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# Antibody Response to *Haemophilus somnus* Fc Receptor

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**To characterize the bovine immune response to an *Haemophilus somnus* antigen known to be recognized by convalescent-phase serum, we studied isotypic antibody titers to the 270-kilodalton protein, which we had previously shown to be an immunoglobulin Fc receptor. With a modified immunodot procedure, an immune response was detected after experimental *H. somnus* abortion, experimental *H. somnus* pneumonia, or vaccination with commercial *H. somnus* vaccine, with the greatest titer found within the immunoglobulin G2 isotype. With protein A peroxidase conjugate, which detects primarily bovine immunoglobulin G2, we showed that cattle with *H. somnus* disease could be distinguished from clinically normal carriers, culture-negative cattle, or cattle with disease due to *Pasteurella haemolytica* or *P. multocida*. Little cross-reactivity between the 270-kilodalton Fc receptor antigen and antigens from other gram-negative bovine pathogens was seen. Thus, this antigen may be a useful diagnostic antigen.**

*Haemophilus somnus* is known to be an etiological agent in several disease syndromes of cattle including thromboembolic meningoencephalitis (1, 9, 15), respiratory disease (5, 12), septicemia (12, 14), arthritis (12, 14), abortion (2, 16), and possibly infertility (16, 19). In addition to being an important pathogen, this organism can be routinely isolated from mucosal surfaces of apparently normal animals. Most bulls carry the organism asymptomatically in the prepuce (10, 11), many cows are vaginal carriers (21, 24), and nasal carriers also occur (21, 24). Thus, it is difficult to determine whether a positive *H. somnus* culture from a mucosal surface indicates an etiologic role or merely a carrier role. A microagglutination test is available for serologic diagnosis, but at least in the northwest United States the majority of animals are positive (5). A more specific serological test that distinguishes between noninfected animals, asymptomatic carriers, and animals with *H. somnus* disease would be very useful. To this end, we have produced experimental bovine pneumonia (7) as well as abortion (25) and collected preinfection- and convalescent-phase serum samples to compare them at defined times postinfection. Subsequent experiments showed that the immunoglobulin G2 (IgG2) antibody response is most diagnostic for *H. somnus* experimental and clinical infections (P. R. Widders, S. C. Dowling, R. P. Gogolewski, J. W. Smith, and L. B. Corbeil, Res. Vet. Sci., in press). We also have shown that convalescent-phase serum passively protected calves against pneumonia (6), and we identified the antigens recognized by protective convalescent-phase serum (3, 6). One of these antigens is a 270-kilodalton molecule (270K molecule) that is also a receptor for the Fc portion of bovine immunoglobulin. We recently purified and characterized this Fc receptor (27). Since the 270K antigen-Fc receptor was recognized by convalescent-phase serum, it appeared likely that the immune response to this antigen may be significant in protection and/or immunodiagnosis. Now we report the antibody response to the 270K Fc receptor in experimentally infected animals, clinical cases, vaccinated animals, asymptomatic carriers, and culturally negative controls.

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## MATERIALS AND METHODS

**Bacterial strain, media, and growth conditions.** *H. somnus* isolate 2336, recovered from a calf with pneumonia, was preserved from the primary culture in 60% glycerol in 0.01 M phosphate-buffered saline (pH 7.4) at −70°C. This isolate was chosen for purification of the 270K Fc receptor antigen because it was shown to be virulent in studies of experimental pneumonia (7) and because antibody against the 270K antigen from this isolate reacted with the 270K antigen of 16 other isolates (27). Bacteria were cultured in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.1% Tris and 0.01% thiamine monophosphate (BHI-TT) as described by Inzana and Corbeil (13) or on Columbia blood agar (Difco) plates made with 10% bovine blood or fetal bovine serum.

