Short Communication

Comparison of antigen detection and quantitative PCR in the detection of chlamydial infection in koalas (*Phascolarctos cinereus)*

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Abstract

The ‘gold standard’ method for detecting chlamydial infection in domestic and wild animals is PCR. However, this technique is not suited to testing animals ‘in the field’ when a rapid diagnosis is frequently required. The objective of this study was to compare the results of a commercially available enzyme immunoassay, the ‘Clearview’ (CLV) *Chlamydia* test, against a quantitative *Chlamydia pecorum*-specific PCR performed on swabs collected from the conjunctival sac, nasal cavity and urogenital sinuses of naturally infected koalas (*Phascolarctos cinereus*). The level of agreement for positive results between the two assays was low (43.2%). The CLV detection cut-off was determined as approximately 400 *C. pecorum* copies, indicating that this assay is sufficiently sensitive to be used for the rapid diagnosis of active chlamydial infections.

Keywords: *Chlamydia pecorum*; Antigen detection; Quantitative PCR; Koala

Members of the family *Chlamydiaceae* are obligate intracellular pathogens and important causes of disease in humans, domestic animals and wildlife (Longbottom and Coulter, 2003). *Chlamydia* infection is typically included as one of the differential diagnosis for a range of presenting clinical signs in animals such as ovine abortion and chronic kerato-conjunctivitis and cystitis in koalas (*Phascolarctos cinereus*) (Sachse et al., 2009). However, subclinical infections can result in 'healthy' shedder animals within populations (Wan et al., 2011), and infection with other pathogens may result in similar clinical signs. In consequence, veterinarians require additional diagnostic techniques to make a definitive diagnosis of chlamydial disease, particularly when disease is subclinical.

The 'Clearview' (CLV) enzyme immunoassay (EIA) is a qualitative, solid-phase direct antigen detection method that employs an antibody directed against the *Chlamydiaceae*-family-specific lipopolysaccharide (cLPS). Compared to 'gold standard' tests such as cell culture and nucleic acid amplification (NAA) for detecting *C. trachomatis* genital tract infections, the CLV is less sensitive but is highly specific for members of the *Chlamydiaceae* in humans (Hislop et al., 2010). Similar, rapid point-of-care tests are rarely performed, however, due to this reduced sensitivity (van Dommelen et al., 2010). Published reports on the use of the CLV to detect chlamydial strains affecting mammals and birds are limited (Wood and Timms, 1992; Vanrompay et al., 1994), and when used in turkeys, significant numbers of false positives and negatives were found (Vanrompay et al., 1994). Despite these limitations, the CLV has significant potential in the diagnosis of chlamydial infection in animals, providing adequate sensitivity and specificity can be demonstrated.

We commonly employ the CLV in field-testing for chlamydial infection in koalas (Wood and Timms, 1992). Of the two species that infect the koala, *C. pecorum* and *C.*

pneumoniae, the former is both more pathogenic and prevalent than the latter (Jackson et al., 1999; Devereaux et al., 2003; Marsh et al., 2011). To evaluate the sensitivity of the CLV in diagnosing *C. pecorum* infection in a 'point-of-care' setting, we collected swabs from the conjunctival **sac**, nasal cavity and urogenital tract of 36 koalas and compared the result of the CLV with the *C. pecorum* DNA copy number, as determined by quantitative PCR (Wan et al., 2011). The sampled koalas were presented to the Australia Zoo Wildlife Hospital, Beerwah, Australia, for veterinary treatment because of injury or illness. Duplicate cotton-tipped swabs for qPCR and CLV were concurrently collected from the conjunctival sac, nasal cavity and urogenital sinus (females), or sequentially from the urethra (males). Duplicate urethral swabs from males were randomly assigned to the 'CLV' or 'PCR' test groups. All swabs were collected by the same clinician, and it was not possible to obtain samples from all sites in several animals.

The CLV was performed within 20 min of sampling in accordance with the manufacturer's instructions. 'Result' and 'control' windows were examined 15 min after applying the extract to the test strips, and were graded semiquantitatively as: 0, negative; 1, weakly positive (but could be clearly discerned); 2, strongly positive (but with less intense banding than the positive control); 3, band intensity ≥ positive control band (Fig. 1). The duplicate swab was stored at -80 °C prior to processing for qPCR as previously described (Wan et al., 2011). Samples with < 30 16S rDNA copies of *C. pecorum* DNA/µL were considered negative.

The geometric mean *C. pecorum* DNA copy number at the sampled sites relative to the CLV result, and the specificity and sensitivity of CLV at each site are detailed in Table 1. As no CLV result was graded as '3', this level of positivity was excluded from the analysis.

