenta. The finding might merely be due to carrier status of the animals and subsequent environmental contamination of the placenta.

While some important abortive agents such as the public health relevant *L. monocytogenes* and *S. enterica* subsp. *enterica* as well as opportunistic septicemia causing bacteria, e.g. *E. coli* and *S. uberis* could be revealed by bacteriological culture, the clarification rate by culture only (18 cases, 23.4%) is limited. In addition, difficult to grow bacteria, such as *C. burnetii*, *C. abortus* and *Leptospira* spp., require molecular diagnostic methods to be put in evidence (Vidal *et al.*, 2016).

The real-time PCR for *C. burnetii* and *C. abortus* showed higher sensitivity compared to the end-point PCRs with detection rates of 49.3% and 32.5% compared to 2.5% and 14.3%, respectively (Table 2). Real-time PCR is known to be more sensitive than end-point PCR in general and especially for *C. burnetii* this was previously reported for small ruminants in the United Kingdom (Jones *et al.*, 2016). Stamp's modification of the Ziehl-Neelsen was only positive in ten cases and is hence clearly insufficiently sensitive for the detection of *C. burnetii* and *C. abortus*.

None of the sera was positive for *C. burnetii* by ELISA whereas 49.3% of the cases were positive in real-time PCR. Serology is known to be of little use for diagnosing abortion due to coxiellosis in individual animals because shedding can occur prior to seroconversion or seroconversion may never occur despite infection and shedding of *C. burnetii* over a long time (Berri *et al.*, 2002, 2007; Rousset *et al.*, 2009). Along these lines a previous study focused on *C. burnetii* in the Swiss small ruminant population observed a seroprevalence at animal-level of 1.8% in healthy sheep and of 3.4% in healthy goats (Magouras *et al.*, 2017). When applying real-time PCR to abortion material Magouras *et al.* (2017) detected DNA of *C. burnetii* in 44.4% and 44.2% of the ovine and caprine samples, respectively, with a high shedding rate in 13.4% of the samples that highlights the zoonotic risk posed to humans in contact with these animals. Of note is that in herds or flocks with abortion problems due to *C. burnetii* also animals that have not aborted may shed the bacterium in high numbers (OIE, 2015).

In the present study, serological detection of antibodies against *C. abortus* correlated better with the molecular findings (33.3% and 32.5%). It is important to keep in mind that the mere presence of antibodies is generally not indicative of an ongoing infection and hence does not corroborate a cause of abortion. Nonetheless, in the specific case of brucellosis serology remains the first-line method of choice for presumptive diagnosis, especially since PCR may lead to false-positive results and is considered unsuitable for assessing individual animal freedom from infection (OIE, 2016).

Regarding leptospirosis our data show that it probably plays a minor role in abortion of sheep and goats. Interestingly, one case was positive by real-time PCR and seroconversion had not yet occurred resulting in a negative MAT result. This indicates an early stage of infec-

Table 5: Cohen's kappa (κ) coefficient with standard errors (SE) and 95% confidence intervals (CI) showing the degree of agreement between molecular and serological techniques for the diagnosis of *C. burnetii*, *C. abortus* and pathogenic *Leptospira* spp. SE and CI are not calculated when $\kappa = 0$.

	C. burnetii	*C. abortus*	*Leptospira spp.*
Observed agreement	45.95%	72.22%	74.19%
Expected agreement	45.95%	48.15%	71.07%
κ	0.000	0.464	0.108
SE of κ	-	0.130	0.162
95% CI	-	0.209 to 0.720	-0.209 to 0.425
Level of agreement	Poor	Moderate	Poor

tion and emphasizes the usefulness of molecular detection for prompt detection of leptospirosis.