**Bovine sera.** Serum samples from 39 animals (29 cows, 10 bulls) showing no signs of *H. somnus* disease were collected once from beef or dairy herds. The genital and nasal mucosae of each animal were cultured for *H. somnus* just before bleeding. Culture-negative cows came from the Washington State University dairy herd, which had no history of *H. somnus*-related disease. On two occasions 20 cows were cultured, but no *H. somnus* was detected on either nasal or vaginal mucosae from any animal. Therefore, we consider this an *H. somnus*-negative herd. The other 19 clinically normal animals (9 cows and 10 bulls from herds other than the Washington State University dairy herd) were culture positive for *H. somnus* and therefore classified as asymptomatic carriers. Twenty sera from animals with *H. somnus* disease (3 abortions, 17 pneumonia cases) and 12 sera from animals with disease due to *Pasteurella haemolytica* or *P. multocida* came from accessions at the Washington State Animal Disease Diagnostic Laboratory. For studies of response to vaccination, eight dairy heifers in late pregnancy were inoculated intramuscularly with a commercial *H. somnus* killed whole-cell vaccine (Somnugen; Bio-Ceutic Laboratories, Inc., St. Joseph, Mo.) given according to the manufacturer's instructions at 0 and 21 days. Serum samples were collected before vaccination and 21 days after the second inoculation. Last, 20 serum samples collected at weeks 0 and 5 were assayed from each of 10 animals

TABLE 1. Clinical and cultural data of animals under study

| Group[a] | No. of animals | Sex[b] | Disease | *H. somnus* culture results | |
|---|---|---|---|---|---|
| | | | | No. positive/ no. tested | Source of culture |
| Negative | 20 | F | None | 0/20 | Nasal cavity or vagina |
| Carriers | 9 | F | None | 9/9 | Vagina |
| Carriers | 10 | M | None | 10/10 | Prepuce |
| Disease | 3 | F | Abortion | 3/3 | Fetus or uterus |
| Disease | 17 | M and F | Pneumonia | —[c] | Lung |
| Disease | 10 | M and F | Pneumonia | −[d] | Lung or nasal cavity |
| Disease | 2 | F | Abortion | −[d] | Fetus or placenta |
| Vaccinees (prevaccination) | 8 | F | None | 0/8 | Nasal cavity or vagina |
| Vaccinees (postvaccination) | 8 | F | None | 3/8 | Nasal cavity or vagina |
| Experimental pneumonia (preinfection) | 5 | M | None | 0/5 | Bronchial lavage |
| Experimental pneumonia (postinfection lavage) | 5 | M | Pneumonia | 5/5 | Bronchial lavage |
| Experimental abortion (preinfection) | 5 | F | None | 1/5 | Nasal cavity or vagina |
| Experimental abortion (postinfection) | 5 | F | Abortion or fetal resorption | 4/5 | Fetus or uterus |

[a] Preinfection or prevaccination samples were collected just before inoculation. Postinfection samples were collected 5 weeks after inoculation with *H. somnus*. Postvaccination samples were collected 3 weeks after the second inoculation.
[b] F, Female; M, male.
[c] Serum from sick calves in contact with others in which *H. somnus* pneumonia was confirmed by pulmonary culture and pathologic changes at necropsy.
[d] *H. somnus* was not cultured from these cattle, but *P. haemolytica* or *P. multocida* (or both) was present in large numbers.

challenged experimentally with *H. somnus*. These animals are included in separate reports of experimental *H. somnus* abortion (25) and respiratory disease (7). In the experimental abortion study, five abortions and one fetal resorption occurred among 11 pregnant cows that received a challenge inoculation of $4 \times 10^{10}$ *H. somnus* CFU by the intravenous route. One of eight animals inoculated intrabronchially resorbed her fetus. Serum samples from five of these aborting cows were included in this present study. In a second study with a well-characterized experimental *H. somnus*-induced calfhood pneumonia, serum was collected from five calves challenged at 6 to 8 weeks of age by intrabronchial instillation of $10^7$ *H. somnus* CFU (7). The sources of all the sera listed above are summarized in Table 1.

