Prevalence of *Chlamydia abortus* in Belgian ruminants

Prevalentie van Chlamydia abortus *bij herkauwers in België*

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ABSTRACT

***Chlamydia* (*C.*) *abortus* enzootic abortion still remains the most common cause of reproductive failure in sheep-breeding countries all over the world. *Chlamydia abortus* in cattle is predominantly associated with genital tract disease and mastitis. In this study, Belgian sheep (n=958), goats (n=48) and cattle (n=1849) were examined, using the ID Screen™ *Chlamydia abortus* indirect multi-species antibody ELISA. In the sheep, the highest prevalence rate was found in Limburg (4.05%). The animals of Antwerp, Brabant and Liège tested negative. The prevalence in the remaining five regions was low (0.24% to 2.74%). Of the nine goat herds, only one herd in Luxembourg was seropositive. In cattle, the highest prevalence rate was found in Walloon Brabant (4.23%). The animals of Limburg and Namur tested negative. The prevalence rate in the remaining seven regions ranged between 0.39% and 4.02%.**

SAMENVATTING

Enzoötische abortus veroorzaakt door *Chlamydia* (*C.*) *abortus* is nog steeds de meest voorkomende oorzaak van reproductiestoornissen bij schapen. Bij runderen wordt *C. abortus* geassocieerd met infecties van het geslachtsstelsel en mastitis. In de voorliggende studie werden Belgische schapen (n=958), geiten (n=48) en runderen (n=1849) onderzocht aan de hand van de ID Screen™ *Chlamydia abortus* indirecte multispecies antistof ELISA. Bij de schapen werd de hoogste prevalentie gevonden in Limburg (4,05%). De dieren van Antwerpen, Brabant en Luik waren negatief. De prevalentie in de overige vijf regio's was laag (0,24% tot 2,74%). Van negen geitenboerderijen was slechts één bedrijf uit Luxemburg positief. Bij de runderen werd de hoogste seroprevalentie in Waals-Brabant (4,23%) gevonden. De dieren uit Limburg en Namen waren negatief. De prevalentie in de overige zeven regio's varieerde van 0,39% tot 4,02%.

INTRODUCTION

Chlamydiaceae are gram-negative obligate intracellular bacteria. Recently, the assignment from the single genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila* by Everett et al. (1999) has been reconsidered. Based on comparative genome analysis of several *Chlamydiaceae* genomes, the *Chlamydiaceae* are currently again reunited into a single genus, *Chlamydia* (Kuo and Stephens, 2011).

Ruminants can become infected with *Chlamydia* (*C.*) *abortus*, *C. pecorum*, *C. psittaci* and rarely with *C. suis* (Reinhold et al., 2011). *Chlamydiaceae* infections in cattle (*Bos taurus*) have been known to occur since 1940, when McNutt isolated intracellular organisms from cases of sporadic bovine encephalomyelitis

in feedlot cattle (McNutt and Waller, 1940). Thereafter, a number of studies worldwide reported epizootic bovine abortion in cattle caused by *C. abortus*. The pathogen also caused bovine mastitis, epididymitis and seminal vesiculitis, and was excreted in bull semen (Kaltenboeck et al., 2005; Reinhold et al., 2011). Chlamydial strains from ruminant abortion were first classified as mammalian *Chlamydia psittaci* serotype 1, mammalian *C. psittaci* biovar 1 or *C. psittaci* outer membrane protein A (*ompA*) gene type B577 strains (Kaltenboeck et al., 1993). Later on, the organism was reclassified as *C. abortus* (Everett et al., 1999).

In the previous century, a second chlamydial agent was reported to be associated with growth retardation, abortion, sporadic bovine encephalomyelitis, pneumonia, enteritis, polyarthritis, keratoconjunctivitis, nephritis or purulent endometritis in cattle. This chlamydial agent was first identified as mammalian *C. psittaci* serotype 2 or mammalian *C. psittaci* biovar 2. However, it became clear that these disease manifestations were actually induced by a serologically and pathologically diverse new species designated *C. pecorum* (Fukushi and Hirai, 1992; Kaltenboeck et al., 1993). This agent is not known to cause disease in humans.

Thus, *C. abortus* in cattle is predominantly associated with genital tract disease and mastitis. Exposure of pregnant women to *C. abortus* infected ruminants may lead to abortion or stillbirth (Hadley et al., 1992; Pospischil et al., 2002; Longbottom and Coulter, 2003; Baud et al., 2008).