PCR has been reported as a reliable method for the detection of *N. caninum* and *T. gondii* in abortion material (Collantes-Fernandez *et al.*, 2002; OIE, 2017). The finding of a positive case of *N. caninum* underlines its sporadic implication in abortion in small ruminants. *N. caninum* is commonly attributed to cattle only, and even though cases in small ruminants have been previously described (Hässig *et al.*, 2003) it usually remains undiagnosed. The positivity rate of *T. gondii* in our study (10%) is slightly below previously reported levels (16.5%) in abortion in Swiss small ruminants (Chanton-Greutmann *et al.*, 2002).

Overall, broad-spectrum culture only identified a possible abortive agent in 18 cases (23.4%), whereas combination with molecular techniques raised this number to 58 (75.3%) underlining that PCR has become an invaluable tool to diagnose infectious diseases. Equipment acquisition and reagents may be costly and not readily available in some countries, yet, the possibility of automation, improved precision and reduced labor requirements along with the continuously decreasing cost and increasing accessibility for laboratories countervail the disadvantages (Pestana *et al.*, 2010). PCR is currently considered the most reliable tool for the diagnosis of infectious abortions (Sidi-Boumedine *et al.*, 2010). Thus, the application of molecular techniques appears to be the best choice for cost-efficient and timely diagnostics of the main abortive agents. However, without the backup of the pathological examination this would lead to overestimation of some agents, considering that it is not possible to differentiate between viable and non-viable bacteria and between environmental contamination and infection (Roest *et al.*, 2012; van den Brom *et al.*, 2015). A selection of analyses upon triage by the pathologists seems to be a good approach for abortion diagnostics. It is however important to notice, that the combination of macroscopical and histopathological examination of placenta and fetal tissues in our study do not always allow etiological diagnosis as pathological changes in different infections may be very similar.

Interestingly, our results indicate a high number of mixed presence of possible abortive agents (31.2%) implying that many abortions could be multifactorial events due to yet unknown interactions of classical and/or opportunistic abortive agents (Table 4). This result also shows the complexity of abortion diagnostics leading to difficulties in assigning an etiological agent when investigations are incomplete.

While the identification of opportunistic abortive causes is relevant for the exclusion of other epizootic diseases, one has to keep in mind that its implications for the farm management are minor. For the surveillance of abortions at national level we would therefore propose a stepwise approach to abortion diagnostics; of note is that this requires complete material submission. We recommend the combination of the pathological examination with legally prescribed tests and cost-effective real-time PCR methods and, if no infectious cause is revealed, subsequent analysis of culturable bacteria. This approach would allow for the detection of a broad spectrum of abortive agents with a focus on those important in terms of epizootic and zoonotic risk such as the bacteria *C. abortus*, *C. burnetii*, pathogenic *Leptospira* spp., *L. monocytogenes* and *S. enterica* and the parasites *N. caninum* and *T. gondii*.

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Examens bactériologiques, mycologiques, parasitologiques et pathologiques des avortements chez les petits ruminants entre 2012 et 2016

Les avortements représentent un problème à la fois clinique et économique avec des conséquences en matière d'épizooties et un risque de zoonose pour certains agents. Diverses bactéries, champignons et parasites peuvent causer des avortements mais le diagnostic de routine, orienté sur les coûts, se concentre sur les principaux agents épizootiques. Afin d'avoir une vision large sur les agents d'avortements les plus fréquents et sur ceux qui sont sous-diagnostiqués, nous avons examinés 41 avortements de moutons et 36 de chèvres au moyen d'une coloration de Ziehl-Neelsen modifiée selon Stamp, de cultures ciblant les agents d'avortements classiques et opportunistes, d'une PCR en temps réel ciblant *C. burnetii*, *C. abortus*, les leptospires pathogènes, *Toxoplasma gondii* et *Neospora caninum*. Lorsque du sérum de la mère était disponible, nous avons procédé à une recherche d'anticorps contre *B. melitensis*, *C. burnetii*, *C. abortus* et *Leptospira* spp. Dans 37 cas, on disposait d'assez de tissu placentaire pour des examens pathologiques. Sur les 77 cas, 11 (14.3%) étaient positifs à la coloration alors que la PCR en temps réel démontrait la présence de *C. burnetii* et de *C. abortus* dans 49.3% respectivement 32.5% des cas. On a trouvé des anticorps contre *C. abortus* und *Leptospira* spp. dans 33.3% respectivement 26.7% des cas. Dans 23.4% des cas, on a pu mettre en évidence des pathogènes bactériens cultivables. Un avortement mycotique a été confirmé dans 1.3% des cas. Dans 44.2% des cas, un seul agent abortif était présent et dans 31.2% des cas, on trouvait plusieurs agents potentiels. Notre étude indique que le plus haut taux de diagnostic ne peut être atteint qu'en combinant diverses méthodes et montre le rôle possible de multi infections dans l'origine des avortements.

