

## Serologic Response in Human Fasciolopsiasis

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**OBJECTIVE:** In most helminthic infections in which serologic responses have been demonstrated, there is considerable invasion of host tissues by the parasite. The extent to which such tissue involvement is necessary for the production of antibody is not well understood. It is therefore of interest to study antibody production in helminthiasis in which there is little tissue invasion. *Fasciolopsis buski* ranks low among intestinal parasites with respect to the degree of tissue invasion accompanying infection. The organism has no extraintestinal developmental forms and is believed to maintain its position in the bowel by the muscular acetabulum alone, feeding on intestinal contents rather than host tissue. We therefore examined sera from patients with *F. buski* for antibody reactive with antigens of the worm.

**DESCRIPTION:** Sixty-two sera were collected from individuals previously shown to be infected with *F. buski*. Control sera were obtained from Americans and Thais with no history of parasitic infection and from people infected with the liver fluke, *Opisthorchis viverrini*. The latter specimens were employed to investigate reactivity of *F. buski* antigen with sera from people infected with another species of trematode which has a greater potential for the induction of an immune response.

A crude antigen was prepared from whole adult *F. buski* collected from infected pigs and stored at  $-70^{\circ}\text{C}$ . The frozen worms were thawed and weighed, added to 4 ml of saline for each gram of worm and extracted in a high speed steel bladed homogenizer for 10 minutes in an ice bath. The preparations were centrifuged for 30' at  $11,500 \times g$  and the supernatant stored at  $-70^{\circ}\text{C}$ .

The sera were studied by a quantitative complement fixation test essentially as described by Levine<sup>1</sup>. Antigen concentration was varied and the serum dilution kept constant at 1/400. This high serum dilution was necessary to minimize anticomplementary effects noted with some sera in preliminary experiments. The extent of lysis was determined by absorption measurements at 412 m $\mu$ . (Soret band of hemoglobin) on supernatants from reaction mixtures. The difference in absorption ( $\Delta A_{412}$ ) between experimental mixtures and complement controls (i. e. mixtures without serum or antigen) was taken as an estimate of complement fixation. The mixtures were prepared so that complete lysis gave an absorption reading of 1.5 units and the complement controls gave 80–90% lysis. Sera which gave  $\Delta A_{412}$  values of greater than 0.15 in the absence of antigen were considered anticomplementary. All measurements were performed on duplicate reaction mixtures.

**PROGRESS:** Six experiments were performed comparing individual sera in each of the four categories described above. A representative experiment is summarized in Fig. 1. Complement fixation was observed with all four sera, but was much greater with the *F. buski* serum than in the controls. These experiments are summarized in Table I; the figures are for the highest antigen concentration employed. In all but one case, greatest fixation was observed at the highest antigen level. The exception was a serum from an American which gave strong fixation ( $\Delta A_{412} = 0.595$ ) at a relative antigen concentration of 0.005 ml/ml but essentially none at 0.05 ml/ml.

Tests of a total of 62 *F. buski* sera, including those employed above, were also tested at four antigen concentrations up to and including 0.1 ml/ml. At least one serum from a normal Thai was tested in each experiment, and a total of 14 such control sera were studied. All *F. buski* sera induced a progressive increase in complement fixation with increasing antigen. However, 10 sera showed significant anticomplementary

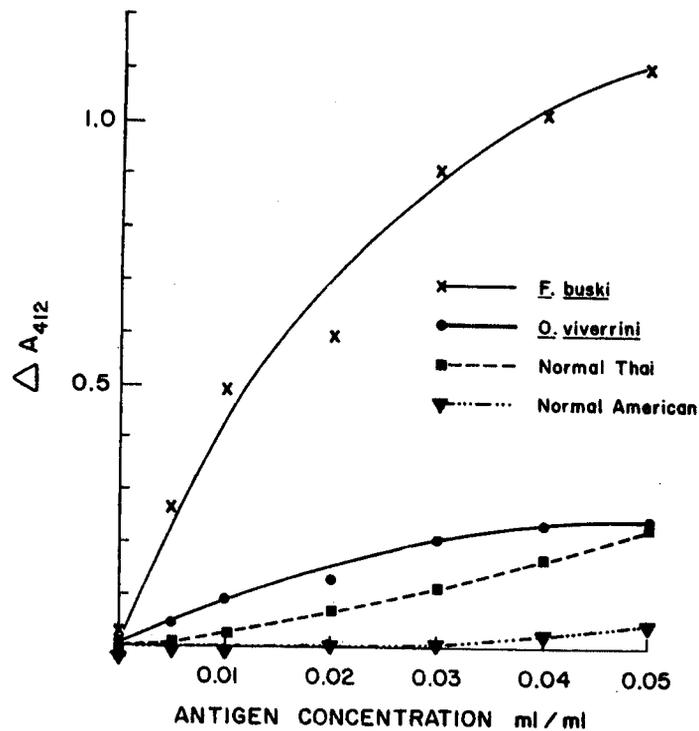


Fig. 1 Complement fixation by four sera and *F. buski* antigen.

activity so that estimates of complement fixation might be unreliable. The remaining 52 sera gave  $\Delta A_{412}$  values ranging from 0.748–1.389 with the 0.1 ml/ml lever of antigen. Seven of the 14 controls also showed some fixation, but the maximum  $\Delta A_{412}$  was 0.371.

The fact that many of the control sera have some reactivity is not surprising in view of the complexity of the worm extract used as antigen. Since the lowest degree of fixation observed with any *F. buski* serum was higher than that obtained with any of the controls, the data indicate that there is a serologic response to the infection. Serum dilution titrations are in progress.

**SUMMARY:** Tests on 52 sera from individuals with *F. buski* infections showed greater complement fixation than in any of 32 control sera from normal individuals (26 sera) or people with *O. viverrini* infections (6 sera).

**REFERENCE:** Levine, L., "Micro-complement Fixation" in Weir, D.M., Ed., "Handbook of Experimental Immunology" F.A. Davis, Philadelphia, 1967.

Table I. Complement fixation by human sera and crude *F. buski* antigen.

Serum	$\Delta A_{412}$ , Range in six experiments
<i>F. buski</i>	0.667–1.260
<i>O. viverrini</i>	0.066–0.553
Normal Thais	0–0.372
Normal Americans	0–0.257