Over the years, *Chlamydia* diagnosis in animals has improved (Sachse and Longbottom, 2013), and recent use of highly specific and sensitive nucleic amplification methods has also identified *C. psittaci*, albeit less frequently (Borel et al., 2006; Twomey et al., 2006; Kauffold et al., 2007; Pantchev et al., 2009; Kemmerling et al., 2009), and rarely *C. suis* in cattle (Teankum et al., 2007; Pantchev et al., 2009). Birds and swine are respectively, the main hosts for these two species. *C. psittaci* causes reproductive failure and respiratory disease in cattle, while the clinical significance of the occurrence of *C. suis* in cattle remains unclear (Longottom and Coulter, 2003; Reinhold et al., 2011).

Chlamydiaceae infections in sheep and goats are caused by *C. abortus* and *C. pecorum*. Despite the existence of commercially available vaccines, *C. abortus*-induced lamb loss and enzootic abortion during the last third of gestation still remain the most common causes of reproductive failure in sheep-breeding countries all over the world. *C. abortus* causes major economic losses in affected flocks. Enzootic abortion in ewes (OEA) is a notifiable disease in Belgium and is also notifiable to the OIE. *C. pecorum* occasionally causes abortion in small ruminants. Especially in lambs, *C. pecorum* can induce, according to the subtype, pneumonia, polyarthritis, conjunctivitis, enteritis or clinically inapparent infections (Rodolakis et al., 1998).

The authors could not find any data on zoonotic transfer of *C. abortus* in Belgium; perhaps, because of unawareness by physicians, which is due to the absence of an efficient risk assessment.

It is difficult to conduct a zoonotic risk assessment, as epidemiological data on *C. abortus* in Belgium are lacking. Therefore, in the present study, the seroprevalence of *C. abortus* on Belgian sheep, goats and cattle farms is examined.

MATERIALS AND METHODS

Transversal seroepidemiological study in small and large ruminants

Sheep (n=958), goats (n=48) and cattle (1849) sera were provided by the biobanks of the following governmental institutions ARSIA (Association Régionale de Santé et d'Identification Animales, Ciney), DGZ (Dierengezondheidszorg Vlaanderen, Drongen) and CODA-CERVA (Veterinary and Agrochemical Research Centre, Brussels). For sheep and cattle, the examined herds were proportional to the number of herds in each Belgian province. The survey included 95 sheep herds from the list of volunteers enrolled in the Visna-Maedi certification program. For cattle herds (n=129), samples originated from the whole Belgian population. In addition, available sera of nine goat herds were serologically examined. The available sera of cattle were limited to animals ageing at least 24 months, while sheep and goat sera were not restricted by age category. None of the examined animals were vaccinated against *C. abortus*.

Sample collection and preparation were as follows: blood was collected by vene puncture during the winter of 2009-2010, incubated overnight at room temperature, centrifuged (325 x g, 4°C, 10 min), and serum was collected and stored at -20 °C. All sera were tested for the presence of antibodies against *C. abortus* using the same batch of the ID Screen™ *Chlamydia abortus* indirect multi-species ELISA (IDVET Innovative Diagnostics, Montpellier, France). The assay was performed according to the instructions of the manufacturer. Samples were tested at a dilution of 1/100. For each sample, the S/P was calculated, which is 100 times the OD_{450} of the sample/mean value of the positive control OD_{450}. Samples presenting a S/P of greater than or equal to 60% were considered positive.

Statistics

All data were recorded in a MS Access® relational database. In sheep, the following variables were recorded at animal level: identity, disease status, probability of being sampled among the population herd of origin (Dohoo et al., 2010). At herd level size (two categories: ≤ and > 20 animals), disease status, geographical location, probability of being sampled and

province were recorded. In cattle, the same variables were recorded but four categories in herd size were considered (1-10, 11-50, 51-120 and >120 animals). At animal level, the age category was added and at herd level the production type (pure beef production versus other) was recorded.

Both at animal and herd levels, logistic regressions were used to assess apparent seroprevalences of *C. abortus* and to analyse potential multivariate relationships between risk factors (recorded variables) and individual disease status using STATA SE (StataCorp 4905 Lakeway Drive, College Station, Texas 77845, USA) also providing 95% confidence intervals (CI). Herd clustering was taken into account as random effect (Dohoo et al., 2010).