Esami batteriologici, micologici, parassitologici e patologici negli aborti di piccoli ruminanti tra il 2012 e il 2016

Gli aborti rappresentano un problema clinico ed economico con conseguenze sulla salute degli animali e sui rischi di zoonosi dovuti a certi agenti patogeni abortivi. Vari batteri, funghi e parassiti possono provocare aborti ma la diagnostica di routine è orientata ai costi e copre solo gli agenti patogeni delle più importanti malattie degli animali. Per poter coprire un ampio spettro di patogeni abortivi comuni e sotto diagnosticati, abbiamo esaminato 41 aborti di pecora e 36 di capra mediante colorazione modificata di Ziehl-Neelsen secondo Stamp, culture di comuni e opportunisti patogeni abortivi, PCR real-time sui patogeni *C. burnetii*, *C. abortus*, *Leptospira* spp., *Toxoplasma gondii* e *Neospora caninum*. Nel caso che il siero della madre fosse a disposizione è stata eseguita l'individuazione degli anticorpi contro *B. melitensis*, *C. burnetii*, *C. abortus* e *Leptospira* spp. In 37 casi la quantità di tessuto placentare era sufficiente per degli esami patologici. Dei 77 casi, 11 (14.3%) erano positivi alla colorazione, mentre la PCR real-time di *C. burnetii* e *C. abortus* risultava solo nel 49.3% e nel 32.5% dei casi. Inoltre sono stati rilevati anticorpi contro *C. abortus* e *Leptospira* spp. (33.3 e 26.7%). Nel 23.4% è stato rilevato un patogeno batterico coltivabile. Nell'1.3% dei casi l'aborto era causato da un fungo. Un singolo agente abortivo è stato rilevato nel 44.2% dei casi e nel 31.2% erano presenti vari possibili agenti abortivi. Il nostro studio ha dimostrato che l'alto tasso di rilevamento si può ottenere solo con una combinazione di metodi diversi e che vi è un possibile ruolo delle infezioni multiple nella causa abortiva.

References

Abril C., Thomann A., Brodard I., Wu N., Ryser-Degiorgis M. P., Frey J., Overesch G.: A novel isolation method of *Brucella* species and molecular tracking of *Brucella suis* biovar 2 in domestic and wild animals. Vet. Microbiol. 2011, 150: 405-410.

Alton G. G., Jones L. M., Angus R. D., Verger J. M.: Techniques for the Brucellosis Laboratory. Institut National de la Recherche Agronomique, Paris, France, 1988.

Berri M., Rousset E., Champion J. L., Russo P., Rodolakis A.: Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection. Res. Vet. Sci. 2007; 83: 47-52.

Berri M., Souriau A., Crosby M., Rodolakis A.: Shedding of *Coxiella burnetii* in ewes in two pregnancies following an episode of *Coxiella* abortion in a sheep flock. Vet. Microbiol. 2002, 85: 55-60.

Borel N., Frey C., Gottstein B., Hilbe M., Pospischil A., Franzoso F., Waldvogel A.: Laboratory diagnosis of ruminant abortion in Europe. Vet. J. 2014, 200: 218-229.