Of 138 swabs, 81 (58.7%) were qPCR positive and 35 of these 81 were CLV positive, indicating 43.2% agreement. The approximate detection limit of the CLV was 384 *C. pecorum* cells/µL of swab material. Although this level of agreement level is lower than that found in a previous study in koalas where cell culture was used (Wood and Timms, 1992), the findings are consistent with NAA test/CLV comparisons (Hislop et al., 2010). This result is also not surprising considering that qPCR is considered more sensitive than an EIA in detecting chlamydial infection (Sachse et al., 2009). Sampling error, including the potential failure to collect equal numbers of chlamydial cells in both swabs may also have contributed to this finding.

When sampling sites were compared (Table 1), the lowest agreement was observed from swabs collected from the nasal cavity (5 CLV positive vs. 17 qPCR positive; 29.4%), likely reflecting the lower load of *C. pecorum* in these swabs compared to other sites. The CLV was more reliable for swabs of the urogenital region (15 CLV positive vs. 25 qPCR positive; 60%), where the average qPCR copy number was approximately 10 times higher (Table 1). Four 'false positive' CLV results were detected in 57 *C. pecorum* DNA negative swabs, possibly because of the presence of other *Chlamydiaceae*, such as *C. pneumoniae*, which are known to infect wild koalas at a much lower prevalence (Jackson et al., 1999). There is also evidence that other, *Chlamydia*-like organisms infect koalas (Devereaux et al., 2003), although there is no data currently available on the potential for CLV to cross-react with these organisms.

Interestingly, CLV signal semiquantitative grading correlated strongly with the *C. pecorum* infectious load ($P < 0.05$; one-way ANOVA), suggesting that when sufficient *C. pecorum* organisms are present (i.e. approximately 400 *C. pecorum* copies /µL), subjective

reading of the CLV signal at the recommended time could be used to semi-quantify chlamydial infection loads. Based on our recent study examining the relationship between *C. pecorum* infectious load in koalas, and disease severity, this finding suggests the CLV is potentially suitable for confirming chlamydiosis in animals with active disease affecting the eye or urogenital regions, as the infectious load in these animals is within this detection range (Wan et al., 2011). However, the test may not be reliable in: detecting subclinically infected animals with infectious loads < 400 copies/µL; confirming *C. pecorum* infection in animals with chronic, inactive disease, as such animals often shed levels of organisms below the detection threshold of this assay (Wan et al., 2011).

In conclusion, when the portability, turn-around time and ease of use of the CLV are considered, this assay is a useful adjunct clinical test in the diagnosis of chlamydial infection in animals, as demonstrated in our study in the koala. Further work will be required to validate its diagnostic value in other species.

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Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1.

Details of the sensitivity and specificity of the 'Clearview' (CLV) enzyme immunoassay antigen detection test compared with a *Chlamydia pecorum* species-specific qPCR assay carried out on swabs taken from the conjunctival sac (conjun), nasal cavity, and urogenital tract (UGT) of naturally infected koalas.

CLV band intensity 'score'	**No. of swabs**	**qPCR 16S copies** [a]	**No. of PCR positives (*n* = 81) diagnosed as:**		**No. of PCR negatives (*n* = 57) diagnosed as:**		**Sensitivity** [b]	**Specificity** [c]
			CLV positive	**CLV negative**	**CLV positive**	**CLV negative**		
Negative	99	1	-	46	-	53	-	-
+	20	384	18	-	2	-	-	-
++	19	1385	17	-	2	-	-	-
Total (all)	**138**	**-**	**35**	**46**	**4**	**53**	**43.2**	**92.9**
Conjun swabs	69	67	15	25	2	27	37.5	93.1
Nasal swabs	35	36	5	12	1	17	29.4	94.4
UGT swabs	34	291	15	10	1	8	60.0	88.9

[a] Geometric mean copies of 16S rDNA PCR product/µL of DNA/swab.
[b] Sensitivity, % number of positives detected against number PCR positive.
[c] Specificity, % number of negatives detected against number PCR negative.

193 **Figure legend**
194

195 **Fig. 1.**

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197 detection method that employs an antibody directed against the *Chlamydiaceae*-family-
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199 illustrated. ‘Control’ and ‘result’ windows were examined 15 min after applying the extract to
200 the test strips, and were graded as: 0, negative; 1, weakly positive (but could be clearly
201 discerned); 2, strongly positive (but with less intense banding than the positive control); 3,
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