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***Brief Communication***

# IMMUNOFLUORESCENT IMMUNOGLOBULIN DIFFERENTIATION OF VIBRIO FETUS ANTIBODIES FROM BOVINE CERVICO-VAGINAL SECRETIONS

Reports on discrepancies between local and systemic immunity started to appear about 50 years ago (cf. *Tomasi & Bienenstock* 1968). Protection against infections has been shown in many cases to be closely related to the antibody content of external secretions and more or less independent from the serum antibody level.

Three 18—24 months old heifers were inoculated in the cervical canal with material from a Vibrio fetus carrier bull. The heifers were tested bacteriologically according to *Adler* (1957) twice a week before and after inoculation. Nos. 7 and 75 were positive from days 11 and 7 respectively and onwards. No. 24 was constantly found non-infected. Mucus samples were collected by the tampon method (*Szabo* 1951). The proteins were eluted from the tampon with 5.0 ml physiological saline buffered at pH 7.1 by 0.01 M phosphate (*Nairn* 1969). Approx. 2.5 ml extract could be obtained after compressing the tampon with a spatula. The extract was centrifuged at 1800 × g for 20 min., and the supernatant was subsequently filtered through 220 nm filters.

The antisera employed had all been exhaustively absorbed with Vibrio fetus organisms. The goat anti rabbit immunoglobulin had been rendered totally unreactive with bovine immunoglobulins by duplicate absorptions with bovine peripheral leukocytes isolated according to *Aalund et al.* (1970). Following the last absorption the fluorescein-isothiocyanate (FITC) labelled goat anti rabbit immunoglobulin (Behringwerke) was diluted 10 times, centrifuged at 40,000 × g for 15 min. and filtered through a 450 nm filter. All reagents were stored at —20°C in 0.5 ml aliquots.

Monospecific anti bovine IgG-1 and anti IgG-2 reagents were produced by immunizing rabbits with IgG-1 or IgG-2 and absorbing exhaustively with IgG-2 and IgG-1 respectively. Specific rabbit anti IgA and anti IgM were prepared according to *Nansen et al.* (1971).

Smears of Vibrio fetus were dried in the air and fixed in acetone for 10 min. before examination with the immunofluorescence sandwich technique. This included subsequent reactions with tampon extract, rabbit anti bovine immunoglobulin and FITC labelled goat anti rabbit immunoglobulin. Each step of the reaction took place in a moist chamber at 37°C for 30 min. Between the individual reactions

the slides were rinsed thoroughly in phosphate buffered saline (*Nairn*). The cover slips were mounted in phosphate buffered glycerol (*Nairn*). The slides were examined in dark field illumination with a Zeiss WL microscope equipped with an FITC interference exciter

Table 1. Immunofluorescent reactions.

| Time (days) | FITC-score | | | | | |
|---|---|---|---|---|---|---|
| | no. 7 | | no. 24 | | no. 75 | |
| —63 | 0 | | □ | | □ | |
| —21 | □ | | □ | | 0 | |
| 0*) | □ | oestrus | □ | | □ | oestrus |
| 4 | 0 | | 0 | oestrus | 0 | |
| 7 | 0 | | 0 | | 0 | |
| 10 | □ | | □ | | 0 | |
| 14 | + | | + | | □ | |
| 18 | □ | oestrus | □ | | 0 | |
| 21 | + | | ++ | | 0 | oestrus |
| 25 | □ | | □ | | 0 | |
| 27 | □ | | 0 | oestrus | □ | |
| 28 | □ | | □ | | +++ | |
| 30 | ++ | | □ | | □ | |
| 32 | □ | | □ | | ++ | |
| 35 | □ | | □ | | ++ | |
| 39 | +++ | oestrus | 0 | | 0 | |
| 42 | ++++ | | 0 | | □ | oestrus |
| 46 | +++ | | 0 | oestrus | ++++ | |
| 49 | ++ | | | | + | |
| 53 | □ | | | | +++ | |
| 56 | +++ | | | | 0 | |
| 60 | □ | oestrus | | | +++ | |
| 63 | ++++ | | | | 0 | oestrus |
| 67 | ++++ | | | | +++ | |
| 70 | | | | | +++ | |
| 74 | | | | | +++ | |
| 77 | | | | | 0 | |
| 81 | | | | | ++++ | |
| 84 | | | | | 0 | oestrus |
| 88 | | | | | ++++ | |
| 91 | | | | | +++ | |
| 95 | | | | | ++++ | |
| 97 | | | | | 0 | |
| 109 | | | | | ++++ | |
| 112 | | | | | + | |
| 116 | | | | | +++ | |

□ = not done. *) day of inoculation.

filter*), a 520 nm barrier filter and a high pressure 250 watt CSI halogen lamp. The fluorescence was scored + to ++++ according to the brightness of the FITC stained bacteria.

The results are given in Table 1. Nos. 7 and 75 were negative prior to the inoculation. No. 24 was not tested prior to inoculation, but samples from days 4 and 7 were scored 0. Nos. 7 and 24 had become positive at 14 days and no. 75 at 28 days. No. 7 remained positive during the entire observation period, while no. 75 was intermittently negative, to some extent synchronized with oestrus. No. 24 was reactive on days 14 and 21.

Semiquantitative immunoglobulin differentiation on specimens from nos. 7 and 75 (Table 2) demonstrated that the antibody activity was almost equally distributed among the IgA, IgG-1 and IgM entities, while the IgG-2 activity was scored 0.

Table 2. Immunofluorescent immunoglobulin differentiation.

| Animal no. | Time (days, cf. Table 1) | FITC-score | | | |
|---|---|---|---|---|---|
| | | IgA | IgG-1 | IgG-2 | IgM |
| 7 | 63 | ++++ | ++ | 0 | +++ |
| 75 | 46 | ++++ | ++++ | 0 | +++ |

The results are consonant with the reports on IgA as the significant immunoglobulin in several secretions of many mammalian species. *Wilkie* (1970) has reported the exclusive occurrence of IgA mucus agglutinins following cervico-vaginal inoculation of heifers with Vibrio fetus. Contrasting our observations *Wilkie* found IgG and IgM mucus antibodies only after parenteral inoculation with Vibrio fetus. The results may encourage research on the possible application of this procedure to the diagnostics of Vibrio fetus infections.

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