RESULTS

Transversal seroepidemiological study in sheep

From nine different Belgian regions, 958 sheep sera were tested (Table 1). The highest prevalence rate was found in Limburg (4.05%). The animals sampled in Antwerp, Brabant and Liège tested negative. The prevalence in the remaining five regions was rather low, ranging from 0.24% to 2.74%. Prevalence rates for all seropositive regions were statistically the same. The multivariate logistic regression model revealed a seroprevalence rate of 0.68% (95% confidence interval (95 CI), 0.30-1.49) for the tested Belgian sheep population.

Fifteen of 95 (15.7%) sheep herds tested seropositive. The highest percentage of positive herds was found in Hainaut (Table 2). The *C. abortus* herd level prevalence was 6.15% (95% CI, 1.93-17.94), as calculated by the multivariate logistic regression model. Only herds with less than 50 sheep were seropositive. The *C. abortus* prevalence rate was higher in smaller herds (Table 3). However, taken all together, the *Chlamydia abortus* morbidity in the sheep was low.

Transversal seroepidemiological study in goats

Nine goat herds were tested, of which two herds with sample size ≥ 10 and seven herds with sample size <10. None of the seven low-sample size herds tested seropositive. One of the two higher sample size herds tested seropositive. This herd, which was located in Luxemburg, revealed a high intraherd prevalence (52.9%; 9/17).

Transversal seroepidemiological study in cattle

From ten different Belgian regions, 1849 sera were tested (Table 4). The highest prevalence rate was found in Walloon Brabant (4.23%). The animals sampled in Limburg and Namur tested negative. The prevalence rate for the remaining seven regions ranged between 0.39% and 4.02%, without being statistically different

Table 1. *C. abortus* seroprevalence in sheep (N = 958).

Region	Sheep			
	Animals tested (N)	Prevalence (%)	95% CI Inferior limit (%)	95% CI Superior limit (%)
Antwerp	60	0.00	0.00	0.00
Brabant	134	0.00	NC	NC
Hainaut	100	0.79	0.36	6.56
Liège	70	0.00	NC	NC
Limburg	50	4.05	0.92	16.13
Luxemburg	82	2.74	0.37	17.53
Namur	61	0.24	0.01	3.83
East-Flanders	210	0.49	0.15	1.60
West-Flanders	191	0.93	0.22	3.85

Table 2. *C. abortus* seroprevalence in sheep herds (N = 95).

Region	Herds tested (N)	Prevalence (%)	Inferior limit (%)*	Superior limit (%)*
Antwerpen	8	0.0	0	-
Brabant	11	0.0	0	-
Hainaut	9	33.3	11.1	66.7
Liège	6	0.0	0	-
Limburg	9	22.2	5.6	57.9
Luxemburg	5	20.0	2.7	69.1
Namur	6	16.7	2.3	63.1
East-Flanders	25	16.0	6.1	35.7
West-Flanders	16	18.8	6.2	44.7

*Multivariate logistic regression with 95% confidence interval - Impossible to calculate the upper limit of the 95% confidence interval.

Table 3. Number of positive and negative sheep herds in function of herd size and minimum and maximum prevalences in positive herds.

Herd size	Herd status		Intraherd seroprevalence (%) for positive herds*		
	Negative	Positive	Minimum	Mean	Maximum
1 to 10	42	8	5.88	12.23	20.00
11 to 50	32	6	5.88	9.97	16.67
51 to 100	2	0	0.00	0.00	0.00
> 100	5	0	0.00	0.00	0.00

Table 4. *C. abortus* seroprevalence in cattle (N = 1849).

Region	Animals tested (N)	Prevalence (%)	Inferior limit (%)*	Superior limit (%)*
Antwerp	183	3.66	1.83	7.21
Hainaut	247	1.45	0.47	4.39
Liège	246	4.02	0.92	15.90
Limburg	83	0.00	0.00	8.74
Luxemburg	161	3.86	1.22	11.53
Namur	106	0.00	NC	NC
East-Flanders	356	0.41	0.10	1.67
Flemish Brabant	87	0.61	0.07	4.96
Walloon Brabant	30	4.23	2.67	6.63
West-Flanders	350	0.39	0.07	2.16

Table 5. *C. abortus* seroprevalence in cattle herds (N = 129).

