EVALUATION OF AN ENZYME IMMUNOASSAY TEST FOR THE DIAGNOSIS OF CHLAMYDIA PSITTACI INFECTION IN FREE-RANGING KOALAS (PHASCOLARCTOS CINEREUS) IN SOUTHEASTERN QUEENSLAND, AUSTRALIA

Authors: Weigler, Benjamin J., Baldock, F. Christian, Girjes, Adeeb A., Carrick, Frank N., and Lavin, Martin F.

Source: Journal of Wildlife Diseases, 24(2) : 259-263

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-24.2.259

Journal of Wildlife Diseases, 24(2), 1988, pp. 259–263

EVALUATION OF AN ENZYME IMMUNOASSAY TEST FOR THE DIAGNOSIS OF *CHLAMYDIA PSITTACI* INFECTION IN FREE-RANGING KOALAS (*PHASCOLARCTOS CINEREUS*) IN SOUTHEASTERN QUEENSLAND, AUSTRALIA

Benjamin J. Weigler,[1,4] F. Christian Baldock,[2] Adeeb A. Girjes,[3] Frank N. Carrick,[5] and Martin F. Lavin[3]

[1] Department of Animal Sciences and Production, University of Queensland, St. Lucia, Queensland 4067, Australia
[2] Animal Research Institute, Queensland Department of Primary Industries, 665 Fairfield Road, Yeerongpilly, Queensland 4105, Australia
[3] Department of Biochemistry, University of Queensland, St. Lucia, Queensland 4067, Australia
[4] Present address: Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616, USA.
[5] Present address: Department of Zoology, University of Queensland, St. Lucia, Queensland 4067, Australia

ABSTRACT: The IDEIA Chlamydia Test, a commercially available antigen-capture enzyme-linked immunosorbent assay (ELISA) test, based on a monoclonal antibody for the detection of chlamydia in clinical specimens, was evaluated in a population of 65 free-ranging koalas in southeastern Queensland determined to be infected with *Chlamydia psittaci*. Compared to isolation of the organism in tissue culture, the sensitivity of the IDEIA test ranged from 3 to 11%, and the specificity from 90 to 97%. The results indicated that the IDEIA test is unsuitable for use as a diagnostic screening test for *C. psittaci* in free-ranging koalas.

Key words: *Phascolarctos cinereus*, koala, *Chlamydia psittaci*, enzyme-linked immunosorbent assay test, ELISA screening test, evaluation.

INTRODUCTION

Chlamydia psittaci has been found in association with four common syndromes in the koala (*Phascolarctos cinereus*). These are keratoconjunctivitis (Cockram and Jackson, 1981), reproductive tract disease of female koalas (Brown and Grice, 1984; McColl et al., 1984), urinary tract disease (known colloquially as "dirty tail"), and rhinitis/pneumonia (Brown and Grice, 1984, 1986). Previous surveys of koalas for the presence of chlamydial infections have relied upon the detection of antichlamydial complement-fixing antibodies in koala serum (McColl et al., 1984) or radiographic examination of female koalas for cystic reproductive tract lesions (Brown et al., 1984). Isolation of the organism in tissue culture (Grice and Brown, 1985) is laborious, reducing its practicality for use as a survey tool. Results from another study have shown that the complement fixation (CF) test lacks sensitivity in detecting koalas infected with *C. psittaci* (Weigler et al., 1988). Therefore, this study was conducted in search of an alternative test for the detection of chlamydial infection in free-ranging koalas.

The IDEIA Chlamydia Test (Boots-Celltech Diagnostics Ltd., Slough, Berkshire, United Kingdom) is a commercially available enzyme-linked immunosorbent assay (ELISA) test which uses a monoclonal antibody directed against the chlamydial genus-specific lipopolysaccharide (LPS) antigen in combination with an enzyme amplification step (Pugh et al., 1985). The IDEIA test has been applied successfully to the diagnosis of acute chlamydial conjunctivitis in cats (Wills et al., 1986). This paper evaluates the performance of the IDEIA test kit applied to a population of free-ranging koalas, determined to be infected with *C. psittaci*, in southeastern Queensland.

