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Evaluation of two commercial assays for the detection of *Chlamydophila abortus* antibodies

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Abstract

Two commercial enzyme-linked immunosorbent assays (ELISA), the CHEKIT®-CHLAMYDIA which uses inactivated *Chlamydophila psittaci* antigen, and the *Chlamydophila abortus* ELISA produced by the Institut Pourquier which uses a recombinant fragment of the 80–90 kDa protein, were evaluated with the objective to determine whether the new ELISAs would perform as improved alternatives to the complement fixation test (CFT) for the serological diagnosis of ovine enzootic abortion (OEA). The results were compared to those obtained by the CFT and the competitive ELISA (cELISA). The tests were assessed with a panel of 17 serum samples from specific pathogen-free (SPF) lambs experimentally infected with various subtypes of *Chlamydophila pecorum*, with sera from 45 *C. abortus*-infected pregnant sheep and from 54 sheep free of OEA. The *C. abortus* ELISA was identified as being more specific and sensitive than the other tests. The 4 assays were evaluated further with 254 sera from flocks with documented OEA, from flocks with no history of abortion and from animals after abortion of unknown cause. The *C. abortus* ELISA by the Institut Pourquier identified less OEA-positive sera than the other assays though it identified correctly 9 of 10 OEA-positive flocks. The basis of the discordant results is discussed.

Keywords: Ovine enzootic abortion; *Chlamydophila abortus*; *Chlamydophila pecorum*; Serological diagnosis; Indirect ELISA

1. Introduction

Chlamydophila abortus (*C. abortus*) is a zoonotic pathogen that induces infectious abortion in ruminants, termed ovine enzootic abortion (OEA) and is also hazardous for pregnant women (Longbottom and Coulter, 2003). OEA is the most common disease of reproductive failure in sheep- and goat-breeding countries in Europe and has an important economic impact (Aitken, 1993). Infected pregnant ewes and goats abort late in gestation or give birth to weak lambs as a result of affected placenta. Bacteria excreted at abortion are the main source of infection of susceptible animals through ingestion or inhalation of

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the uterine discharge. After the onset of abortion, most of the animals acquire immunity and rebreed successfully. Chronically infected animals that excreted detectable amounts of chlamydial antigen during subsequent estrous cycles and maintained a persistent specific antibody response for up to a period of 2.5 years were observed after experimentally induced chlamydial abortion (Papp et al., 1994).

Early diagnosis of chlamydia as the cause of abortion is important to prevent and limit the spread of infection. Though direct evidence of the pathogen is the ultimate diagnosis, serological assays are more suitable for screening large numbers. The complement fixation test (CFT) is the most widely used test and is recommended by the Organisation Internationale des Epizooties (OIE) (http://www.oie.int). However, it lacks specificity due to its antigen, consisting mainly of the heat-resistant lipopolysaccharide (LPS), common to all *Chlamydiaceae* species (Brade et al., 1987). Ruminants in particular are also largely infected by *Chlamydophila pecorum* (*C. pecorum)*, subspecies of which cause a variety of clinical manifestations like arthritis, conjunctivitis and enteric infections (Kaltenboeck et al., 1993; Fukushi and Hirai, 1992; Anderson et al., 1996).

Several experimental assays have been developed in order to improve chlamydial serology. These include indirect immunofluorescence (Markey et al., 1993), immunoblotting (Jones et al., 1997) and indirect enzyme-linked immunoasays (ELISAs) using whole elementary bodies (EB) or extracts thereof (Cevenini et al., 1989; Markey et al., 1993; Anderson et al., 1995) and purified or recombinant LPS (Sting and Hafez, 1992; Griffiths et al., 1996). More specific assays are based on the major outer membrane protein (MOMP, Kaltenboeck et al., 1997; Hoelzle et al., 2004; Salti-Montesanto et al., 1997; Gut-Zangger et al., 1999; Borel et al., 2004), and the polymorphic outer membrane proteins (POMP) at 80–90 kDa (Buendia et al., 2001; Longbottom et al., 2001, 2002). Few of these assays have gone beyond experimental ''in house'' application, causing urgent need for harmonization. In the present study, we assessed the performance of two commercial ELISAs, the CHEKIT®-CHLAMYDIA which uses inactivated *Chlamydophila psittaci* antigen, and the ''ELISA *C. abortus"* produced by the Institut Pourquier which uses a recombinant fragment of an 80–90 kDa protein. The results were compared to those obtained by the CFT as the primary reference assay and the ''in house'' competitive cELISA (Salti-Montesanto et al., 1997). The objective was to determine whether the new ELISAs would perform as improved alternatives to CFT for the serological diagnosis of OEA.

2. Materials and methods

2.1. Antibody assays

(i) *C. abortus* ELISA (version P00700/04-18/02/05; Institut Pourquier, Montpellier, France) was performed according to the instructions of the manufacturer. The final values were expressed as Sample/Positive control % (S/P %). Sera with S/P% equal to or lower than 50% were considered negative, sera with an S/P% between 50 and 60% were doubtful and sera with an S/P% $\geq$60% were positive for OEA-infection.

(ii) CHEKIT®-CHLAMYDIA ELISA (Dr. Bommeli AG-IDEXX, Switzerland) results were normalized using the positive and negative control sera and were expressed as value (%). Sera with a value below 30% were considered negative, sera with values between 30 and 40% were ambiguous, while sera $\geq$40% were considered positive for OEA-infection according to the instructions in the kit.

(iii) Competitive enzyme-linked immunosorbent assay (cELISA). The assay was performed essentially as described previously and the results were expressed as % inhibition (Salti-Montesanto et al., 1997). Sera with inhibition values lower than 50% were considered negative for OEA. The questionable zone which was originally included in the calculations was omitted from this study for purposes of conformity with the other ELISAs. The selected cut-off predictably increased the specificity and reduced the sensitivity of the test.

(iv) Complement fixation test. The CFT was performed according to published procedures (Stamp et al., 1952) using a Greek *C. abortus* strain as antigen, a two-fold serum dilution, and standard reagents (Virion/Serion, Würzburg, Germany). CF titers were expressed as the