Chanton-Greutmann, H., Thoma, R., Corboz, L., Borel, N., Pospischil, A.: Abortion in small ruminants in Switzerland: Investigations during two lambing seasons with special regard to *Chlamydiae*. Schweiz. Arch. Tierheilk. 2002, 144: 483-492.

Collantes-Fernandez, E., Zaballos, A., Alvarez-Garcia, G., Ortega-Mora, L. M.: Quantitative detection of *Neospora can-*

inum in bovine aborted fetuses and experimentally infected mice by real-time PCR. J. Clin. Microbiol. 2002. 40:1194-1198.

Costa J. M., Pautas C., Ernault P., Foulet F., Cordonnier C., Bretagne S.: Real-time PCR for diagnosis and follow-up of *Toxoplasma* reactivation after allogeneic stem cell transplantation using fluorescence resonance energy transfer hybridization probes. J Clin. Microbiol. 2000, 38: 2929–2932.

Givens M. D., Marley M. S. D.: Infectious causes of embryonic and fetal mortality. Theriogenology. 2008: 70: 270-85.

Harwood L. J., Thomann A., Brodard I., Makaya P. V., Perreten V.: Campylobacter fetus subspecies *venerealis* transport medium for enrichment and PCR. Vet. Rec. 2009, 165: 507-508.

Häsler B., Regula G., Stärk K. D., Sager H., Gottstein B., Reist M.: Financial analysis of various strategies for the control of *Neospora caninum* in dairy cattle in Switzerland. Prev. Vet. Med. 2006, 77: 230-253.

Hässig M., Sager H., Reitt K., Ziegler D., Strabel D., Gottstein B.: Neospora caninum in sheep: a herd case report. Vet. Parasitol. 2003, 117: 213-220.

Jones R. M., Twomey D. F., Hannon S., Errington J., Pritchard G. C., Sawyer J.: Detection of *Coxiella burnetii* in placenta and abortion samples from British ruminants using real-time PCR. Vet. Rec. 2010, 167: 965-967.

Kirkbride C. A.: Laboratory diagnosis of livestock abortion. Iowa State University Press, 1990.

Kirkbride C. A.: Bacterial agents detected in a 10-year study of bovine abortions and stillbirths. J. Vet. Diagn. Invest 1993, 5: 64-68.

Longbottom D., Coulter L. J.: Animal Chlamydioses and Zoonotic Implications. J. Comp. Path. 2003, 128: 217-244.

Magouras, I., Hunninghaus J., Scherrer S., Wittenbrink M. M., Hamburger A., Stark K. D., Schüpbach-Regula G.: Coxiella burnetii Infections in Small Ruminants and Humans in Switzerland. Transbound. Emerg. Dis. 2017, 64: 204-212.

Müller, N., Vonlaufen N., Gianinazzi C., Leib S. L, Hemphill A.: Application of real-time fluorescent PCR for quantitative assessment of *Neospora caninum* infections in organotypic slice cultures of rat central nervous system tissue. J. Clin. Microbiol. 2002, 40: 252-255.

OIE World Organisation for Animal Health: Chapter 2.7.6. Enzootic Abortion of Ewes (Ovine Chlamydiosis) (Infection with *Chlamydophila abortus*). OIE Terrestrial Manual 2012, 1008-1016.

OIE World Organisation for Animal Health: Chapter 2.1.12. Leptospirosis. OIE Terrestrial Manual 2014, 1-15.

OIE World Organisation for Animal Health: Chapter 2.1.16. Q Fever. OIE Terrestrial Manual 2015, 1-15.

OIE World Organisation for Animal Health: Chapter 2.1.4. Bovine Brucellosis. OIE Terrestrial Manual 2016, 1-44.

