COMPARISON BETWEEN STANDARD IN VITRO VIRULENCE ASSOCIATED ASSAYS AND HUMAN COPROANTIBODY SIGA PRODUCTION AS PREDICTORS OF YERSINIA ENTEROCOLITICA AND YERSINIA ENTEROCOLITICA-LIKE ORGANISM ASSOCIATED MOUSE VIRULENCE AND HUMAN DISEASE PRESENTATION

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I. ABSTRACT

A semi-quantitative indirect immunofluorescence assay was developed which distinguishes two types of patients from whom yersiniae are recovered: those who produce a strong yersiniae specific coproantibody secretory IgA (SIgA) response and those who do not. This SIgA response appeared to be yersiniae specific as faecal supernatant controls from patients whose stools where shown to yield negative or positive cultures for Salmonella, Campylobacter, or Clostridia were SIgA negative.

Organisms isolated from patients with high SIgA titers had a higher incidence of virulence associated characteristics although SIgA response was not associated with most other commonly recognized assays of virulence. A strong association was shown to exist between SIgA titre and mouse virulence, the gold standard of bacterial virulence.

Clinical examination of patients culture positive with yersiniae documented a strong association between acute enteric illness and high SIgA titre. This association was not dependant on the cultured yersiniae species.

No single *in vitro* virulence associated assay was found to be a reliable predictor of animal virulence. The virulence of nine *Y.frederiksenii* and one *Y.kristensenii*, previously thought to be non-pathogenic in man, was also documented.

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V. INTRODUCTION

The genus Yersinia consists of gram negative fermentative bacilli belonging to the Enterobacteriacae family. Originally members of the genus Pasteurella, these organisms were assigned to a new genus in 1944 based on their oxidase negativity (1), and later, in 1964, they were separated into three species: Y.pestis, Y.pseudotuberculosis, and Y.enterocolitica (2). Y.pestis is the known causative agent of Bubonic Plague in man, Y.pseudotuberculosis is associated with granulomatous lesions of the intestine, and Y.enterocolitica is associated with a variety of enteric disorders including diarrhea, abdominal pain, gastroenteritis, and mesenteric lymphadenitis (3-6).

A. Versiniae Characteristics

The first report of Y.enterocolitica-related enteric illness came from Carlsson et al in 1964 who recovered the organism from the mesenteric lymph nodes of a patient with acute terminal ileitis (7). In 1966 Winblad et al documented patients culture and sero-positive with Y.enterocolitica who had presented with acute diarrhea or appendicitis-like symptoms (8). Although the majority of Y.enterocolitica isolates reported were primarily restricted to Northern Europe, Canadian isolates were first reported in 1967 (9). Since then Y.enterocolitica has been increasingly associated with acute,

self-limited enteric illness and several post-infection syndromes, such as polyarthritis and erythema nodosum (5,10-13). Y.enterocolitica has also been associated with pseudoappendicitis in children resulting in surgery (14). In addition, severe yersiniae associated septicaemia in children and immunosuppressed patients has been documented (15,16).

In 1980, a series of atypical Y.enterocolitica-like organisms were subclassified on the basis of DNA-DNA hybridization homology (17) and unique biochemical properties into three new species: Y.frederiksenii, Y.kristensenii, and Y.intermedia (18-20). Table I outlines the biochemical properties of these yersiniae and those of two additional species, Y.ruckeri and Y.aldovae (21), solely found as aquatic isolates. Y.enterocolitica does not refer to a single organism. Instead, this species may be subdivided by biochemical reactivity into 8 biotypes (Table II) and by 56 lipopolysaccharide O antigens and 19 flagellar H antigens into serotypes (22). When labelling a strain however, only the biotype and O antigen type are routinely included. Phage typing systems which further differentiate the serotype/biotypes have also been developed, but are usually reserved for epidemiologic studies (23).

Serotype (24) and biotype (25) have both been associated with the organism's pathogenic potential. In 1983, the World Health Organization outlined the syndromes associated with various serotypes (10). Types O:3 and O:9 were termed pathogenic as they were associated only with the "classic" gastroenteritis syndrome in man. "Other" serotypes, ie 4, 5, 6, 7, 8, 13, etc, were found only in healthy carriers or cases of

supra-infection and were therefore considered non-pathogenic. This same report noted that strains belonging to Biotype 1 were not usually associated with human disease. Since then there have been several reports of infection with these "non-pathogenic" strains (26-31), ie Biotype 1 strains have been implicated in septicaemia cases (28), O:5, O:5,27, O:6,30, O:6,31, and O:21 in human enteritis cases (32-35), and O:8 in food bourne outbreaks (36). The varied reports of pathogenic potential may be the result of varied patient ages across the studies, i.e. Caprioli et al noted a rise in the incidence of "other " serotype with increasing patient age. It is important to note that some "pathogenic" strains have also been recovered from healthy human and animal carriers (10,37,38). Currently the most commonly pathogenic strains are O:3, O:5,27, O:8, and O:9.

Yersinia species are psychrotropic and will therefore grow at temperatures lower than competing flora. In 1974 Eiss (45) documented a 40% increase in yersiniae recovery after cold enrichment, however the usefulness of cold enrichment in the recovery of pathogenic organisms has been questioned. Van Noyen (46) reported that cold enrichment did not significantly increase recovery of serotypes O:3 and O:9 although increased recovery of other serotypes was found.

Y.enterocolitica sensu stricto, and the newly defined strains have been isolated from a variety of environmental and clinical sources including human and animal stools, fish, small mammals, water, milk, raw and cooked pork, tofu, and ice cream (39-44). Y.kristensenii, Y.frederiksenii, and Y.intermedia have primarily been isolated from the

environment or skin and wound infections and were not originally associated with enteric illness (18–20). Recently, however, these organisms have been recovered from patients presenting with acute enteric symptoms (6,32).

B. Virulence Associated Characteristics

Virulence of Y.enterocolitica and related organisms has repeatedly been associated with a variety of in vivo and in vitro characteristics. In vivo assays are animal model based, and in vitro assays range from differential media to plasmid analysis.

In vivo virulence has been defined as the ability to cause keratoconjunctivitis in guinea pigs (Sereny Test)(47), to cause mouse death after intraperitoneal injection (48), and to cause mouse diarrhea or to invade the intestine and deep organs (spleen and liver) of rabbits and mice after oral challenge (49–51). The guinea pig Sereny Test is limited in its usefulness as a yersiniae virulence test as some commonly pathogenic strains (i.e. serotypes O:3, O:9, and O:5,27) fail to evoke a conjunctivitis response (50). However, when mice are utilized in place of guinea pigs the assay's sensitivity does increase significantly (52). Overall the mouse is currently the animal model of choice as mouse symptoms following inoculation via numerous routes closely mimic those of humans, i.e. intravenous or intragastric inoculation yields mouse intestinal lesions histologically similar to those seen in humans (50,53). Injection of dilute bacterial suspensions intraperitoneally (i.p.) into mice will detect the virulence of numerous serotypes. The

spectrum of the i.p. assay can be further improved by the addition of iron dextran to the inoculum (54). Oral inoculation mouse models with subsequent mouse diarrhea better parallel the events of human disease, but onset of mouse diarrheal symptoms may be difficult to assess with accuracy. Infiltration of mouse deep organs, liver and spleen, following oral challenge with Yersinia species provides the simplest method of assessing virulence. This model correlates well with animal models, yet has an easier, more definable end point, i.e. <10 orgs/spleen considered avirulent and 50–100 orgs/spleen considered virulent.