**Purification of the 270K Fc receptors.** The *H. somnus* 270K Fc receptor was purified by using a modification of a previously described procedure (27). Briefly, *H. somnus* 2336 was grown for 8 h at 37°C with shaking in 300 ml of BHI-TT. The bacteria were removed by centrifugation for 10 min at 10,000 × *g*, and the supernatant was filtered through a 0.45-μm-pore-size filter. The filtered supernatant was centrifuged at 45,000 rpm (350,000 × *g*) for 2 h at 4°C in a Beckman TI 50.2 rotor. The pellet was solubilized in phosphate-buffered saline (pH 7.4) containing 0.5% sodium dodecyl sulfate before it was applied to a Sepharose 4B column equilibrated in the same buffer. Each fraction was run on a sodium dodecyl sulfate-polyacrylamide gel (17), which was stained with 0.2% Coomassie brilliant blue. Fractions containing only the 270K protein were pooled, dialyzed against 10 mM Tris hydrochloride (pH 7.5), and lyophilized. The lyophilized material (purified 270K Fc receptor) was suspended in $H_2O$ to an optical density of 0.5 at 280 nm to serve as the stock antigen and stored at −20°C.

**Immunodot assay.** Antibody titers to *H. somnus* 270K Fc receptor antigen were measured by using a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, Calif.) and a modification of the manufacturer's recommended procedure. A nitrocellulose sheet soaked in 0.05 M Tris-buffered saline, pH 7.5 (TBS), was placed in the apparatus. Samples of 50 μl of a 1/50 dilution in TBS of stock purified 270K Fc receptor antigen (≈60 ng) were loaded into each well and allowed to flow through the nitrocellulose by gravity for 1 h. After each well was washed with 200 μl of TBS containing 0.1% Tween 20 (TBS-TW), the nitrocellulose was removed; each dot containing Fc receptor antigen was cut from the nitrocellulose and placed in an individual well of a 24-well tissue culture plate (Costar, Cambridge, Mass.). The test serum was doubly diluted in TBS-TW across the wells of the tissue culture plate and incubated with the antigen-coated nitrocellulose for 1 h with shaking. After a wash with TBS-TW, bovine isotype-specific monoclonal antibodies diluted 1/5,000 in TBS-TW were added for 1 h. Bovine IgG1, and IgG2 antibodies were provided by A. Guidry (U.S. Department of Agriculture, Beltsville, Md.), and anti-bovine IgM was from W. Davis (Washington State University, Pullman). The bovine immunoglobulin bound to the antigen was determined with a 1/1,000 dilution of peroxidase-conjugated goat anti-mouse IgG (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) and developed with 4-chloro-1-naphthol (0.05%) plus $H_2O_2$ (0.015%) in 16% methanol. In certain assays, peroxidase-conjugated protein A at a 1/1,000 dilution (Kirkegaard and Perry) was substituted for the anti-immunoglobulin and conjugate. The last dilution with color was the criterion used to determine the titer. Rabbit anit-270K serum with appropriate second antibody was used as a positive control to detect the 270K antigen bound to the nitrocellulose. The nitrocellulose surrounding the dot of 270K antigen served to internally control possible nonspecific binding of the test serum at each dilution.

**Fc receptor activity.** Fc receptor activity was determined by incubating the Fc receptor-coated nitrocellulose with bovine Fc fragments purified by digesting polyspecific bovine IgG with papain for 6 h as previously described (26). Increasing amounts of purified 270K Fc receptor were dotted onto nitrocellulose as described above. These dots were incubated with 200 μl of purified bovine Fc fragments in TBS-TW (0.5 mg/ml) for 2 h with shaking. The concentration of Fc fragments used was approximately equal to the amount of bovine IgG in a 1/20 dilution of bovine serum. Fc fragments which were bound by the 270K Fc receptor were detected by using peroxidase-conjugated goat anti-bovine IgG (1/2,000 dilution) (Cooper Biochemical Inc., Malvern, Pa.) or protein A peroxidase (PAP) (1/1,000 dilution), followed by chloronaphthol plus $H_2O_2$.

