

# California Studies on Diagnostic Procedures, Female Immune Responses and Epidemiology of Bovine Trichomoniasis

R. H. BonDurant, DVM, Dip., ACT

Department of Reproduction,  
School of Veterinary Medicine  
University of California at Davis

## Summary

In spite of concerted efforts to develop a sensitive and specific immuno-diagnostic assay for bovine trichomoniasis, the most reliable method for establishing a diagnosis in an infected herd is the culture of preputial or vaginal secretions on Diamond's medium (or equivalent). Recent improvements in packaging of media have made it possible to expedite the culture procedure without significant loss of sensitivity. Studies on the immune responses of experimentally infected heifers have shown that a large number of antigens of *T. foetus* are recognized by immunoglobulins (especially IgA and IgG<sub>1</sub>) in local secretions of the reproductive tract, with little systemic response seen in the serum.

A survey of randomly selected cow-calf herds in California, in which bulls from each herd were cultured for *T. foetus*, showed that approximately 15 % of all herds, and 5% of all bulls, were infected. Without an effective legal treatment for either cows or bulls, the need for inexpensive accurate diagnostics and effective immunoprophylaxis is urgent.

## Introduction

Trichomoniasis, a venereal disease causing infertility, pyometra and occasional abortion, has been recognized for some time in California (Kimsey et al.; Skirrow et al., 1985). Because a huge proportion of California geography is occupied by foothill, mountain and desert lands, the use of extensive management practices, including natural breeding, is common. This use of natural breeding in areas of "wide open spaces," has no doubt contributed to the prevalence of trichomoniasis in the state. But because the classical control method for any venereal disease - artificial insemination - is impractical under these geographic conditions, the control of trichomoniasis in such rangelands requires an increased ability to identify infected animals, and an immunoprophylactic means of protecting against infection. To these ends, we have studied the natural history of infection in virgin heifers; we have also surveyed the state to accurately estimate the prevalence of trichomoniasis.

## Diagnostic Procedures

In a preliminary set of experiments (Skirrow and BonDurant, 1989), four virgin beef heifers were infected by intravaginal introduction of  $7 \times 10^6$  *T. foetus* organisms. Secretions from the vagina, cervix and uterus were collected weekly thereafter, for a minimum of 14 weeks. All secretions were examined by both wet mount/direct microscopic examination, and by inoculation of secretions into Diamond's medium, with examination of the medium for motile trichomonads every day for 7-9 days. Infection of the vagina and uterus occurred within the first week; clearance of protozoa occurred at 13 to 28 weeks. Eight weeks after clearance, the four heifers were re-inoculated with  $10^5$  *T. foetus*: Only two could be infected a second time, with re-infections maintained for up to 4 weeks.

The diagnostic sensitivity of wet mount examination of the reproductive tract secretions was 30.4% while culture on Diamond's medium had a diagnostic sensitivity of 78.1% for culture of the secretions. (This compares with a diagnostic sensitivity of about 81% for culture of preputial smegma from infected bulls [Skirrow et al., 1985]). Culture of cervical and vaginal mucus provided the most reliable method of diagnosis of trichomoniasis during experimental infection.

In a similar experiment, 24 heifers were infected with varying doses of *T. foetus*, and secretions collected and cultured weekly in standard Diamond's media tubes. Duplicated samples from each heifer were inoculated into a two-chambered proprietary packet ("Inpouch-TF") containing a selective growth medium similar to Diamond's. Pouches were then sealed and incubated at 37° C in parallel with inoculated tubes of Diamond's medium. Each pouch was removed every day from the incubator, mounted on a plastic slideframe (provided by the pouch manufacturer), examined under 400 X magnification for motile trichomonads. Our results showed that the pouch was almost identical in sensitivity to the Diamond's tubes after 7 days, i.e. the total agreement between the two tests was nearly 99%. The Diamond's medium tubes were somewhat more likely to show *T. foetus* organisms earlier: the predictive value of a positive "pouch test" was about 90% after the initial observation (usually 24 hours after collection-

inoculation), while the predictive value of a negative test at this examination was 88.5%. If the manufacturer's claims of a prolonged shelf life are accurate (we did not test this), the pouches may provide a highly practical and accurate means by which veterinarians can diagnose trichomoniasis in female cattle. As the bull is often long-gone by the time diagnostic efforts are initiated, the convenience of a system for culturing females should be beneficial.