In vitro virulence associated characteristics include a requirement of calcium for growth (55), ability to autoagglutinate (56), absorption of a haemin type dye, Congo Red (57), hydrophobicity (58), expression of outer membrane proteins (74), and carriage of a 40–48 Megadalton (Mda) DNA plasmid (59), all of which are expressed in virulent strains at $37^{o}C$ but not at $25^{o}C$.

The requirement of calcium for growth by virulent Yersinia species was first reported in 1959 by Higuchi et al (60). They documented virulent Y.pestis strains requiring the cation for growth in liquid media at $37^{o}C$. When starved for calcium these virulent strains undergo restriction, a slow down of metabolism, involving a reduction in stable RNA and nucleotide triphosphate formation and decreased adenylate energy stores. Growth on calcium deficient media at $37^{o}C$ is therefore pin-point in nature. Although no mechanistic role for calcium dependance has been elucidated, this phenomenon may relate to the low intraphagosomal Ca^{++} and Mg^{++} ion concentrations encountered during

ingestion by mammalian cells (55). Since 1959 calcium dependance of yersiniae has been frequently associated with the organism's ability to cause disease in animals (55) and man (61). More recently this characteristic has been assigned to a specific, highly conserved 9kb region on the virulence associated plasmid (62). It is important to note that this characteristic has been detected in environmental non-pathogenic isolates as well as clinical isolates (63,64).

Autoagglutination of Yersinia species in tissue culture media at 37^{o}C was reported by Laird and Cavanaugh in 1980 (56). They noted that virulent strains had a greater tendency to clump at 37^{o}C than avirulent strains. In 1985 Prpic et al (58) described autoagglutination by Laird and Cavanaugh's method as a reliable predictor of mouse virulence in organisms carrying an additional virulence associated characteristic, Congo Red absorption (see below). Autoagglutination has been associated with the expression of two outer membrane proteins, V and W antigens, originally defined in Y.pestis and is thought to result from development of a protein fiber matrix linking the cells together (62,65). As with calcium dependance, autoagglutination has been associated with the virulence plasmid although in this case no specific loci has been elucidated (65,66).

Y.enterocolitica species do not generally produce exogenous siderophores and therefore rely on the presence of free ferric iron in the environment, or uptake of siderophores released by other organisms in their vicinity, i.e. desferrioxamine released by Streptomyces pilosus (17). Absorption of Congo Red is thought to parallel iron absorption,

a characteristic with great advantages to organisms attempting to survive in the mammalian host. Absorption was originally associated with virulence in Y.pestis (67,68), but has more recently been documented in Y.enterocolitica species (57). In 1987, the expression of a series of high molecular weight Iron Regulated Proteins (IRPs) on the outer membrane of Yersinia species under iron starvation conditions was documented (69). Such expression could account for iron or congo red uptake although absorption studies on these organisms were not included in their report. In addition, aerobactin producing strains of Y.frederiksenii, Y.kristensenii, and Y.intermedia have recently been reported (70). Although Y.enterocolitica and Y.pseudotuberculosis strains examined in the same study did not produce the hydroxymate siderophore, its role in potential iron absorption should not be ignored.

Several studies have documented a family of 40–48 Mda plasmids present in Y.enterocolitica strains that can be associated with virulence (48,52,60,71,72,73,74). The first of this series was documented during a milk related yersiniosis outbreak in 1980 (47). Its association with virulence and tissue invasiveness was established through ethidium bromide curing experiments (47), and at this time it was found that repeated subculture at 37^{o}C also resulted in curing of the plasmid. Since 1980, 50–100% DNA homology has been shown to exist within Y.enterocolitica plasmids and 50% DNA homology between Y.enterocolitica and Y.pestis plasmids, perhaps indicating a common ancestral origin (75). In addition to calcium dependance and

autoagglutination plasmid carriage has been associated with expression of a unique series of outer membrane proteins (74), cytotoxicity to human epithelial cells (73,75), and inhibition of phagocytosis and chemiluminescence of normal human neutrophils (76). It is important to note that non-pathogenic strains have also been shown to carry plasmids within this size range, and that plasmid-less pathogenic strains have been documented (77). A recent study has suggested the existence of movable genetic elements in Yersinia species that could account for the presence of plasmid characteristics in the absence of a plasmid (78).

Each of the in vitro characteristics discussed above has some ability to differentiate organisms with potential virulence in man (79), but none considers the human response to the organism.

The **first objective** of this study was to compare the in vitro virulence associated characteristics of Y.enterocolitica and Y.enterocolitica-like organisms recovered from symptomatic patients to animal virulence, the gold standard of bacterial virulence, and to the disease presentation in humans.

C. Coproantibody Secretory Immunoglobulin A

In the cells of the intestinal lamina propria (Gut Associated Lymphoid Tissue) there is a predominance of Immunoglobulin A (IgA) producing plasma cells, accounting for >80% of the Ig secreting cells (79). Two IgA monomers are linked via disulfide bonds to a 15,000mw J-chain and actively transported out of the lamina propria (79).

Epithelial cells synthesize a glycoprotein or secretory component which in turn acts as a basal receptor for the IgA dimer. This complex (SIgA) is then transported through the epithelial cell layer to the gut lumen and released. This type of active transport ensures its presence in large quantities in intestinal secretions.

Of the immunoglobulins, SIgA is the best suited to deal with antigenic material in the gut lumen as it resists phagocytosis and proteolysis by intestinal enzymes, is quadravalent, and has an affinity for the mucosa (80). SIgA is thought to be the most important resistance mechanism for bacterial elimination as it can both agglutinate the organisms and act as an immune barrier in the intestine by preventing adherence and thereby invasion of the organisms (81). This type of blocking activity is preferred in the gut as it does not activate any further antimicrobial activity, i.e. complement activation, that could lead to degranulation and tissue damage.

Several recent studies have documented a rise in serum titre of yersiniae specific antibody early in infection (82–84) primarily accounted for by IgA class antibodies in particular the 11S or dimer form normally found in secretions, however the reliability of serological diagnosis of yersiniae infection remains controversial. Agglutination assays, either by tube or microtitre, have great potential for cross–reactivity as whole organisms are utilized (85). In addition, although high antibody titers can be considered diagnostic, low antibody titers may result from some underlying illness, i.e. co–infection or immunodeficiency, and cannot exclude yersiniosis.