OIE World Organisation for Animal Health: Chapter 2.9.9. Toxoplasmosis. OIE Terrestrial Manual 2017, 1-12.

Pantchev A., Sting R., Bauerfeind R., Tyczka J., Sachse K.: New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue *samples*. Vet. J. 2009, 181: 145-150.

Pestana, E. A. B. S., Diallo A., Crowther J. R., Viljoen G. J.: Early, Rapid and Sensitive Veterinary Molecular Diagnostics - Real Time PCR Applications. 2010. Springer Netherlands.

Pospischil A., Thoma R., Hilbe M., Grest P., Zimmermann D., Gebbers J.-O.: Abort beim Menschen durch *Chlamydophila abortus* (*Chlamydia psittaci* serovar1). Schweiz. Arch. Tierheilk. 2002, 144: 463–466.

Reitt K., Hilbe M., Voegtlin A., Corboz L., Haessig M., Pospischil A.: Aetiology of bovine abortion in Switzerland from 1986 to 1995 - a retrospective study with emphasis on detection of *Neospora caninum* and *Toxoplasma gondii* by PCR. J. Vet. Med. A Physiol Pathol. Clin. Med. 2007, 54(1): 15-22.

Roest, H. J., van Gelderen B., Dinkla A., Frangoulidis D., van Zijderveld F., Rebel J.,van Keulen L.: Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. PLoS One. 2012, 7: e48949.

Roest H. I., Bossers A., van Zijderveld F. G., Rebel J. M.: Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis and control of the zoonotic disease Q fever. Vet. Q. 2013, 33: 148-60.

Rousset E., Berri M., Durand B., Dufour P., Prigent M., Delcroix T., Touratier A., Rodolakis A.: Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl. Environ. Microbiol. 2009, 75: 428-433.

Scheidegger A., Vonlaufen N., Naguleswaran A., Gianinazzi C., Müller N., Leib S. L., Hemphill A.: Differential effects of interferon-gamma and tumor necrosis factor-alpha on *Toxoplasma gondii* proliferation in organotypic rat brain slice cultures. J. Parasitol. 2005, 91: 307-315.

Scott P. R.: Sheep medicine, second edition. CRC Press, 2015, 52.

Sidi-Boumedine K., Rousset E., Henning K., Ziller M., Niemczuck K., Roest H. I. J., Thiéry R.: Development of harmonised schemes for the monitoring and reporting of Q-fever in animals in the European Union. EFSA Scientific Report on Question No EFSA-Q-2009-00511 2010, 1-48.

van den Brom R., Lievaart-Peterson K., Luttikholt S., Peperkamp K., Wouda W., Vellema P.: Abortion in small ruminants in the Netherlands between 2006 and 2011. Tijdschr. Diergeneeskd. 2012, 137: 450-7.

Vidal Lopez S., Greub G., Aeby S., Perreten V., Rodriguez-Campos S.: Neglected zoonotic agents in cattle abortion: molecular and serological screening of difficult to grow bacteria. J. Bacteriol. Parasitol. 2016, 7:6 (Suppl).

Villumsen S., Pedersen R., Borre M. B., Ahrens P., Jensen J. S., Krogfelt K. A.: Novel TaqMan(R) PCR for detection of *Leptospira* species in urine and blood: pit-falls of in silico validation. J. Microbiol. Methods 2012, 91: 184-190.

von Tavel L., Fivian R., Kirchhofer M., P. Boujon, Hirsbrunner G.: Abort beim Schaf: *Salmonella* Abortusovis Epidemie 2005 in der Westschweiz. Schweiz. Arch. Tierheilk. 2005, 147: 445–452.

Willems, H., Thiele, D., Frolichritter, R., Krauss, H.: Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). J. Vet. Med. B. Zbl. Vet. B. Infect. 1994, 41: 580-587.

Williams E., O'Donovan J.: Ovine abortion: an overview. Ir. Vet. J., 2009, 62: 342-346.

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