

Predominance of the ac Variant in K88-Positive *Escherichia coli* Isolates from Swine†

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Monoclonal antibodies to K88ac and K88ab were used in enzyme-linked immunosorbent assays on *Escherichia coli* cultures known to produce K88 pili. A total of 415 K88-positive *E. coli* isolates from nine states were all found to be the K88ac variant. The cultures tested were isolated during the years 1976 to 1985.

Adherence of *Escherichia coli* to epithelial cells and the concurrent ability to cause disease have been associated with the presence of structures called pili or fimbriae. These pili were first described in 1961 and were called K antigens on the basis of serological differentiation of *E. coli* isolated from pigs showing signs of "edema disease" or enteritis (6). Analysis of purified K88 antigen revealed that it was a protein as opposed to the previously described K antigens, which were known to be polysaccharides (9, 10). Using specific antisera, Orskov et al. (7) demonstrated two variants of the K88 antigen, which they called K88ab and K88ac. The K88ab variant was associated with the original isolate from enteritis in England (6), whereas K88ac was associated with isolates from Ireland and the German Democratic Republic (8). In 1979 another K88 variant (ad) was found to be associated with enteropathogenic *E. coli* from the Netherlands (3). Emergence of these variants may be the result of selective pressure due to widespread immunization with K88 antigen-containing vaccines (3). Another view holds that the increased numbers of pigs without receptors for a certain pilus type has caused a selection toward *E. coli* with a variation in the pili to match the presence of receptor sites on the epithelial cells of the porcine gut (2). Currently, the most common K88 pilus types isolated from infected pigs have been identified as K88ac or K88ad to the exclusion of the K88ab variant (3, 11). It has been suggested that there was a pattern developing toward the disappearance of the K88ab antigen (2).

Monoclonal antibodies specific for K88ac pili were obtained from Molecular Genetics (Minnetonka, Minn.). The monoclonal antibodies specific for the K88ab pili were produced by the Department of Biology at Kansas State University. *E. coli* K88 antigen was purified by a modification of the method of Stirm et al. (9) by using a K88ab culture obtained from the *E. coli* Reference Center (Pennsylvania State University). BALB/c mice were injected intraperitoneally at four weekly intervals with 50 µg of purified intact K88 pili followed by 10 µg intravenously 2 weeks later. Spleen cells from the immunized mice were fused with P3X63Ag8.653 myeloma cells by using the fusion protocol of

Fazekas de St. Groth and Scheidegger (1). After 8 to 10 days, supernatants from the hybridomas were screened for specific antibody production by using a modification of the enzyme-linked immunosorbent assay (ELISA) described by Mills et al. (4, 5).

A total of 585 *E. coli* cultures isolated from 1976 to 1985, which were previously shown to produce a heat-labile enterotoxin or K88 pili, were surveyed to identify isolates which retained the ability to express the K88 antigen. Cultures were collected from nine swine-producing states (see below) so that isolates from a wide geographical area could be compared with those sent to the Kansas State University Department of Veterinary Diagnosis. The cultures were first grown on Tergitol 7 medium (BBL Microbiology Systems, Div. Becton Dickinson and Co., Cockeysville, Md.), from which a single colony was picked and inoculated onto blood agar (5% sheep blood). Cultures were tested for K88 pili by the polyclonal ELISA as previously described (4, 5). A total of 415 isolates were found to be producing K88 pili. The numbers of isolates from each of the nine states were: Kansas, 273; South Dakota, 46; Iowa, 39; Minnesota, 20; Nebraska, 13; Illinois, 12; Pennsylvania, 3; Indiana, 1; and Missouri, 1; along with 7 isolates for which the state of origin was unknown. Bacterial suspensions used in the polyclonal ELISA were then checked by two monoclonal ELISAs, one which was specific for the b component and one which was specific for the c component. The specificity of the monoclonal ELISAs was demonstrated by determining the reaction of the assays to various pilus types. *E. coli* suspensions of the three pilus types were standardized by using a nephelometer. Absorbance values (Biotek Instruments, Burlington, Vt.) are listed in Table 1 and show the specificity of the monoclonal ELISAs.

The 415 isolates tested by monoclonal ELISAs were all found to produce the K88ac pili. The only *E. coli* cultures found to produce the K88ab antigen were the four K88ab

TABLE 1. Mean light absorbances from specific K88ab and K88ac ELISA reactions

Variant of standardized <i>E. coli</i> suspensions	ELISA reading ^a	
	ac specific	ab specific
ab	0.003	0.174
ac	0.445	(-).004
ad	0.007	0.002

^a The reader was blanked on wells without bacteria. Readings shown are the average A₄₁₄s for 10 wells.

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cultures obtained from the *E. coli* Reference Laboratory at Pennsylvania State University.

This survey suggests that K88ac is the predominant K88 variant in the major pork-producing region of the United States. These data also support our contention that K88ac should be the variant used for various K88 pilus vaccines and for production of monoclonal antibodies used in diagnosis of colibacillosis caused by K88 *E. coli*.

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