In addition, agglutination assays detect primarily IgM and IgG as IgA does not agglutinate. In fact, the presence of IgA may even decrease the apparent titre of IgM and IgG by binding to antigenic sites and blocking IgM and IgG interactions (84). Although ELISA assays provide a more sensitive method for detecting class specific antibody, they still do not overcome the problems associated with IgA blocking (85,86). In addition to methodological problems, no criteria exist for ascribing significance to an increased IgA titre (87), i.e. healthy individuals often exhibit high background antibody levels to yersiniae. Therefore the diagnostic applications of serum IgA levels in a case of suspected yersiniosis are limited, and any serology for this purpose requires at least one sera from both acute and convalescing phases (13,88). Overall, in spite of the difficulties discussed, an increased IgA titre has been implicated in the prediction of post-infection aseptic arthritis, thereby remaining important (85,87).

In 1971 Reed and Williams (89) documented the presence of Secretory IgA (SIgA) class shigella agglutinating antibodies in faeces from patients with recent onset diarrhea. In the past 25 years several other investigators have reported coproantibody responses to enteric organisms in rectal mucosa and faeces using indirect immunofluorescence assays (90–92). Although no mechanistic role has been suggested, a good association between coproantibody production and stage of enteric illness was clearly demonstrated.

The **second objective** of this study was to determine whether or not an immunological marker of human immune response, coproantibody SIgA,

correlated first with human disease presentation and secondly with any of the established virulence associated markers including mouse virulence.

It was suggested by Sarasombath et al in 1987 (93) that systemic antibody responses and intestinal SIgA responses were linked during the course of S.typhi infections. Such a linkage potentially exists during yersiniae infection. The **third objective** of this study was to compare organisms eliciting varied human intestinal immune responses to serological titers in a mouse model.

VI. METHODS

A. SPECIMENS AND ORGANISMS

Faecal specimens were received in the UBC-HSCH Microbiology Laboratory in Cary-Blair Transport Media or sterile containers and routinely processed for detection of enterics as previously described (6). Briefly, faecal material was plated on CIN Yersinia Selective Agar (PML Microbiologicals) for 18-24 hours at 37°C. Remaining specimen was incubated at 4°C in broth culture and subcultured after 1, 3, 7, and 14 days. Colonies with the characteristic bulls-eye appearance were screened on Triple Sugar Iron, Urea, Lysine, Indole, and motility assays (PML Microbiologicals). Strains were further characterized on API 20E Enterobacteriacae System (API Laboratory Products). All Y.enterocolitica strains were then biotyped and serotyped by an independent reference laboratory. Faecal specimens were simultaneously examined for the presence of parasites, but viral cultures were not included.

Original faecal specimens were maintained at -4°C until bacteriology was completed. When a Yersinia species was recovered, the faeces was processed for coproantibody SIgA detection. Organisms were maintained at -70°C in Glycerol and Dimethylsulfoxide (DMSO) until assayed for virulence. Clinical history of all patients was reviewed and is presented in Table 3.

In cases where the organisms were isolated and frozen prior to the onset of this study, faecal supernatant was not available. These organisms were included in the virulence testing for comparison purposes, but were not tested for SIgA.

B. ORGANISM VIRULENCE ASSOCIATED ASSAYS

1. Autoagglutination

Interpretation of autoagglutination is commonly based on subjective observation of complete tube clearing. Several authors have expressed difficulty in assessment of autoagglutination with Laird and Cavanaugh's method (56), i.e. strains failing to grow in tissue culture media, high rate of false positives, varying results dependant on inoculum size, and faulty interpretation as a result of partially cleared tubes (94,95). For these reasons the method of Laird and Cavanaugh (56) was modified to include a spectrophotometric analysis of clearing. Organisms were grown overnight in tryptone yeast extract broth (pH 7.0) (Difco) at $25^{o}C$, centrifuged, washed in phosphate buffered saline (PBS), and resuspended to McFarlane Standard 3 (10^{9} orgs/ml). The culture was divided, incubated 18 hours at $25^{o}C$ or $37^{o}C$, and the optical density (O.D.) read at 460 nm. Known autoagglutination positive and negative strains (Strains 700L and 700S, Dr.C.Pai, Calgary) were included in ten experimental

runs and the difference between twice their Standard Error of the Mean (Appendix A) values was calculated to be 0.1 O.D. units. Strains where the O.D. at 25°C exceeded that at 37°C by 0.1 were interpreted as autoagglutination positive.

2. Calcium Dependance

Direct plating on Magnesium Oxalate Agar (Appendix B) as described by Higuchi and Smith (96) was used to detect calcium dependant strains. Colonies from organisms requiring calcium for growth (Calcium Dependant) were <1 mm diameter after 36 hours at 37°C and those not requiring calcium (Calcium Independent) were >1 mm diameter.

3. Congo Red Absorption

The Congo Red Acidmorpholinepropanesulfonic Acid agar (Appendix B) described by Prpic et al (57) was used to assess absorption of Congo Red. Organisms that actively take up the dye form red colonies after 36 hours at 37°C and are scored as positive, those that do not form white or slightly pink colonies and are scored as negative.

4. Plasmid Profile

Presence of a 40–48 Mda plasmid was determined with a modified Birnboim and Doly Alkaline Lysis Extraction method (97). Organisms were grown overnight in beef heart infusion broth (Difco) at 25°C and harvested by centrifugation, washed, and resuspended to 10^9 orgs/ml in PBS. Cells were resuspended in 100ul fresh, chilled Lysis Buffer (50mM Glucose, 10mM EDTA,

25mM Tris-HCl (pH 8.0), and 4mg/ml Lysozyme) and allowed to stand 5 minutes at room temperature to weaken the cellular walls. 200ul of fresh, chilled Alkaline Buffer (0.2 N NaOH and 1% SDS) was added, mixed twice by inversion, and allowed to stand 5 minutes on ice to lysis cells. 150ul fresh, chilled Acid Buffer (60 ml Potassium Acetate, 11.5 ml Glacial Acetic Acid and 28.5 ml distilled H_2O) was added, inverted and gently vortexed for 10 seconds, and allowed to stand 5 minutes on ice to precipitate chromosomal DNA. Samples were centrifuged (12,000 xg for 5 min) and the supernatant added to an equal volume of Phenol/Chloroform. Samples were then vortexed and centrifuged (12,000 xg for 2 min) to extract contaminating proteins. The supernatant was removed and allowed to stand in 2 volumes of 70% ethanol at $-20^{o}C$ for 20 minutes to precipitate plasmid DNA. Samples were recentrifuged, additional ethanol poured off, and the pellet air dried. Pellets were resuspended in 40ul of RNase Buffer (30mM Tris (pH 8.0), 5mM EDTA, 50mM NaCl, and 20 ug/ml RNase) to eliminate contaminating RNA. 15ul of DNA solution and 5ul of Stop Mix (50 ml Glycerol, 7ml EDTA (0.5 M), 5mg Bromphenol Blue, in 100 ml Distilled H_2O) were loaded on a Tris Borate Buffered Agarose Gel (8.9mM Tris Base, 8.9mM Boric Acid, 0.2mM EDTA, and 0.6% agarose in distilled H_2O). Gels were electrophoresed for 2 hours at 50mV on a Mini-Gel Apparatus (Bethesda Research Laboratories) and post-stained in 5ug/ml