Region	Herds tested (N)	Prevalence (%)	Inferior limit (%)*	Superior limit (%)*
Antwerp	14	35.71	15.70	62.37
Hainaut	16	25.00	9.71	50.82
Liège	16	12.50	3.14	38.60
Limburg	7	0.00	0.00	64.24
Luxemburg	9	22.22	5.60	57.90
Namur	8	0.00	0.00	68.62
East-Flanders	27	7.41	1.86	25.25
	7	14.29	1.97	58.06
Walloon Brabant	3	33.33	4.34	84.65
West-Flanders	22	9.09	2.28	29.96

Table 6. Number of positive and negative cattle herds in relation to herd size and minimum and maximum prevalences in positive herds.

Herd size	Herd status		Intraherd seroprevalence (%) for positive herds*		
	Negative	Positive	Minimum	Mean	Maximum
1 to 10	21	0	0.0	0.0	0.0
11 to 50	20	4	5.0	7.4	12.5
51 to 120	41	7	5.0	8.3	17.6
> 120	28	8	5.0	10.6	30.0

(Table 4). The prevalence rate was higher (albeit not statistically) in 24- to 48-months-old animals (prevalence 2.32%, 95% CI, 1.07-4.95), than in animals older than 48 months (prevalence 1.07%, 95% CI, 0.56-2.04). The prevalence rate was also higher (albeit not statistically) in beef cattle (prevalence 1.82%, 95% CI, 0.70-4.62), as compared to dairy or cross bred cattle (prevalence 1.59%, 95% CI, 0.72-3.46). For the cattle population, the multivariate logistic regression model revealed a seroprevalence rate of 1.69% (95% CI, 0.91-3.10).

Nineteen of 129 (14.72%) cattle herds tested seropositive. Looking at herd level, the highest percentage of positive cattle herds was found in Antwerp (Table 5). The *C. abortus* prevalence on Belgian herd level was 14.7% (95% CI, 9.60-21.90), as calculated by the multivariate logistic regression model. Again, no significant differences were observed regarding

age or cattle breed. Interestingly, all positive herds consisted out of at least eleven animals (Table 6). Larger herds (11 to 50, 50 to 120 or <120 animals) were more frequently positive (albeit not significantly) and the number of positive animals per herd augmented with an increasing herd size (although not significantly) (Table 6). Morbidity due to *Chlamydia abortus* in cattle was rather low, but albeit 2.5 times higher than in sheep.

DISCUSSION

C. abortus is frequently isolated from ruminants and is responsible for abortion, infertility, keratoconjunctivitis, pneumonia, enteritis, mastitis and arthritis (Reinhold et al., 2011). Transmission occurs orally or sexually. *C. abortus* is a zoonotic pathogen. Several cases of abortion have been reported in pregnant women after contact with sheep (McKinlay et al., 1985; Herring et al., 1987; Longbottom and Coulter, 2003; Walder et al., 2005; Janssen et al., 2006) and goats (Pospischil et al., 2002; Meijer et al., 2004). Human infection is characterized by acute flu-like symptoms followed by abortion and in some cases renal failure, hepatic dysfunction and extensive intravascular coagulation resulting in death. No information is available on the *C. abortus* infection status in Belgian ruminants or on the risk for human health. The authors therefore conducted a seroprevalence study in cattle, sheep and goats. The ID Screen™ *Chlamydia abortus* indirect multi-species ELISA was used (ID-VET Innovative Diagnostics, Montpellier, France), since it is the only commercially available *C. abortus* ELISA, which uses microwells coated with a *C. abortus*-specific synthetic peptide from the major outer membrane protein (MOMP). The specificity of the test is 99.5%, while the sensitivity for small ruminants is expected to be 80% (difficult to find a large infected population) (P. Pourquier, personal communication, 2012). However, in cattle, the sensitivity is even more difficult to determine, because of very low confirmed *C. abortus* cases in cattle (P. Pourquier, personal communication, 2012).

