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Application of Immunohistochemistry and ELISA for the Diagnosis of Neospora-Infected Cattle

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Abstract
Studies were undertaken to adapt diagnostic methods for use in our laboratory for detection of Neospora sp. infection in cattle. An immunohistochemical (IHC) test was used for detection of Neospora sp. antigen in tissues of aborted bovine fetuses. Neospora sp. antigen was detected most frequently in fetal brain tissue. Polyclonal antibodies were tested for specificity and sensitivity of the IHC. Sera were obtained from Neospora sp. infected dairy herds for use as positive and negative controls in the continuing development of an enzyme-linked immunosorbent assay (ELISA).

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Application of Immunohistochemistry and ELISA for the Diagnosis of *Neospora*-Infected Cattle

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Summary

Studies were undertaken to adapt diagnostic methods for use in our laboratory for detection of *Neospora* sp. infection in cattle. An immunohistochemical (IHC) test was used for detection of *Neospora* sp. antigen in tissues of aborted bovine fetuses. *Neospora* sp. antigen was detected most frequently in fetal brain tissue. Polyclonal antibodies were tested for specificity and sensitivity of the IHC. Sera were obtained from *Neospora* sp. infected dairy herds for use as positive and negative controls in the continuing development of an enzyme-linked immunosorbent assay (ELISA).

Introduction

Neosporosis is a recently described protozoal infection proposed to be a major cause of abortion in dairy cattle and to a lesser extent in beef cattle.[1,3] The pathogenesis is not completely known, but vertical transmission has been indicated.[2] The bulk of the data collected has been from Southwest and West Coast drylot dairy herds. Although neosporosis has been identified as a causative agent in Midwestern bovine abortions, tests for definitive diagnosis or for epidemiological studies have not been available in Iowa.[3] Here we explore the adaptation and reliability of the IHC and ELISA for diagnosis of neosporosis at the ISU Veterinary Diagnostic Laboratory.

Materials and Methods

Immunohistochemistry

Archival fetal tissues were tested with streptavidin-biotin peroxidase (ABC) immunohistochemistry (IHC) using goat anti-*Neospora caninum* polyclonal antibody. Protocol used followed those described by Halbur et al with the following minor exceptions.[4] Phosphate-buffered saline (PBS), pH 7.2, was used as wash and as diluent. Antibody was diluted at 1:3500 and was incubated on the tissues for two hours at room temperature. Eleven of the known *Neospora* sp. infected tissues also were tested with mouse anti-*N. caninum* monoclonal antibody (MAB 6G7, isotype IgG2a).[5]

All tissues tested were fixed in formalin and embedded in paraffin.

ELISA

Sera from three known *Neospora* sp. infected Iowa dairy herds were collected. Sample size ranged from 45 to 60 milking cows. Cows with a history of a recent abortion were included with the random sampling of non-aborting cows. ELISAs were performed by California Veterinary Diagnostic Laboratory Systems (CVDLS) and interpreted as follows: optical density (OD) values: <.45, were considered negative by CVDLS; .45-.7, were 65% likely to be *Neospora*- infected; and, >.7, were 100% likely to be *Neospora*- infected.

N. caninum was successfully cultured in our laboratory for use as an antigen in our ELISA. Protocol followed that of Pare et al.[6] In our laboratory, we inoculated Vero cell monolayers at 80-100% confluency with 3-5 drops of *N. caninum*, approximately $7x10^6$ tachyzoites. Cell cultures were passed and harvested at 80% cell lysis. Antigen preparation and ELISA followed the protocol of CVDLS, using filtered, washed, and sonicated antigen. Our laboratory used *N. caninum* instead of the bovine isolate (BPA-1) since no notable differences have been detected in Western blot or in the CVDLS ELISA.[6]

Results and Discussion

Immunohistochemistry

IHC sensitivity and specificity were determined through the use of 17 known *Neospora* sp. infected (positive controls) and 10 known infectious bovine rhinotracheitis (IBR) infected cases (negative controls). All positive controls were positive and all negative controls were negative by IHC. Results illustrated a high correlation of IHC positive and known *Neospora*-infected cases.

Twenty-seven *Neospora* sp. infected fetal tissues, 17 known positive cases, and 10 suspect cases diagnosed as "consistent with protozoal causes", were used to study frequency of finding antigen in various tissue types. Availability of tissue type varied with each case. Results of IHC using goat anti-*N. caninum* polyclonal antibody are summarized in Table 1. The consistent detection of *Neospora* sp. antigen in fetal brains supports previous studies.[1,5]

Table 1. Frequency of immunohistochemistry (IHC) detection of *Neospora* sp. antigen within aborted fetal tissues.

Tissue	# positive/n	Tissue	# positive/n
brain	21/26	spleen	2/7
kidney	3/9	muscle	0/3
heart	3/8	intestine	0/4
liver	4/7	placenta	0/6
lung	2/10	thymus	0/1

Seven of the 10 cases diagnosed "consistent with protozoal causes" tested positive by IHC, although not all had brain tissue available for testing.

In addition, 11 of 11 known *Neospora*-infected fetal brain tissues tested with mouse anti-*N. caninum* monoclonal antibody were also positive. Preliminary comparison of monoclonal and polyclonal antibodies indicate that more consistent results were obtained with the use of the monoclonal antibody.

ELISA

Serology results from three *Neospora*-infected Iowa dairy herds are summarized in Table 2. All cows that had aborted had sera antibody levels >.5 (considered positive). All cattle testing positive had aborted.

Sera from cows that were confirmed *Neospora* sp. positive or negative by CVDLS ELISA were used as controls and for

comparison in the continuing refinement of our ELISA serology test. Serology tests are useful in detection of *Neospora* sp. infected fetuses, fetal fluids, and cows.

A fourth Iowan herd that had a *Neospora* sp. diagnosed abortion also was tested. Random sampling of 30 of 175 milking Holsteins, the aborted cow not included, revealed three positive cows by CVDLS ELISA. This is a calculated 13% herd seroprevalence.[6] The 95% confidence interval estimates the infection rate between 2-24%.

Table 2. Incidence of *Neospora-sp.* antibody seroconversion for three Iowa dairy herds. See text for value interpretation.

	O.D. Values		
	>.7	.45-.7	<.45
Herd #1	4	2	9
Herd #2	4	1	11
Herd #3	1	0	13

Implications

Neosporosis has been identified as a significant cause of abortion in Iowa. Immunohistochemistry in fetal brain tissue is useful for the diagnosis of neosporosis in both dairy and beef cattle. More information is needed about transmission and incidence of *Neospora* sp. in cattle. Continued development of serology testing will provide further information on incidence and disease prevalence within Iowa herds. It will also aid in diagnosis when fetal brain tissue is not available.

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