MATERIALS AND METHODS

The swabs of koala tissues used in the present study were taken during an epidemiological in-

vestigation of chlamydial infection in koalas, reported earlier in this issue (Weigler et al., 1988). All koalas examined during the course of that study were sampled at the same time for this investigation, providing a total of 65 conjunctival and 65 urogenital specimens for IDEIA testing. Details regarding the study population and the procedures of other tests performed are given in Weigler et al. (1988). Briefly, swabs were collected from the conjunctival sac and urogenital canal or penile urethra of koalas and these specimens were inoculated onto buffalo-green monkey (BGM) kidney cells (Flow Laboratories, Inc., McLean, Virginia 22102, USA) grown in coverslip cultures for the isolation of chlamydiae. Aerobic bacterial and fungal cultures were also done on swabs which had been taken from both sites, and koala serum was taken for CF testing.

Urogenital and conjunctival samples for IDEIA testing were collected using sterile, cotton-tipped, plastic or aluminum-shafted swabs (Medical Wire and Equipment Company, Corsham, Wiltshire, United Kingdom) and placed in 1 ml of IDEIA transport medium contained inside polystyrene vials (Nunc Cryotubes, Roskilde, Denmark). Specimens were transported on water-ice to the laboratory within 2–9 hr of collection. Vials containing swabs and transport medium were vortex mixed for 15 sec with sterile glass beads, the swabs were extracted, and the vials were then frozen in liquid nitrogen (−196 C) for 1–4 wk prior to testing.

The IDEIA Chlamydia Test was performed according to the manufacturer's instructions and processed in batches of approximately 40 samples. Frozen specimens were thawed at room temperature, vortex mixed for 15 sec, boiled for 15 min and vortex mixed for an additional 15 sec. After cooling, 200 μl of each specimen was added to a single well on the monoclonal antibody-coated plate provided. Following a 2-hr incubation, 50 μl of the enzyme-conjugated monoclonal antibody supplied in the kit was added to each well, and the plate was incubated for 1 hr. The contents of each well were then hand-aspirated and wells were washed four times using the wash buffer supplied. An aliquot (100 μl) of freshly reconstituted substrate was added to each well and allowed to incubate for 40 min, after which 200 μl of fresh amplifier was added. Following a final 10 min incubation, 50 μl of the supplied stopping solution was added and the plates were read at 492 nm on a Titertek Multiscan spectrophotometer (Flow Laboratories, Inc., McLean, Virginia 22102, USA).

The IDEIA kit provides a positive control of McCoy cells infected with chlamydiae and negative controls are taken from reserved transport medium. In addition to these, we included tissue culture propagated koala conjunctival and urogenital isolates of *C. psittaci* as positive controls and growth medium from noninfected tissue culture cells as negative controls. The cutoff value for positive samples was calculated according to the manufacturer's recommendations, adding 0.05 to the mean of the three standard negative control wells.

RESULTS AND DISCUSSION

The sensitivity of a diagnostic test indicates the frequency of positive test results in animals with a particular disease, whereas test specificity indicates the frequency of negative test results in animals without that disease (Galen, 1982). Sensitivity and specificity estimates of the IDEIA test were calculated by comparison with the isolation of *C. psittaci* in tissue culture from conjunctival and urogenital tract specimens, and then combined to give estimates regardless of site for each koala. These determinations are presented in Table 1, along with lower and upper bounds of the 95% confidence interval (Fleiss, 1981). Overall, the sensitivity of the IDEIA test was only 11%, detecting only five of the 46 koalas found to be infected with *C. psittaci* by tissue culture isolation. However, overall specificity was 90%; 17 of the 19 koalas with negative tissue culture results for *C. psittaci* were also negative on the IDEIA test. The wide confidence intervals reflect the low sample sizes in each group.

Combining the results from both tissue sites gave the highest sensitivity in the present study (11%), but this level is still unacceptably low. By interpreting in parallel the results from two or more diagnostic tests, sensitivity is increased at the expense of specificity. With parallel testing using two tests, an animal is classified as positive if a positive result occurs on either or both tests employed (Fletcher et al., 1982). Therefore, animals are classified as negative only when both test results are negative. When the combined IDEIA data presented here were interpreted in parallel with the results from a CF test conducted on sera taken at the same time as tissue

TABLE 1. Sensitivities and specificities for the IDEIA Chlamydia Test performance measured against tissue culture for *Chlamydia psittaci* infection in conjunctival and urogenital tissues of 65 free-ranging koalas (*Phascolarctos cinereus*) in southeastern Queensland, Australia.

