ANCILLARY TESTS FOR DETECTING TUBERCULOSIS IN DEER

G.W.de Lisle,K.C.Corrin,C.E.Carter

INTRODUCTION

The prevalence of false negative reactions to the intradermal cervical skin test is not great and the sensitivity of the test in deer appears to be similar to that for cattle.However,field investigations by Beatson(1) have demonstrated that some Mycobacterium bovis infected deer are not detected by intradermal skin testing.False negative reactions to the skin test have been seen in clinically normal deer which have had lesions in only one lymph node and in heavily infected deer,some of which developed clinical signs of tuberculosis.These findings have prompted a search for ancillary tests to identify such animals.Possible ancillary tests include those which detect the bacterium and those which have an immunological basis.

BACTERIOLOGICAL EXAMINATION OF NASAL SWABS

This experiment and the serological examinations were conducted on deer kept at Flock House.Details of these animals are summarised in the preceding article in this proceedings(3).Deer were experimentally infected with M.bovis by intratracheal inoculation.These animals were the source of infection for the naturally infected "incontact" deer.An attempt was made to isolate M.bovis from the nasal cavities of deer.Cotton tipped swabs were inserted approximately 12 cm into the nasal cavity of known infected deer.Swabs were then agitated in 10 ml of sterile saline which was then decontaminated with 5% H_2SO_4.Following centrifugation the deposits were inoculated onto culture media.M.bovis was not isolated from any of the 185 swabs collected from known infected deer.However 2 isolates of M.smegmatis,2 of M.chelonei subsp. abscessus and one of M.diernhoferi were obtained from the swabs.These mycobacteria are saprophytes and are commonly found in the environment(5).Their importance lies in their potential to sensitise deer to tuberculin and cause false positive skin test reactions(6).A similar isolation procedure has been used successfully in tuberculous cattle(4).Our findings indicate that M.bovis was not present in large numbers in the nasal cavities of known infected deer.Bacteriology is too insensitive and takes too long to produce a result for it to be a useful ancillary diagnostic test to detect tuberculous deer.

SEROLOGY

Skin testing in the foreseeable future will remain the principal diagnostic test for detecting tuberculosis in deer.This test is based on the cell mediated immune(CMI) response to M.bovis.CMI develop early in mycobacterial infections and this is reflected in the high sensitivity of the intradermal

skin test.On the other hand,the antibody response early in infection is meager and develops maximally in the more heavily infected animals(2).Thus one should not expect serological tests for tuberculosis to be as sensitive as skin tests.Serological tests may have a role as ancillary tests which can detect the classical anergic animal.These animals are heavily infected and thus are a very important source of infection for other deer.

False positive reactions are a major problem with serological tests for tuberculosis.Reagents such as Purified Protein Derivative(PPD) contain a number of antigens some of which are common to a wide range of different species of mycobacteria and closely related genera. Sensitisation by saprophytic mycobacteria may induce an antibody response which can be detected with bovine PPD.In our previous studies we demonstrated seroconversion in experimentally M.bovis infected deer by an enzmye linked immunoabsorbent assay(ELISA) using an antigen made from M.phlei(7).The following results illustrate both the problems and the potential of serology as an ancillary diagnostic test for detecting tuberculosis in deer.Details of the methods used in this study will be published in a separate article.

An immunodiffusion(ID) test was used to examine the serological response of 50 deer from Flock House(3) to one of the antigens in bovine PPD. The pre-inoculation or pre-exposure sera and the samples taken prior to slaughter were examined with an ID test.A positive reaction was one which had a line of identity with a serum from animal 25,a known M.bovis infected deer.The same sera were also examined with an indirect ELISA test in which bovine PPD was used as an antigen.Doubling dilutions of sera(1/5 to 1/640) were examined.

The results are summarised in Table 1.The post exposure or post inoculation sera have been classified according to the bacteriology and histopathology findings on the slaughtered deer.Interpretation limits have not yet been established for the ELISA.Two different criteria have been selected as a demonstration of the conflict between sensitivity and specificity when one uses PPD as an antigen in the ELISA.No false positive reactions were recorded when sera were examined at 1/320 dilution with an ELISA.However,when sera were examined at a 1/80 dilution there were 5/50 false positive reactions.The number of true positive reactions in the ELISA test was similar to the number of true positives recorded with the comparable skin test.One should note that small skin test reactions(<2.5mm) were also recorded in the noninfected deer.