ethidium bromide for 20 minutes. Standards included T4, T5, T7, and lambda phage DNA (Sigma) and a strain known to carry the 40Mda plasmid (700S). Those strains with plasmids banding in the same region as the control strain were considered plasmid carrying.

5. Mouse Virulence

For animal virulence assessment the method of Bakour et al (50) was modified. Adult Swiss mice (Charles River) were dehydrated for 24 hours then allowed to drink ad lib from a 0.1% peptone broth containing 10^9orgs/ml, from an overnight tryptone soy agar plate (Difco), for 36 hours. Mice were sacrificed by carbon dioxide and their spleens homogenized then plated on CIN yersinia selective agar (PML Microbiologicals) for 24 hours at 37°C. Colonies with the characteristic bulls-eye appearance were further characterized on TSI and Urea slants (PML Microbiologicals). A strain was defined as mouse virulent if the homogenized spleen contained more than 100 organisms.

C. HUMAN COPROANTIBODY SIGA RESPONSE

Intestinal SIgA response was assessed with a newly established semi-quantitative indirect immunofluorescence assay. Faecal samples were stored at -4°C until a yersiniae species was recovered. Faecal specimens were then diluted 1:1 with PBS, centrifuged (30 min at 15,000xg) and the supernatant stored at -70°C. 200 ul of serial

supernatant dilutions (1/10,1/50,1/100) were incubated with 40 ul of 10^9orgs/ml in PBS on coverslips (18 hours at 25°C). Coverslips were air dried, dip washed in distilled water, and incubated with 120 ul of FITC labelled goat anti-human IgA (Sigma) (30 min at 37°C). Slips were dip washed, air dried, inverted and mounted for fluorescence microscopy. Results were defined as follows: no fluorescence - ; single organisms 1+; homogeneous microagglutination 2+ (Figure 1).

D. MURINE SEROLOGY

Adult Swiss mice were orally inoculated with IgA 2+, 1+, and - strains as described above. Animals were sacrificed 7 days after inoculation and their heart blood immediately withdrawn. Blood was centrifuged and sera stored at -70°C until time of assay. Formalinized antigen was prepared as described by Winblad (98). Organisms were grown 1-2 days on Blood Agar plates (PML Microbiologicals) at room temperature, harvested by centrifugation, and washed in saline before suspending in 10% Buffered Formalin. A loopful of antigenic material was streaked on Blood Agar plates and incubated overnight at 37°C to check sterility. Prior to the microagglutination assay the antigenic material was washed in saline and resuspended to McFarlane Standard 5. 0.5 ml of sera was serially diluted in duplicate round bottom microtitre plates (Becton Dickinson) and 0.5 ml of formalinized antigen was added to each well. Plates were spun at 700

rpm for 10 minutes. The inverse of the last dilution showing halo-type agglutination was recorded as the antibody titre.

E. DIAGNOSTIC TEST EVALUATION

Clinical evaluation of any diagnostic test is based on the test's accuracy, that is, how close is the test measurement to the true value (99). The true value in many cases is unreadable, therefore new tests are compared against established or "gold standard" tests. In this study, two gold standards of organism virulence were utilized. Animal virulence is a widely accepted standard of organism pathogenicity, and association with acute human enteric symptoms further approximates the true human pathogenic potential of the organism.

When selecting a test for use it becomes important to know the probability that the test will be positive when applied to an organism that is truly virulent, i.e. its sensitivity, and the probability that the test will be negative if applied to an organism that is truly avirulent, i.e. its specificity. Although these values are useful when selecting a test for use, they are of limited use in evaluating a single test result. In that case it is necessary to know the probability that an organism will be virulent if the test result is positive, that is, the predictive value of the test needs to be calculated. Positive predictive value refers to the ratio of true positives to test positives and negative predictive value refers to the ratio of true negatives to test negatives. Predictive values are influenced not only by the

sensitivity and specificity of the test, but also by the probability of virulence in the population prior to testing (99). Testing of a population with a high incidence of positives increases the predictive values. Appendix C shows sample calculations of positive predictive value, negative predictive value, sensitivity, and specificity for the Coproantibody SIgA assay. However, since this study was concerned primarily with the probability that an organism is pathogenic in man when the test value is positive, only positive predictive values for remaining assays are reported in the text.

VII. RESULTS

Figure 1 illustrates typical fluorescence microscope fields for a 2+, a 1+, a negative, and a negative in phase contrast. A 2+ result is defined as large clusters of agglutinating organisms fluorescing bright apple green and is indicative of a strong specific IgA class antibody response (Figure 1A). A 1+ result is defined as single fluorescent organism and is indicative of a weak specific antibody response (Figure 1B). When the cultured yersiniae species stimulates no antibody response, the field is apparently empty (Figure 1C). This absence of fluorescence can not be accounted for by an absence of bacteria however as organisms are detected in phase contrast of the same field (Figure 1D). Supernatant only and organism only control slides (photos not included) showed some homogeneous background fluorescence, but no organism-like structures.

Faecal supernatants from salmonella (Pt# 64), campylobacter (Pt# 65, 66, 67), or clostridia (Pt# 68) associated diarrhea patients and asymptomatic, organism-negative patients (Pt# 69, 70, 71) were examined for cross-reactive agglutination with yersinia. No specific agglutinating cross-reactivity was found. However, non-agglutinating halo-type staining of single organisms was detected when supernatants were tested against control organisms including salmonella, campylobacter, clostridia, and Escherichia coli.