Site	% Sensitivity (number IDEIA positive and culture positive/total number culture positive)[a]	95% Confidence interval[c] of sensitivity	% Specificity (number IDEIA negative and culture negative/total number culture negative)	95% Confidence interval of specificity
Conjunctiva	3 (1/31)	(0, 16)	97 (32/33)	(81, 100)
Urogenital	10 (3/31)	(1, 24)	91 (31/34)	(74, 97)
Combined[b]	11 (5/46)	(3, 23)	90 (17/19)	(63, 98)

[a] Cut-off value of 10 or more chlamydial inclusion bodies per coverslip culture.
[b] Shown as a positive test result from either tissue site sampled.
[c] Lower and upper bounds of the 95% confidence interval.

specimens for the IDEIA test, the sensitivity, though greater than either test alone, was still a low 16% (6, 28). In addition, specificity remained at 90% (63, 98).

The proportion of koalas with clinical disease consistent with chlamydial infection, including three cases each of keratoconjunctivitis and "dirty tail," was six of 65 (9%). The overall prevalence of *C. psittaci* infection as determined by tissue culture isolation of the organism was 46 of 65 (71%). However, the apparent prevalence of *C. psittaci*, as measured by the IDEIA test, was only seven of 65 (11%). *Chlamydia psittaci* was isolated in tissue culture from all koalas with clinical signs of disease, but only three of these were positive using the IDEIA kit.

Two koalas which were positive on the IDEIA kit were negative on tissue culture. These discordant results could be due to the presence of interfering substances in the original specimens. Some strains of *Staphylococcus aureus* are known to contain protein A, which is capable of binding nonspecifically to the Fc portion of monoclonal antibodies and thus may create false positive results. Other workers have cautioned about this when using rapid diagnostic kits for the detection of chlamydiae in humans (Krech et al., 1985; Rothburn et al., 1986). However, bacterial culture in this study isolated no *S. aureus* in association with positive IDEIA test results. Alternatively, nonviable chlamydiae may have been present in the corresponding two samples which were negative on tissue culture.

The low sensitivity of the IDEIA test can likely be explained by the low concentration of *C. psittaci* present in most koalas. Many of the conjunctival and urogenital swabs taken during this survey produced low numbers (<25) of intracytoplasmic chlamydial inclusions per coverslip culture, possibly indicating that the volume of chlamydial LPS present was below the level of detection provided by the IDEIA test kit. In a comparative titration study, Wills et al. (1986) demonstrated the superior sensitivity of cell culture over the IDEIA test when the concentration of chlamydial organisms was low.

The IDEIA test manufacturer recommends storage of specimens at 2–8 C for no longer than 7 days prior to testing, contrary to the regime used for the present study. Comparative trials indicating the long-term viability of *C. psittaci* under different combinations of temperature and storage media are lacking. However, a study by Sherman and Jordan (1985) compared cryopreserved human semen specimens containing *C. trachomatis*, and found high agreement between the results of a direct immunofluorescence test and tissue culture isolation of the organism after a storage period of 6 mo. The present study compares IDEIA test results to the isolation of *C. psittaci* in tissue culture from specimens which had been stored under the same combination of time and tem-

perature. Thus, only differences between the two storage media on the preservation of group-specific chlamydial antigen could account for the low sensitivity of the IDEIA test.

Most chlamydial infections exist in a well-balanced host-parasite relationship. The organisms are excreted by healthy carriers, and they sometimes persist in latently infected hosts during a noninfectious stage (Schachter et al., 1973). Because diagnostic tests for the detection of chlamydiae are developed for clinically-affected populations, the sensitivity of other tests currently available may not prove substantially higher than the results reported in the present study. One alternative test, the IMAGEN Chlamydia Test (Boots-Celltech Diagnostics Ltd., Slough, Berkshire, United Kingdom SL1 4ET), is a direct fluorescent antibody test which is based on the same monoclonal antibody as the IDEIA test. Therefore, this test is unlikely to be significantly more sensitive when applied to this koala population.