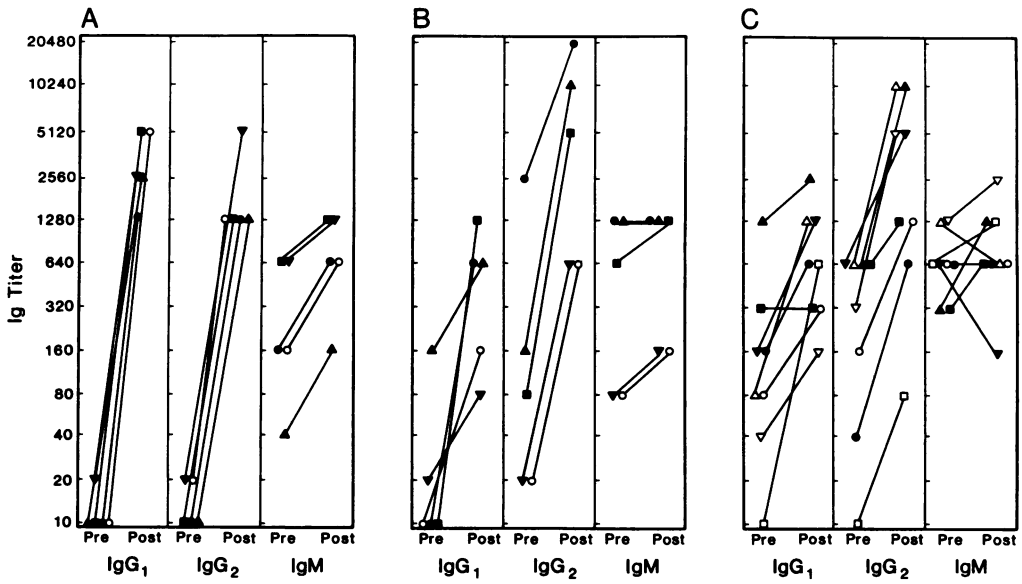


FIG. 1. Isotypic antibody response after experimental infection or vaccination with *H. somnus*. (A) Experimental pneumonia; (B) experimental abortion; (C) vaccinated cattle. Each symbol represents a single animal with a line drawn between the pre- and postinfection (or pre- and postvaccination) titers to show the response of each animal.

**Gel electrophoresis and Western blotting (immunoblotting).** Preparations of whole cells or pellets from high-speed centrifugation of culture supernatants were analyzed by electrophoresis under denaturing conditions in polyacrylamide gels containing sodium dodecyl sulfate by the method of Laemmli (17). The separated proteins were electroblotted onto nitrocellulose (23) and probed with rabbit antiserum against the 270K Fc receptor prepared by cutting the 270K band from sodium dodecyl sulfate-polyacrylamide gel electrophoresis, emulsifying in Freund complete adjuvant, and inoculating into rabbits as described previously (27). Antigen-antibody reactions were developed with goat anti-rabbit IgG (Kirkegaard and Perry), followed by chloronaphthol plus $H_2O_2$.

**Statistics.** Statistical analyses were performed by using Student's *t* test or by one-way analysis of variance. Individual comparisons of groups were performed by using Fisher's protected least significant difference. *P* values of $\leq 0.05$ were considered significant.

## RESULTS

**Isotypic antibody response to the 270K Fc receptor antigen.** The antibody titers of three groups of sera were compared to determine which isotype responded best to the 270K Fc receptor. With the modified immunodot blot procedure, pre- and postinoculation (5 weeks) serum titers for bovine IgG1, IgG2, and IgM were determined for experimental serum samples from cattle with pneumonia (Fig. 1A), serum from cows subjected to experimental abortion (Fig. 1B), and serum from vaccinated cattle (Fig. 1C). There was no significant increase in IgM titers for sera from vaccinated cattle or cows subjected to experimental abortion ($P > 0.05$); however, the IgM titer for sera from calves with pneumonia increased slightly ($P < 0.05$). Both IgG1 and IgG2 titers increased in all three groups ($P < 0.005$). Although the IgG2 titers and the net increase in IgG2 titers were greater with sera from cows after experimental abortion and vaccinated cattle compared with the IgG1 titers, there was no significant difference between the IgG1 and IgG2 response ($P > 0.05$).