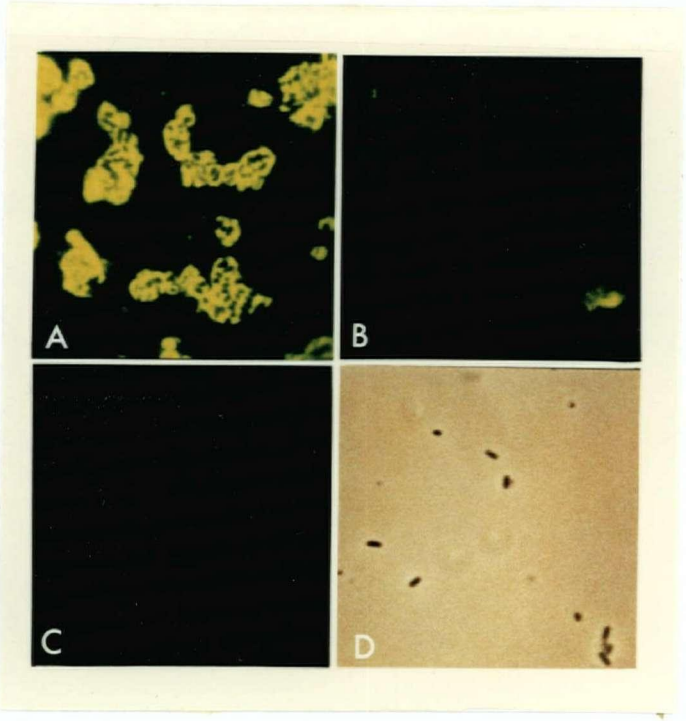

Figure 1 – Typical Indirect Immunofluorescence SIgA Assay Results. A, ++, Homogenous Microagglutination B, +, Single Organism and Non-Specific Background; C, –, No Specific Fluorescence; D, – in Phase Contrast, Non-clumping Organisms Coating Slide.

Table III contains a complete listing of all patients and organism characteristics used in this research and referred to in the text.

Table IV documents the ability of the virulence associated assays to differentiate organisms from patients with acute enteric symptoms. Although not absolute, the positive predictive value of SIgA response for acute disease (P.P. – 85.7%) was greater than that of any single in vitro (Autoagglutination – 56.0%, Calcium Dependance – 56.5%, Congo Red – 58.5% , Plasmid Carriage – 53.7%) and paralleled that of mouse virulence (P.P. – 73.7%). Of note, exceptions to this association included patients # 6, 8, and 47 who suffered inflammatory bowel disease

yet elicited a strong SIgA response (see discussion) and patients #9, 10, 24, and 25 who presented with acute diarrhea yet had a low or absent SIgA response. Patient #10 was shown to be Clostridium difficile toxin positive, although the bacterium was not cultured, and patient #9 carried B. hominis.

When organisms recovered from patients with inflammatory bowel disease are excluded from data analysis on the basis that these patients may be presenting with diarrheal symptoms as a result of underlying illness, SIgA production remains a better predictor of acute enteric symptoms (P.P. - 75.0%) than any other in vitro assay (Autoagglutination - 52.2%, Calcium Dependance - 52.3%, Congo Red - 55.3% , Plasmid Carriage - 51.4%) and still parallels the gold standard of mouse virulence (P.P. - 70.6%)(Table V).

Overall positive predictive values (P.P.) of the in vitro virulence associated assays for mouse virulence were: Autoagglutination - 44.0%; Calcium Dependance - 39.1; Congo Red Absorption - 34.1%; and Plasmid Carriage - 29.3% (Table VI), although these values fluctuated slightly with the yersiniae species. These results agree with those of Prpic et al (58), which found autoagglutination to be the most reliable predictor of mouse virulence in congo red positive organisms. The newly established coproantibody SIgA assay predicted mouse virulence better than any of the established virulence associated assays (P.P. - 85.7%)(Table VI) and if organisms recovered from patients with inflammatory bowel disease are again excluded the SIgA assay appears to be an absolute predictor of mouse virulence (Table VII).

Organisms cultured from SIgA positive (2+) patients have a higher incidence of some virulence associated characteristics. Table VIII documents no significant difference between SIgA 2+ and SIgA 1+/- strains in expression of congo red absorption (X^2=0.00), calcium dependance (X^2=0.14), and plasmid carriage (X^2=0.15), however, a significant difference was apparent with mouse virulence (X^2=17.65; $p<0.002$) and autoagglutination (X^2=5.39; $p<0.05$).

Table IX relates the ability of organisms to stimulate a human coproantibody response to their ability to elicit a serological response in another animal, the mouse, similiarly orally challenged. Although no definite conclusions could be drawn from only four organisms, it was noted with interest that organisms coming from SIgA producing patients (2+) elicited high antibody responses in the mouse whereas those coming from low SIgA producers (1+ or -) elicited little or no antibody response. These findings are consistent with those of Sarasombath et al (93) that a correlation between intestinal and systemic antibody responses does exist. Simultaneous human sera was not available from these patients, therefore similar experiments in man were not included in the study.

Table X compares the days cold enrichment required for recovery to the type of SIgA stimulation. The number of SIgA stimulators did not increase or decrease substantially from 0 to 14 days and although a slight increase in SIgA non-stimulators was seen in the first week, this increase did not persist into the second week. Overall, 58.3% of the mouse virulent, antibody-inducing yersiniae were recovered only after cold enrichment.

VIII. DISCUSSION

The virulence test results in this study agree with those of Kay et al (100) that no single virulence associated assay correlates well with yersiniae virulence as assessed by the mouse model. In addition, this study demonstrates a relationship between coproantibody SIgA stimulation, mouse virulence, and acute human enteric illness.

As discussed earlier, Y.frederiksenii, Y.kristensenii, and Y.intermedia were not originally considered enteric pathogens but have recently been recovered from patients presenting with acute diarrhea. Prpic et al (58) found that some Y.enterocolitica-like organisms expressed some of the in vitro virulence associated characteristics, but were still mouse avirulent. Similarly, this study documents the expression of one or more in vitro characteristics in Y.frederiksenii, Y.intermedia, and Y.kristensenii species. It also documents the existence of Y.frederiksenii, Y.kristensenii, and atypical Y.enterocolitica strains (i.e. O:41,43, O:36, etc) which are mouse virulent and immunostimulatory in humans. This is consistent with the previously discussed observation that strains of Y.enterocolitica other than O:3, O:5,27, etc, may be pathogenic in man.

Although the usefulness of cold enrichment techniques is still in question, this study found it to be important in recovery of some enterically pathogenic yersiniae, i.e. 58.3% of the mouse virulent,

immunostimulatory yersiniae were recovered only after cold enrichment.

The existence of coproantibody SIgA in faecal specimens following enteric infections was documented as early as 1947 when Harrison & Banvard (101) reported the detection of agglutinating antibody in faecal specimens from patients with both acute and chronic bacillary dysentery. Since then several studies have reported the presence of high coproantibody titers in faeces (102) and intestinal mucosa (90) of patients with ulcerative colitis and in volunteers orally immunized with live S. typhi vaccine (91,92). In the current investigation the presence of high SIgA titers in faecal specimens has been documented and strongly associated with acute enteric illness and carriage of mouse virulent organisms. Of note, when patients with inflammatory bowel disease were excluded from analysis because their underlying disease represented confounding data, the positive predictive value of SIgA for mouse virulence became 100%. In addition, this association was not dependent on the cultured yersiniae species.