The low sensitivity of the IDEIA test found in this study indicates it is unsuitable for screening free-ranging koalas for *C. psittaci* infection. To detect a high proportion of truly infected animals, screening tests for infectious diseases should have a very high sensitivity (Galen, 1982). High sensitivity is especially important when there is a substantial penalty for failing to detect infected animals; for example, prior to the introduction of free-ranging koalas to known chlamydiae-free colonies. Isolation of the organism remains as the most effective method of *C. psittaci* diagnosis in free-ranging koalas until alternative tests have proven adequate.

LITERATURE CITED

BROWN, A. S., AND R. G. GRICE. 1984. Isolation of *Chlamydia psittaci* from koalas (*Phascolarctos cinereus*). Australian Veterinary Journal 61: 413.

———, F. N. CARRICK, G. GORDON, AND K. REYNOLDS. 1984. The diagnosis and epidemiology of an infertility disease in the female koala *Phascolarctos cinereus* (Marsupialia). Veterinary Radiology 25: 242–248.

———, AND R. G. GRICE. 1986. Experimental transmission of *Chlamydia psittaci* in the koala. *In* Chlamydial infections, D. Oriel, G. Ridgway, J. Schachter, D. Taylor-Robinson, and M. Ward (eds.). Proceedings of the Sixth International Symposium on Human Chlamydial Infections. Cambridge University Press, Cambridge, England, pp. 349–352.

COCKRAM, F. A., AND A. R. B. JACKSON. 1981. Keratoconjunctivitis of the koala, *Phascolarctos cinereus*, caused by *Chlamydia psittaci*. Journal of Wildlife Diseases 17: 497–504.

FLEISS, J. L. 1981. Statistical methods for rates and proportions, 2nd ed. John Wiley and Sons, New York, New York, 321 pp.

FLETCHER, R. H., S. W. FLETCHER, AND E. H. WAGNER. 1982. Clinical epidemiology—The essentials. Williams and Wilkins, Baltimore, Maryland, 223 pp.

GALEN, R. S. 1982. Application of the predictive value model in the analysis of test effectiveness. Symposium on test selection strategies. Clinics in Laboratory Medicine 2: 685–699.

GRICE, R. G., AND A. S. BROWN. 1985. A tissue culture procedure for the isolation of *Chlamydia psittaci* from koalas (*Phascolarctos cinereus*). Australian Journal of Experimental Biology and Medical Science 63: 283–286.

KRECH, T., D. GERHARD-FSADNI, N. HOFMANN, AND S. M. MILLER. 1985. Interference of *Staphylococcus aureus* in the detection of *Chlamydia trachomatis* by monoclonal antibodies. The Lancet (Letters) i: 1161–1162.

MCCOLL, K. A., R. W. MARTIN, L. J. GLEESON, K. A. HANDASYDE, AND A. K. LEE. 1984. Chlamydia infection and infertility in the female koala (*Phascolarctos cinereus*). The Veterinary Record 115: 655.

PUGH, S. F., R. C. B. SLACK, E. O. CAUL, I. D. PAUL, P. N. APPLETON, AND S. GATLEY. 1985. Enzyme amplified immunoassay: A novel technique applied to direct detection of *Chlamydia trachomatis* in clinical specimens. Journal of Clinical Pathology 38: 1139–1141.

ROTHBURN, M. M., H. MALLINSON, AND K. J. MUTTON. 1986. False-positive ELISA for *Chlamydia trachomatis* recognised by atypical morphology on fluorescent staining. The Lancet (Letters) i: 982.

SCHACHTER, J., J. STORZ, M. L. TARIZZO, AND K. BOGEL. 1973. Chlamydiae as agents of human and animal diseases. Bulletin of the World Health Organization 49: 443–449.

SHERMAN, J. K., AND G. W. JORDAN. 1985. Cryosurvival of *Chlamydia trachomatis* during cryopreservation of human spermatozoa. Fertility and Sterility 43: 664–666.

WEIGLER, B. J., A. A. GIRJES, N. A. WHITE, N. D. KUNST, F. N. CARRICK, AND M. F. LAVIN. 1988. Aspects of the epidemiology of *Chlamydia psit-*

taci infection in a population of koalas (*Phascolarctos cinereus*) in southeast Queensland, Australia. Journal of Wildlife Diseases 24: 282–291.

WILLS, J. M., W. G. MILLARD, AND P. E. HOWARD. 1986. Evaluation of a monoclonal antibody based ELISA for detection of feline *Chlamydia psittaci*. The Veterinary Record 119: 418–420.

Received for publication 14 July 1987.