**Titers determined with PAP.** PAP was used in our next series of experiments because of the greater IgG2 titers in most of the sera in the above study, and because protein A is known to react primarily with IgG2 at the pH and molarity used in our immunodot assay (4). The reason for choosing PAP rather than anti-IgG2 in this study was the stronger color produced by the PAP reagent. Also, PAP should be more widely available than monoclonal anti-IgG2 antibody, and the assay can be completed with one less step. Using PAP, we determined the anti-270K antigen titers in serum from culture-negative asymptomatic animals, culture-positive asymptomatic animals, and clinical cases (cattle with *H. somnus* abortion or pneumonia). The results of a typical experiment with a representative serum sample from each group of cattle are presented in Fig. 2. By using this immunodot procedure, titers in serum from animals were determined (Fig. 3). Of the asymptomatic animals surveyed, the 20 culture-negative cows had a slightly lower (but not statistically significant) mean IgG2 titer than the 19 culture-positive cows and bulls, although all three mean titers were below 1,000. No significant difference was found between male and female culture-positive animals. PAP titers in sera from the 25 *H. somnus* disease-associated cattle were significantly ($\alpha$ [significance level of the test] = 0.05) elevated over the titers from clinically normal animals. Sera from both cattle with experimental abortion and those with pneumonia had mean IgG2 titers of 16,000. Sera from cattle infected with *P. haemolytica* or *P. multocida* were also tested. The mean titer for these sera was 1,000, which was significantly ($\alpha = 0.05$) below the titer for cattle infected with *H. somnus*.

**Fc receptor activity.** Since the 270K antigen also has Fc receptor activity, we designed experiments to differentiate between Fc binding and whole immunoglobulin binding to antigen by the Fab portion of the molecule. When Fc fragments of IgG were used in the immunodot assay, no color was seen unless at least four times the original concentration of 270K protein was used and only when the concentration of Fc fragments was equivalent to or greater

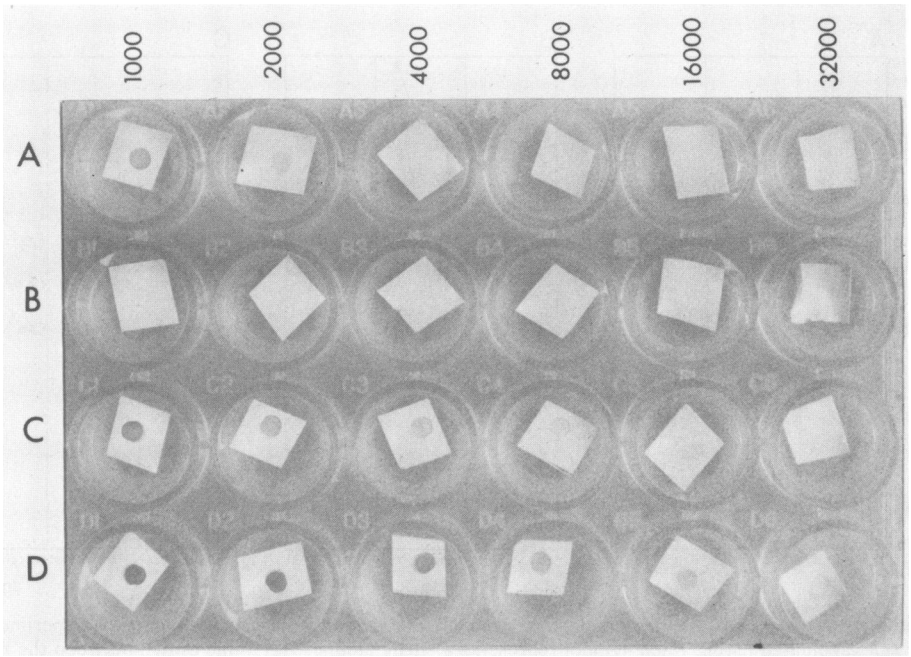


FIG. 2. Anti-270K titers of representative sera with the modified immunodot procedure. Rows: A, serum from an asymptomatic culture-positive bull; B, serum from a culture-negative cow; C, serum from a cow that aborted; D, serum from a cow with pneumonia. The antibody response to the 270K antigen was detected by using PAP.

than the amount of IgG in a 1/20 dilution of bovine serum (approximately 0.5 mg/ml) (data not shown). When PAP was used to detect bound Fc fragments instead of peroxidase-conjugated anti-bovine IgG, eight times the original concentration of 270K antigen was needed to detect Fc receptor activity (data not shown).