In 1951 Barksdale et al (102) noted that certain strains of coliform bacilli contained antigenic components common to certain shigella and salmonella species. Such cross-reactions could conceivably limit the diagnostic value of a coproantibody assay, therefore appropriate controls, including supernatants from salmonella, campylobacter, or clostridia diarrhea patients and enteropathogen negative asymptomatic patients, were included in this study. Since these specimens were negative, a strong agglutinating SIgA response appeared to be yersiniae specific. However, as the number of control supernatants in this study was low, no overall specificity could be

determined.

Two exceptions to the association between SIgA response, acute disease, and mouse virulence in the current study were found in patients with Crohn's Disease and acute colitis, both of whom exhibited a strong SIgA response to an apparently avirulent yersiniae strain. This observation was consistent with Barksdale et al's case of a chronic ulcerative colitis patient from whom neither shigella nor salmonella were isolated, yet whose coproantibodies agglutinated three salmonella species and four or more shigella species (102). Toivanen et al (103) suggested that persistence of yersiniae within the intestine, i.e. in epithelial or lymphoid tissues, could provide a stimulus for prolonged antibody production in cases of reactive arthritis. Such a mechanism may also be involved in maintenance of antibody production in inflammatory bowel disease. Monteira et al (92) suggested that strains of enteric bacteria which would produce only transient reactions in non-colitic patients may be able to elicit antibody production in mucosa of colitic patients. Further explanations include the possibility that mouse-virulent organisms could lose their temperature-dependent virulence plasmid while in the gut lumen or that strains of yersiniae may exist which are immunostimulatory in humans yet inactive in the mouse model. The second explanation is supported by Hancock et al's (104) finding that certain strains of mice are naturally resistant to some yersiniae strains. For the aforementioned reasons, the coproantibody SIgA assay as outlined here may produce false positives and should not be relied on to predict the animal virulence potential of

yersiniae strains recovered from patients with known inflammatory bowel disease .

Carriage of yersiniae in the presence of another causal agent would account for cases of acute diarrhea where low or absent SIgA responses were detected. The mouse avirulent nature of the cultured yersiniae species in these cases and the observation that some of these serotypes are normally found only in carriers or in suprainfections (10) supports this concept. Two such patients (Pt# 9, 10) carried bacterial or parasitic pathogens which may have accounted for their symptoms. No additional organisms were cultured from the remaining patients (Pt# 24, 25), however, since electron microscopy of faecal samples was not done virally induced enteritis could not be excluded.

A number of patients culture positive with a mouse virulent yersiniae carried a concurrent parasite, i.e. B.hominis, D.fragilis, or I.butchlii. These patients may represent concurrent yersiniae and parasitic infection, or infection with one organism predisposing the patient to the other. The actual sequence of infection, however, could not be determined from a single faecal specimen. In these cases the coproantibody SIgA assay may be of use in differentiating transient non-immunostimulatory yersiniae isolates from those eliciting a strong intestinal immune response.

IX. SUMMARY

In summary this thesis has outlined the use of a semi-quantitative indirect immunofluorescence assay which facilitates the performance of a serological assay without the need for repeat specimens. This assay was shown to distinguish two types of patients from whom yersiniae are recovered: those who produce a strong yersiniae specific coproantibody secretory IgA (SIgA) response and those who do not. A positive (2+) SIgA response was a better predictor of acute human enteric illness and mouse virulence than any of the established *in vitro* assays of virulence and appeared to be yersiniae specific. In addition, the virulence of nine *Y.frederiksenii* and one *Y.kristensenii*, previously thought to be non-pathogenic in man, was documented.

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APPENDIX A

EVALUATION OF AUTOAGGLUTINATION STANDARDS

Value	Autoagglutination	
	−	+
Number of Runs	10	10
Mean Value	0.0425	0.1268
Standard Error of the Mean	0.0156	0.0224
Upper Boundary	0.0737	
Lower Boundary	−	0.1729
Twice the Standard Error of the Mean	0.0992	= 0.10

APPENDIX B

MAGNESIUM OXALATE AGAR

880 ml Distilled Water

40 gm Blood Agar Base

Boil, Cool, and Add:

40 ml $MgCl_2 - 6H_2O$

80 ml Sodium Oxalate

Autoclave 20 min at 121°C.

CRAMP AGAR

15 gm Noble Agar

2 gm Galactose

2 gm Casamino Acids

1000 ml Distilled Water

10 ml Congo Red (5,000 ug/ml)

pH 7.0

Autoclave 25 min at 121°C

APPENDIX C

DIAGNOSTIC TEST EVALUATION
SAMPLE CALCULATIONS

2 x 2 DATA TABLE

		Gold Standard Value +	Gold Standard Value −
Diagnostic Test Value	+	a	b
	−	c	d

a = # True Positives
b = # False Positives
c = # False Negatives
d = # True Negatives

Positive Predictive Value = a/a+b
Negative Predictive Value = d/d+c
Sensitivity = a/a+c
Specificity = d/d+b

EXAMPLE:

2 x 2 DATA TABLE FOR SIGA ASSAY

		Gold Standard Value +	Gold Standard Value −
Diagnostic Test Value	+	12	2
	−	0	14

Positive Predictive Value = 12/12+2 = 85.7%
Negative Predictive Value = 14/14+0 = 100%
Sensitivity = 12/12+0 = 100%
Specificity = 14/14+2 = 87.5%

TABLE I

BIOCHEMICAL CHARACTERISTICS OF YERSINIA SPECIES[21,105]

Characteristic	Y.pestis	Y.pseudo-tuberculosis	Y.entero-colitica	Y.inter-media	Y.freder-iksenii	Y.kris tensenii	Y. ruckeri	Y. aldovae
Motility	-	+	+	+	+	+	v	+
Lysine Decarboxylase	-	-	-	-	-	-	+	-
Ornithine Decarboxylase	-	-	+	+	+	+	+	+
Urease	-	+	+	+	+	+	-	+
Citrate	-	-	-	+	v	-	+	+
Voges-Proskauer	-	-	+	+	+	-	v	+
Indole	-	-	v	+	+	v	-	-
Rhamnose	-	+	-	+	+	-	-	+
Sucrose	-	-	+	+	+	-	-	-
Cellobiose	-	-	+	+	+	+	-	-
Melibiose	v	+	-	+	-	-	-	-
Sorbose	-	-	+	+	+	+	-	-
Sorbitol	-	-	+	+	+	+	-	+

TABLE II

BIOCHEMICAL FEATURES OF YERSINIA BIOGROUPS[64]