Attempts to develop a *T. foetus* specific antigen-detecting ELISA have been hampered by lack of specificity (Yule et al., 1989). In particular, affinity-purified polyclonal antibodies have been shown to cross react with antigens found in secretions of non-infected (virgin) heifers and bulls. At this time, we don't know if this cross reaction represents true antigen mimicry (i.e. the production of host-like antigens by the protozoan), or simply the absorption of host proteins onto the surface of the parasite. In either case, the high *in vitro* sensitivity of the antigen-trapping ELISA (10 ng antigen detectable) was rendered useless when field samples were tested, due to cross reaction.

#### Local Immunity to *T. foetus*

Secretions from the vagina, cervix and uterus were

collected weekly from experimentally infected heifers. These secretions were examined for isotype-specific antibodies to *T. foetus*, using an ELISA, with whole *T. foetus* antigen used to bind immunoglobulins (IgA, IgG<sub>1</sub>, IgG<sub>2</sub> and IgM) to polystyrene plates. Sera from the same heifers were also examined for antibodies to *T. foetus*, using the same assay system. The vaginal and cervical antibody responses were characterized by significantly increased *T. foetus*-specific IgA and IgG<sub>1</sub> at 7-9 weeks of infection, whereas significant increases in uterine IgA and IgG<sub>1</sub> activity were detected at 10-12 weeks. Serum responses were more muted (i.e. they did not change significantly relative to pre-infection values), but those responses that were detectable were in the IgG<sub>1</sub> and IgG<sub>2</sub> subclasses. In all reproductive tract regions IgA and IgG<sub>1</sub> persisted the longest, usually at least until the time of *T. foetus* clearance. Parasite-specific IgG<sub>2</sub> was seen only transiently in local secretions, but was significantly above pre-infection levels in the uterus in most animals; very little IgM, relative to pre-infection, was detected in any secretion or serum, although cervical secretions had the greatest amount. Anamnestic responses, especially in the IgA and IgG<sub>1</sub> isotypes, occurred after re-exposure of "cleared" tracts to *T. foetus* (Skirrow and BonDurant, 1989b).

## Research Summaries

Papers presented at the Research Summaries Session will appear in the 1990 Bovine Practitioner.

## For Your Library

### Essential Pathology

Emanuel Rubin,  
John Farber

This textbook, edited by two Doctors of Medicine at the Jefferson Medical College, Philadelphia is a concise and selective reference and review for students of pathology. It represents a summary of contemporary general and systemic pathology. A discussion of normal anatomy and physiology and the description of less frequently encountered diseases have been omitted. The book presents all the key concepts of the evolution and expression of disease and assigns priorities based upon the clinical importance

and heuristic relevance of individual disorders.

Because graphic images take advantage of pattern recognition, one of the most fundamental characteristics of the human brain, they are powerful tools for communicating abstract and complex material. The majority have, therefore, been retained from their first book *Pathology*. These color drawings provide a "road map" to the essential concepts of pathology.

In spite of enormous strides in biology, molecular genetics etc. the authors remain dedicated to Virchow's original concept that "the cell is really the ultimate morphological element in which there is any manifestation of life." They conclude "The marriage of classical morphological descriptions of disease and contemporary concepts summarized in this book serves to join traditional pathology with the modern revolution in biology."

This textbook is recommended as an excellent reference book also for veterinary medical students. It has 880 pages with 650 outstanding illustrations; price \$42.95.

Further information from Rosalind Mazur, J.B. Lippincott Company, East Washington Square, Philadelphia, PA 19105-9961.