**Stability of the 270K antigen on nitrocellulose.** When the 270K antigen was coated on nitrocellulose and air dried or

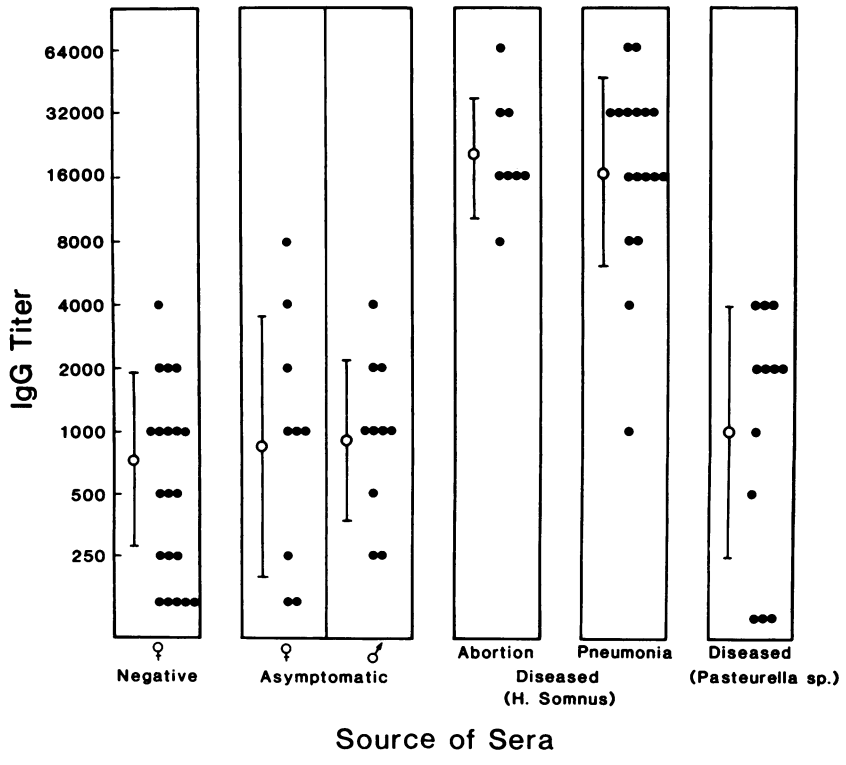


FIG. 3. Anti-270K serum titers from culture-negative cattle, asymptomatic culture-positive cattle, or diseased cattle. Symbols: ●, individual animals; ○, geometric mean titers (vertical bars represent the standard errors).

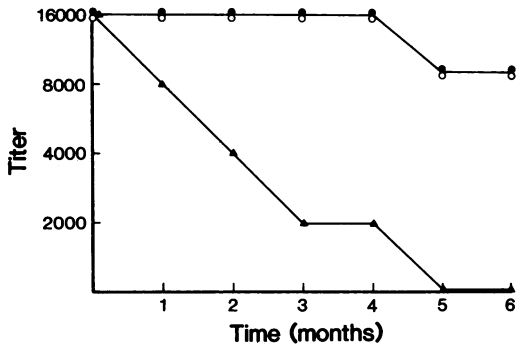


FIG. 4. Stability of 270K antigen on nitrocellulose. Stability was measured with rabbit anti-270K serum (1/2,000 to 1/16,000 dilution) and then detected with goat anti-rabbit IgG peroxidase (1/2,000 dilution). Symbols: ●, 270K antigen-coated nitrocellulose air dried and stored at room temperature; ○, 270K antigen-coated nitrocellulose treated with 70% ethanol for 5 min, air dried, and stored at room temperature; △, 270K antigen-coated nitrocellulose stored at 4°C in TBS-TW.

treated with 70% ethanol before air drying, it was stable for up to 4 months and retained 50% of the original titer at 6 months (Fig. 4). Activity was lost more rapidly, however, when antigen-coated nitrocellulose was stored at 4°C in TBS-TW (Fig. 4).

**Cross-reactivity with related species.** Since other bacterial species may have proteins antigenically related to the 270K antigen, we examined 12 gram-negative bacterial species for cross-reactivity with the 270K antigen. Whole cells and culture supernatant pellets were prepared as described above for purification of the 270K Fc receptor. Antigens from several bacterial species (Table 2) were studied by Western blot analysis with monospecific rabbit anti-270K antisera. Cross-reactivity was seen with a 27K antigen from *Haemophilus influenzae* type b. Also, a 32K antigen from *P. haemolytica* and a low-molecular-weight band (less than 10,000) in both *S. typhimurium* and *K. pneumoniae* reacted weakly with the anti-270K antiserum. None of these antigens from other bacteria gave a reaction as intense as that of the homologous 270K antigen. The 120K antigen seen in *H. somnus* whole-cell preparations has been previously shown to be an Fc receptor that is antigenically related to the 270K antigen (27). The 270K antigen is known to reside in the culture supernatant pellet (27).