Characteristic	Biogroup						
	1	"American" Strains	2	3	3A[1] 3B[2]	4	5
Lipase Activity (Hydrolyzes Tween)	+	+	-	-	-	-	-
Esculin Hydrolysis	+/-	-	-	-	-/+	-	-
Indole Production	+	+	+	-	-	-	-
Acid Production From Xylose	+	+	+	+	+	-	v
Voges-Proskauer Test	+	+	+	+	-	+	+
Ornithine Decarboxylase, Acid Production from Trehalose, and Nitrate Reduction	+	+	+	+	+	+	-
Pyrazinamidase Activity	+	-	-	-	+	-	-

1 - Positive for acid production from sorbose and inositol
2 - Negative for acid production from sorbose and inositol

TABLE III - SUMMARY OF PATIENT AND ORGANISM CHARACTERISTICS

Patient #	Species	Biotype Serotype	Symptoms	IgA Response	Mouse Virulence	Calcium Dependance	Congo Red Absorption	Autoagglu -tination	Plasmid Profile	Other Organism
1	Y.enterocolitica	1,0:6,31	Acute Diarrhea	++	+	-	+	+	-	D.fragilis
2	Y.enterocolitica	1,0:6,36	Abdominal Pain	++	+	-	-	+	+	-
3	Y.enterocolitica	1,0:7,13	Acute Diarrhea	++	+	-	-	-	-	-
4	Y.enterocolitica	4,0:3	Acute Diarrhea	++	+	+	+	+	+	-
5	Y.enterocolitica	4,0:3	Acute Diarrhea	++	+	+	+	+	+	-
6	Y.enterocolitica	1,nt	Acute Colitis	++	+	-	-	-	-	-
7	Y.enterocolitica	1,nt	Acute Diarrhea	++	+	-	-	-	-	-
8	Y.enterocolitica	1,0:5	Crohn's Disease	++	-	-	-	-	-	-
9	Y.enterocolitica	1,0:4,32	Acute Diarrhea	+	-	+	+	-	+	B.hominis
10	Y.enterocolitica	1,0:6,30	Acute Colitis	+	-	-	-	-	-	C.diff.Toxin
11	Y.enterocolitica	1,0:7,8	Fever NYD	+	-	-	-	-	-	-
12	Y.enterocolitica	1,0:7,13	Crohn's Disease	+	-	+	-	-	+	-
13	Y.enterocolitica	1,0:36	Weight Loss	+	-	-	+	+	-	B.hominis
14	Y.enterocolitica	1,0:41,43	Abdominal Pain	+	-	-	-	+	-	-
15	Y.enterocolitica	1,nt	Rectal Bleeding	+	-	-	+	-	-	D.fragilis
16	Y.enterocolitica	1,0:5	Abdominal Pain	-	-	-	-	-	-	-
17	Y.enterocolitica	1,0:7,8	Abdominal Pain	-	-	-	+	-	-	Salmonella
18	Y.enterocolitica	1,0:5,27	Septicaemia	ND	+	+	+	-	+	-
19	Y.frederiksenii	ND	Acute Diarrhea	++	+	+	+	+	+	-
20	Y.frederiksenii	ND	Acute Diarrhea	++	+	-	+	-	+	-
21	Y.frederiksenii	ND	Hyponatremia	++	+	+	+	+	+	-
22	Y.frederiksenii	ND	Acute Diarrhea	++	+	-	-	+	-	-
23	Y.frederiksenii	ND	Acute Diarrhea	++	+	+	+	+	+	-
24	Y.frederiksenii	ND	Acute Diarrhea	+	-	+	+	-	+	-
25	Y.frederiksenii	ND	Acute Diarrhea	-	-	+	-	-	+	-
26	Y.frederiksenii	ND	Abdominal Pain	-	-	+	-	-	+	Trophozoites
27	Y.frederiksenii	ND	Chronic Diarrhea	-	-	-	-	-	-	-
28	Y.frederiksenii	ND	Crohn's Disease	-	-	-	+	-	-	-
29	Y.frederiksenii	ND	Reactive Arthritis	ND	+	+	+	+	+	-
30	Y.frederiksenii	ND	Acute Diarrhea	ND	+	-	+	-	-	B.hominis
31	Y.frederiksenii	ND	Acute Diarrhea	ND	+	-	+	+	-	-
32	Y.frederiksenii	ND	AIDS	ND	+	+	+	-	+	I.butchlii
33	Y.frederiksenii	ND	Acute Colitis	ND	+	-	+	-	+	-
34	Y.frederiksenii	ND	Acute Diarrhea	ND	-	-	+	+	+	B.hominis
35	Y.frederiksenii	ND	Acute Diarrhea	ND	-	-	+	-	-	S.meuchin
36	Y.frederiksenii	ND	Abdominal Pain	ND	-	-	-	+	+	-

TABLE III - SUMMARY OF PATIENT AND ORGANISM CHARACTERISTICS - CONT'D

Patient #	Species	Biotype Serotype	Symptoms	IgA Response	Mouse Virulence	Calcium Dependance	Congo Red Absorption	Autoagglu -tination	Plasmid Profile	Other Organism
37	Y.frederiksenii	ND	Acute Diarrhea	ND	-	-	+	-	-	-
38	Y.frederiksenii	ND	Abdominal Pain	ND	-	+	+	+	+	-
39	Y.frederiksenii	ND	Chronic Diarrhea	ND	-	-	+	-	+	-
40	Y.frederiksenii	ND	Chronic Diarrhea	ND	-	-	+	+	+	-
41	Y.frederiksenii	ND	Abdominal Pain	ND	-	+	+	+	+	B.hominis
42	Y.frederiksenii	ND	Acute Diarrhea	ND	-	-	+	+	-	Isospora
43	Y.frederiksenii	ND	Abdominal Pain	ND	-	+	+	+	+	-
44	Y.frederiksenii	ND	Acute Diarrhea	ND	-	-	+	-	-	Giardia
45	Y.frederiksenii	ND	Acute Diarrhea	ND	-	-	-	-	+	-
46	Y.frederiksenii	ND	Acute Diarrhea	ND	-	+	-	+	+	-
47	Y.kristensenii	ND	Acute Colitis	++	-	+	-	+	+	-
48	Y.kristensenii	ND	Acute Diarrhea	ND	+	+	+	+	+	B.hominis
49	Y.kristensenii	ND	Abdominal Pain	ND	-	+	-	-	+	-
50	Y.kristensenii	ND	Acute Diarrhea	ND	-	+	+	+	+	-
51	Y.kristensenii	ND	Rectal Bleeding	ND	-	-	+	-	+	C.jejuni
52	Y.kristensenii	ND	Acute Diarrhea	ND	-	-	-	-	+	-
53	Y.kristensneii	ND	Abdominal Pain	ND	-	-	+	-	-	-
54	Y.kristensenii	ND	Abdominal Pain	ND	-	+	-	+	+	-
55	Y.kristensenii	ND	Acute Appendicitis	ND	-	-	+	-	+	S.typimurium
56	Y.intermedia	ND	Nausea	ND	-	-	+	-	+	-
57	Y.intermedia	ND	Acute Diarrhea	ND	-	-	+	-	+	-
58	Y.intermedia	ND	Acute Colitis	ND	-	-	+	+	+	-
59	Y.intermedia	ND	Acute Diarrhea	ND	-	+	+	-	+	-
60	Y.intermedia	ND	Abdominal Pain	ND	-	-	+	-	+	D.fragilis
61	Y.intermedia	ND	Acute Diarrhea	ND	-	-	-	-	+	-
62	Y.intermedia	ND	Acute Diarrhea	ND	-	-	+	-	+	-
63	Y.intermedia	ND	Abdominal Pain	ND	-	-	+	-	+	-
64	S.enteridiidis	ND	Acute Diarrhea	-*	ND	ND	ND	ND	ND	-
65	C.jejuni	ND	Acute Diarrhea	-*	ND	ND	ND	ND	ND	-
66	C.jejuni	ND	Acute Diarrhea	-**	ND	ND	ND	ND	ND	-
67	C.jejuni	ND	Acute Diarrhea	-**	ND	ND	ND	ND	ND	-
68	C.difficile	ND	Acute Diarrhea	-**	ND	ND	ND	ND	ND	-
69	E.coli	0157:H7	Acute Diarrhea	-**	ND	ND	ND	ND	ND	-
70	Negative	ND	Asymptomatic	-*	ND	ND	ND	ND	ND	-
71	Negative	ND	Asymptomatic	-*	ND	ND	ND	ND	ND	-
72	Negative	ND	Acute Colitis	-*	ND	ND	ND	ND	ND	-