No positive immunodiffusion reactions were observed in the sera taken from the pre-inoculation or pre-exposure sera.However in 5 of these sera precipitin lines were found which did not form a line of identity with the positive control serum(deer 25).The serological and skin test results were not correlated.Two of the M.bovis infected animals which had positive immunodiffusion reactions were completely unresponsive to the comparable skin test.Six of the immunodiffusion positives from M.bovis infected animals had increases in skin thickness of less than 2.5mm to the comparable skin test.

DISCUSSION

The sensitivity of the skin test results shown in Table 1 may have been reduced by short interval repetitive testing(3).The important point is that some infected animals which were unresponsive to intradermal skin testing were positive to the serological tests.This is a reflection of the different immunological basis of the two tests.The 3 clinically affected deer did not react to their last skin test(skin thickness increase <2.5mm)but all had high levels of antibody to bovine PPD.Use of PPD as the antigen in the ELISA does not allow full advantage to be taken of this test.An unacceptable number of false positive reactions were recorded with ELISA when serum dilutions of less than 1/80 were examined.A more highly purified antigen preparation could improve the specificity of the ELISA and make it a useful routine diagnostic test.Although immunodiffusion tests are very insensitive they can be used to analyse the antibody response to individual antigens.Ten infected deer reacted in the immunodiffusion test to one of the antigens in PPD.No uninfected deer have been found that react in the immunodiffusion test to this antigen.An ELISA test using a purified antigen is being developed.

ACKNOWLEDGEMENTS

Bacteriological examinations were done by P.F.Havill and G.F.Yates and the serology by E.P.Wall.Their expert technical contribution is gratefully acknowledged.

REFERENCES

(1) Beatson,N.S.:Personal communication.
(2) Brown,I.N.(1983):Animal models and immune mechanisms in mycobacterial infection.The biology of the mycobacteria,volume2.(ed.C.Ratledge and J.Stanford)Academic Press.p172-234.
(3) Corrin,K.C.;Carter,C.E.;de Lisle,G.W.(1984):Evaluation of the tuberculin test in deer.This proceedings.
(4) de Kantor,I.N.;Bioch,D.;Roswurm,J.D.(1978):Mycobacteria isolated from the nasal secretions of tuberculin test reactor cattle.Am.J.vet.Res.39:1233-4.
(5) Kazda,J.F.(1983):The principles of the ecology of mycobacteria.The biology of the mycobacteria,volume 2.(ed.C.Ratledge and J.Stanford)Academic Press.p.323-41.
(6) Shield,M.J.(1983):Importance of immunologically effective contact with environmental mycobacteria.The biology of the mycobacteria,volume 2.(ed.C.Ratledge and J.Stanford)Academic Press.p.345-415.
(7) Sutton,P.J.;Wall,E.P.;de Lisle,G.W.(1983)Serology of tuberculosis in deer.Biology of deer production.(ed.P.Fennessy and K.Drew) Royal Society of New Zealand.

TABLE 1

SEROLOGICAL AND SKIN TEST RESULTS

	ID	ELISA (1/80)	ELISA (1/360)	SKIN T. (a)	SKIN T. (b)
PRE-EXPOSURE PRE-INOCULATION	0/50	5/50	0/50	18/50	0/50
POST EXPOSURE POST INOCULATION					
Bact-ve Hist-ve	0/6	1/6	0/6	3/6	0/6
Bact+ve,Hist-ve Bact-ve,Hist+ve	0/4	3/4	2/4	3/4	2/4
Bact+ve,Hist+ve AFO'S-ve	2/12	8/12	5/12	8/12	3/12
Bact+ve,Hist+ve AFO'S+ve	6/25	20/25	15/25	23/25	15/25
Clinical Tb.	2/3	3/3	3/3	2/3	0/3

ID=immunodiffusion,ELISA positive OD>0.150
SKIN T.positive(a)=presence of a reaction
SKIN T.positive(b)=increase in skin thickness of >2.5mm
AFO'S+ve=acid fast organisms seen histological section.