TABLE 2. Cross-reactivity of bacterial proteins with 270K antiserum in Western blots

| Species | Antiserum reactivity (antigen) | |
|---|---|---|
| | Whole cells | Culture supernatant pellets[a] |
| *Haemophilus somnus* | ± (120K) | +++ (270K) |
| *Haemophilus agni* | − | − |
| *Haemophilus influenzae* type b | + (27K) | − |
| *Pasteurella haemolytica* | − | ± (32K) |
| *Pasteurella multocida* | − | − |
| *Actinobacillus equulii* | − | − |
| *Actinobacillus lignieresii* | − | ND[b] |
| *Escherichia coli* JL9 | − | − |
| *Pseudomonas aeruginosa* | − | − |
| *Salmonella typhimurium* | ± (<10K) | − |
| *Brucella abortus* 2308 | − | − |
| *Campylobacter fetus* subsp. *fetus* | − | ND |
| *Klebsiella pneumoniae* | ± (<10K) | − |

[a] The pellet was recovered from filtered culture supernatant after centrifugation at 350,000 × *g* at 4°C for 2 h.
[b] ND, Not determined.

## DISCUSSION

In this study, we have used an immunodot blot technique to monitor the antibody response to the purified 270K *H. somnus* Fc receptor antigen. The use of a purified *H. somnus* antigen eliminates the cross-reactivity that is sometimes seen when whole bacteria are used as the antigen. By using this approach, we found that the IgG1 and IgG2 titers to the 270K antigen increased significantly after vaccination or experimental infection, but the IgM titers remained approximately the same. In this study, preinfection IgG1 and IgG2 titers were much lower in the pneumonia group than in the other two groups because of the age of the animals. Experimental pneumonia was produced in calves at 6 to 10 weeks of age when passive immunity had waned but active immunity had not yet reached adult levels (7). This was done because calves are most susceptible to pneumonia at that time. The abortion and vaccination experiments, on the other hand, were done with adult cattle, which would be expected to have higher cross-reactive natural antibody titers. Regardless of the initial titers, however, the increases in titer after infection or vaccination are consistent with our reported isotypic antibody response against *H. somnus* whole cells in that both IgG1 and IgG2 titers were increased after challenge, but no significant change was seen with IgM (6; Widders et al., in press). It is of interest that cattle respond immunologically to this Fc receptor, suggesting that binding of the Fc moiety of immunoglobulin does not mask its antigenic moiety or that Fc is removed during antigen presentation. Lack of a systemic immune response in asymptomatic carriers, on the other hand, could be explained by lack of antigenic stimulation of the systemic immune system. It is known that bulls and cows that carry *Campylobacter fetus* subsp. *venerealis* in their genital tracts do not have increases in serum antibody to this pathogen (4). This is thought to be due to confinement of antigen to the surface of the genital mucosa, so perhaps the same thing is true with *H. somnus* genital carriers.

The above studies comparing defined prevaccination or preinfection sera with sera collected 5 weeks after *H. somnus* vaccination or infection showed that most animals had the greatest titer with the IgG2 subclass. Although Schmerr et al. (20) showed that protein A can bind to bovine IgG1 at high pH and high phosphate concentrations, most investigators (using neutral pH and lower molarity) demonstrate that protein A binds bovine IgG2 almost exclusively (8, 18). Thus, we used PAP in this assay (at neutral pH and low molarity) to detect antibody against the *H. somnus* 270K antigen because of its preferential binding to bovine IgG2. Although the use of monoclonal anti-bovine IgG2 followed by peroxidase-conjugated anti-mouse IgG should increase the sensitivity of the assay by amplification, PAP detects nanogram quantities of antibody (serum titers of 64,000 were measured) and also eliminates an extra step in the assay. PAP detected antibody that distinguished between clinically diseased animals and clinically normal animals whether the latter were culturally negative or asymptomatic carriers of *H. somnus* (Fig. 3). This may be partly due to the fact that natural antibody to *H. somnus* is mostly of the IgM or IgG1 isotype as shown in our previous studies (6, 25; Widders et al., in press). However, preinfection- or prevaccination-phase sera from adult cattle (groups B and C) did have a wide