Current results revealed a seroprevalence rate of 1.69% for the Belgian cattle population. Seroprevalence in positive herds was relatively low, with generally only 1 or 2 seropositive animals on 10 to 20 tested animals per herd. This may explain a much higher rate of seronegativity at herd level in herds for which < 10 animals were tested. Wilson et al., (2012) performed a study in Irish cattle (100 herds, 20 samples/herd) using a soluble chlamydial antigen (detergent treated *Chlamydia* elementary bodies) ELISA detecting antibodies against both *C. abortus* and *C. pecorum* and reported a seroprevalence rate of 6.04% at animal level. Their results are comparable to the low seroprevalence found in the present study. However, the prevalence at herd level is much higher in Irish herds (60%). The seropositive rate in Belgian cattle herds in the current study could be an underestimation of the real level of infection of cattle in Belgium as the sensitivity of the ID VET ELISA could not yet be tested due to very few confirmed *C. abortus* cases in cattle. This might also explain the apparent absence of infected herds in the provinces of Limburg and Namur. Consequently, the human exposure rate may be underestimated. Seroprevalence studies on cattle in most other countries have reported higher infection rates at animal level (Reinhold et al., 2011). For instance, Wehrend et al. (2005) used a genus-specific enzyme-linked immunosorbent assay (ELISA). They found a seroprevalence of 41.5% in 445 dairy cows from 34 German farms, which is probably not abnormal as the ELISA detected antibodies against all members of the genus *Chlamydia.* Cavirani et al. (2001) examined 671 dairy cows of the Po Valley of Northern Italy using a commercial indirect ELISA (CHEKIT, Bommeli AG- IDEXX), and found a prevalence rate of 24.0%. These two studies focused only on dairy farms with fertility problems, which could explain the higher infection rates than in Belgian cattle, as this subpopulation has probably higher infection levels than the general population. Then again, results of Godin et al. (2008) suggest that *C. abortus* is rare in Swedish dairy cows with reproductive disorders. These authors examined 525 cows in 70 Swedish dairy herds by the use of both the CHEKIT ELISA and the commercial Pourquier *C. abortus* ELISA (Institut Pourquier, Montpellier, France). The Pourquier ELISA, in which a recombinant fragment of an 80–90 kDa polymorphic outer membrane protein as antigen is used, revealed a seroprevalence rate of 0.4% at animal level. The CHECKIT ELISA however, showed a seroprevalence of 28% (Godin et al., 2008). These and other experimental results indicate a lower specificity of the CHECKIT ELISA than of the Pourquier ELISA (Vretou et al., 2007; Godin et al., 2008, Wilson et al., 2009). The authors of the present study decided to use the Pourquier *C. abortus* ELISA. However, it has recently been removed from the market.

In the current study, serological examination of the sheep revealed a seroprevalence rate of 0.68%. As for cattle, the occurrence of *C. abortus* antibodies appears low in Belgian sheep as compared to most other reports on seroprevalence rates in non-vaccinated sheep: Switzerland (9.2 to 19.0%) (Borel et al., 2004, 2006; Blumer et al., 2012), Ireland (11%) (Markey et al., 1993) and Germany (15.1 to 94.0%) (Lenzko et al., 2011; Runge et al., 2012). Nevertheless, large variations in reported seroprevalence rates may in part be due to differences in sensitivity and/or specificity of serological tests used and/or to the size of the population under study. One of two Belgian goat herds that were examined at sample size ≥ 10, tested seropositive, revealing an intraherd seroprevalence of 52.9% (9/17). The seroepidemiological study in goats should be expanded to a larger population.

It is noteworthy that in the UK, the number of reported cases in sheep is considerably higher in farms

holding more than 150 animals (47.6%) than in smaller herds (< 150 animals) (9.4%) (Longbottom et al., 2012). The authors of the present study obtained opposite results: no infection in flocks of larger size. Moreover, *C. abortus* morbidity in Belgian sheep seems to be low given the low prevalence in infected herds. This poses questions about the size of the examined population, which might have been too small. It is therefore possible that the rate of infection in sheep is underestimated. A study of management conditions and risk factors, as initially planned, could answer these questions. It is therefore regrettable, albeit understandable, that the sector was not very willing to provide an active contribution to this study, as it was difficult to collect enough sheep serum samples.

The obtained data suggest a limited infectivity for *C. abortus* in sheep and cattle, as the intraherd seroprevalence in the positive herds was rather low. Wilson et al., (2012) observed the same. In the light of the current results, further research on the development of sensitive and specific *C. abortus* serological assays would be useful. These tests could also be helpful to define the actual exposure level for the human population at risk. In the future, it would also be worthwhile to perform molecular diagnoses on samples obtained from herds with clearly documented clinical data to get insight into the number of conjunctivitis, mastitis and reproductive failure cases in Belgium, where *C. abortus*, perhaps in conjunction with other pathogens, might be involved.

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