* - Tested against yersinia isolates from patient # 1.
** - Tested against yersiniae isolates from patients # 2, 4, 11, 18.

TABLE IV

RELATIONSHIP BETWEEN IN VITRO YERSINIA VIRULENCE ASSOCIATED ASSAYS AND ACUTE ENTERIC DISEASE
(All Isolates Included)
(# True Positives/ Test Positives)

Species	Autoagglu -tination	Congo Red Absorption	Calcium Dependance	Plasmid Carriage	SIgA Response	Mouse Virulence
Y.enterocolitica	3/6	4/8	4/5	4/6	7/8	6/8
Y.frederiksenii	7/14	13/21	5/12	9/19	4/5	7/10
Y.kristensenii	3/4	3/5	3/5	5/8	1/1	1/1
Y.intermedia	1/1	4/7	1/1	5/8	ND	ND
Total	14/25	24/41	13/23	23/41	12/14	14/19
Positive Predictive Value (%)	**(56.0)**	**(58.5)**	**(56.5)**	**(53.7)**	**(85.7)**	**(73.7)**

TABLE V

RELATIONSHIP BETWEEN IN VITRO YERSINIA VIRULENCE ASSOCIATED ASSAYS AND ACUTE ENTERIC DISEASE
(Excluding Strains Isolated From Patients With Inflammatory Bowel Disease)
(# True Positives/ Test Positives)

Species	Autoagglu -tination	Congo Red Absorption	Calcium Dependance	Plasmid Carriage	SIgA Response	Mouse Virulence
Y.enterocolitica	3/6	4/8	3/4	3/5	5/6	5/7
Y.frederiksenii	7/14	11/19	5/12	8/19	4/5	6/9
Y.kristensenii	2/3	3/5	2/4	4/7	0/1	1/1
Y.intermedia	ND	3/6	1/1	4/7	ND	ND
Total	12/23	21/38	11/21	19/37	9/12	12/17
Positive Predictive Value (%)	**(52.2)**	**(55.3)**	**(52.3)**	**(51.4)**	**(75.0)**	**(70.6)**

TABLE VI

RELATIONSHIP BETWEEN IN VITRO YERSINIA VIRULENCE ASSOCIATED ASSAYS AND MOUSE VIRULENCE
(All Isolates Included)
(# True Positives/ # Test Positives)

Species	Autoagglu -tination	Congo Red Absorption	Calcium Dependance	Plasmid Carriage	SIgA Response
Y.enterocolitica	4/6	4/8	3/5	4/6	7/8
Y.frederiksenii	6/14	9/21	5/12	7/19	5/5
Y.kristensenii	1/4	1/5	1/5	1/8	0/1
Y.intermedia	0/1	0/7	0/1	0/8	ND
Total	11/25	14/41	9/23	12/41	12/14
Positive Predictive Value (%)	**(44.0)**	**(34.1)**	**(39.1)**	**(29.3)**	**(85.7)**

TABLE VII

RELATIONSHIP BETWEEN IN VITRO YERSINIA VIRULENCE ASSOCIATED ASSAYS AND MOUSE VIRULENCE
(Excluding Strains Isolated From Patients With Inflammatory Bowel Disease)
(# True Positives/ # Test Positives)

Species	Autoagglu -tination	Congo Red Absorption	Calcium Dependance	Plasmid Carriage	SIgA Response
Y.enterocolitica	4/6	4/8	3/4	4/5	6/6
Y.frederiksenii	6/14	8/19	5/12	6/18	5/5
Y.kristensenii	1/3	1/5	1/4	1/7	ND
Y.intermedia	ND	0/6	0/1	0/7	ND
Total	11/23	13/38	9/21	11/37	11/11
Positive Predictive Value (%)	**(47.8)**	**(34.2)**	**(42.9)**	**(29.7)**	**(100)**

TABLE VIII

COMPARISON OF POSITIVE VIRULENCE ASSOCIATED CHARACTERISTIC EXPRESSION BETWEEN IGA POSITIVE (2+) AND NEGATIVE (1+ OR –) STRAINS
(# of Isolates With Positive Reaction)(%)

IgA Response	# Organisms	Autoagglutination	Congo Red Absorption	Calcium Dependance	Plasmid Carriage	Mouse Virulence
2+	14	9(64.3)	6(42.9)	7(50 0)	7(50.0)	12(85.7)
1+/0	14	2(14.3)	5(35.7)	7(50.0)	5(35.7)	0(0)

TABLE IX

COMPARISON OF MOUSE SEROLOGICAL RESPONSE
TO HUMAN INTESTINAL RESPONSE

SIgA Response	Species	Serotype	Murine Serum Antibody Titre
2+	Y.enterocolitica	O:6,36	1/1024
2+	Y.enterocolitica	O:7,13	1/2048
1+	Y.enterocolitica	O:5	1/1
0	Y.frederiksenii	ND	0

TABLE X

COMPARISON OF DAYS COLD ENRICHMENT
TO RECOVERY OF SIGA STIMULATING ORGANISMS

# Days	IgA Stimulators Recovered	IgA Non-Stimulators Recovered
0	5	2
1–7	5	7
8–14	4	5

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