range of IgG2 titers (10 to 2,560) (Fig. 1). Thus, the range of titers detected by PAP in sera from culture-negative or asymptomatic carriers is likely to be due to an IgG2 response to *H. somnus* 270K antigen (in the carriers) or cross-reactive antigens (in culture-negative animals). PAP does not appear to give a high background, since controls were negative and some animals had very low titers. In the PAP study, the low titers of two animals in the pneumonia group could be due to the fact that there were young calves in the acute phase of the disease and this serum may have been collected before an immune response was well developed. This is a problem with any diagnostic assay based on immune responses. However, in the first study, anti-IgG2 does detect the greatest net increase with preinfection- and postinfection-, or vaccination-phase sera. In the second study, titers detected with PAP were significantly higher than titers from clinically normal animals or animals infected with other closely related gram-negative bacteria (*P. haemolytica* or *P. multocida*) with diseases clinically similar to *H. somnus* disease (pneumonia or abortion). Thus, the PAP assay favors detection of antibodies produced in response to clinical disease and may be useful in differential diagnosis between pasteurellosis and the *H. somnus* complex.

The immunodot procedure described herein is a simple and quick method for diagnosing the disease status in cattle. With the present technique, results can be obtained within a few hours after receiving the test sera, and this time could be shortened considerably. Also, because a purified antigen is used and primarily the IgG2 response is detected with PAP, the problem of natural cross-reactive antibodies resulting in false-positives is practically eliminated. Use of the 270K antigen in this assay has several advantages. First, this antigen has been purified and characterized (27). Second, because the antibody response to the 270K antigen is very high (an average titer of 18,000 in this study), only a small amount of this protein (approximately 60 ng) is needed per assay. Although this protein also has Fc receptor activity, the low amount of protein in the assay eliminates the problem of the Fc receptor binding the IgG through the Fc region since Fc binding is only detected when four times this concentration of Fc receptor is used. Thus, all the activity reported in Table 2 and Fig. 1 through 3 is due to antigen-antibody complexing via the Fab portion of the immunoglobulin molecule. Third, the 270K antigen is stable on nitrocellulose at room temperature for up to 4 months, providing an acceptable shelf life for a diagnostic test. Fourth, a simple two-step purification procedure results in a high yield of purified 270K Fc receptor. Last, the assay is specific; there was very little cross-reactivity of the 270K antigen with other bacteria (Table 2). Only *H. influenzae* type b showed any significant reactivity with anti-270K antibody, but this should not interfere with diagnosis because *H. influenzae* is not usually a bovine pathogen. Furthermore, sera from cattle with pasteurellosis gave results comparable to those with clinically normal animals in the immunodot assay with the 270K antigen, providing additional evidence of the specificity of the assay. These sera from animals with pasteurellosis did show strong reactivity with *Pasteurella* antigens in Western blots (S. Kania and L. B. Corbeil, unpublished data), indicating that the animals did have an immune response to the infecting organisms. Thus, we have shown that the immunodot assay with the *H. somnus* 270K antigen can distinguish between animals with an immune response due to disease or vaccination with *H. somnus* and those animals which are asymptomatic carriers, culture negative, or infected with closely related bacteria. Although the 270K antigen does not distinguish between vaccinates and diseased animals, our research group is investigating subunit vaccines that will not contain the 270K antigen. When this is accomplished, it should be possible to distinguish between subunit-vaccinated animals and diseased animals as well as between diseased animals and carriers or noninfected animals.

In summary, by using a modified immunodot procedure with purified *H. somnus* 270K antigen, we detected an immune response after experimental *H. somnus* abortion, experimental *H. somnus* pneumonia, or after vaccination with commercial *H. somnus* killed vaccine. In most animals, the greatest titer was found within the IgG2 isotype. By using PAP, because it reacts preferentially with bovine IgG2, we showed that diseased cattle could be distinguished from clinically normal carriers or culture-negative cattle.

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