CHARACTERIZATION OF *Campylobacter jejuni* ISOLATED FROM PIGS AND MAN

By

P. V. RAJU

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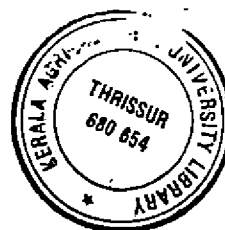

THESIS

Submitted in partial fulfilment of the requirement for the degree

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Department of Microbiology
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DECLARATION

I hereby declare that the thesis entitled **"Characterization of *Campylobacter jejuni* isolated from pigs and man"** is a bonafide record of research work and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Mannuthy,

26.2.1994.

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CERTIFICATE

Certified that the thesis entitled **"Characterization of Campylobacter jejuni isolated from pigs and man"** is a record of research work done independently by **Shri. P.V. Raju**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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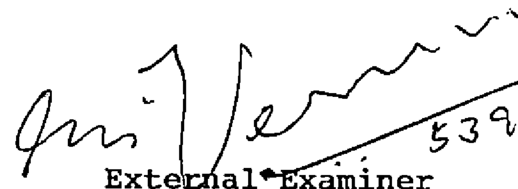

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Introduction

INTRODUCTION

During the last two decades, *Campylobacter jejuni* infections have become increasingly important both in animal and human health. The improved isolation techniques developed by Dekeyser *et al*. (1972) led to the explosion of *C. jejuni* enteritis incidence from obscurity to be recognized as a major enteric pathogen. Even now many mysteries and intrigues associated with the organism is challenging the creative efforts of scientists working in this field (Walker *et al*., 1986).

C. jejuni was recognized as a major cause of enteric disease in man. The incidence of *C. jejuni* enteritis usually exceeds than that of better known enteric pathogens of the genus Salmonella. The economic losses due to Campylobacter infections was estimated to be over 10 million/year in England and Wales. *C. jejuni* associated diarrhoea is recorded to be three to eleven per cent in the developed countries compared to 8.8 to 13.4 per cent in developing countries (Butzler, 1984).

Of late *C. jejuni* is considered as one of the important cause of food poisoning which causes concern to public health authorities (Walton, 1988). Zoonotic importance

of campylobacteriosis is becoming increasingly evident with the isolation of *C. jejuni* from almost every warm blooded species including zoo animals.

Pig, chicken, cattle and sheep act as reservoirs of human Campylobacteriosis. The close association between human beings and domestic animals, particularly in developing countries like India will result in the persistence of infection (Chattopadhyay *et al.*, 1992). Transmission of this enteropathogenic organism can also occur through food products like pork, chicken, beef, mutton, milk etc.

Considering the importance of *C. jejuni* in the causation of enteritis/diarrhoea in man and animals, The World Health Organization has established a collaborating centre for *C. jejuni* at Brussels. A couple of reports from India indicated the association of this organism with enteritis/diarrhoea in animals and its potentiality of being transmitted to man. None of these studies specifically implicated pig as a source, eventhough pork is regarded as a source of *C. jejuni*. In view of this lacuna, the present investigation was undertaken to:

1. assess the association of *C. jejuni* with swine and human enteritis/diarrhoea.

2. study the antimicrobial sensitivity patterns of the *C. jejuni* isolates and to recommend the drug of choice for infection in pigs and man.

3. ascertain the degree of antigenic relationship between *C. jejuni* isolates of porcine and human origin.

4. monitor *C. jejuni* antibodies by passive haemagglutination (PHA) and *Staphylococcus aureus* protein-A antibody mediated haemagglutination assay (SAPA-AMHA) in the field serum samples collected from pigs and man.

Review of Literature

REVIEW OF LITERATURE

Incidence in pigs

Doyle (1944) purported the association of microaerophilic vibrios in the causation of swine dysentry and named the organism as *Vibrio coli*. Due to inadequate biochemical and biotyping schemes to differentiate *C. jejuni* from *C. coli*, in many literature both were grouped together. Doyle (1948) in another study confirmed the association of *C. jejuni* with swine dysentry.

Association of *C. jejuni* with enteric disease in swine herds in Britain was established by Prescott and Bruin-Mosch (1981). The disease syndrome was associated with blood and mucus in faeces and was often fatal. Persson (1981) in another study to detect the occurrence of porcine Campylobacters in Sweden isolated 12 *C. jejuni* from the caecal contents of 108 pigs. Sticht-Groh (1982) recovered *C. jejuni* from the intestine of healthy pigs during slaughter.

Rosef (1981) succeeded in isolating *C. jejuni* by enrichment method from 58 per cent of bile/gall bladders and bile duct samples from 50 clinically healthy pigs. In contrast Svedhem and Kaijser (1981) could obtain an isolation rate of 95 per cent from 138 faecal samples screened.

Stern (1981) reported that the recovery rate of *C. jejuni* from livestock carcasses was higher than that of Salmonella organisms. He demonstrated that 38 per cent of the swine carcasses (22/58) and 87 per cent of the faeces (33/38) contained *C. jejuni*. Conversely, only 20 per cent of the pork was contaminated with organisms belonging to genus Salmonella.

Taylor and Olubunmi (1981) reported the isolation of *C. jejuni* from small intestine of 17 piglets below six weeks of age of which sixteen had enteritis. The changes observed on post-mortem examination were congestion of the small intestinal mucosa, reduction in the height of villi, thickening of the terminal ileum and histological evidence of inflammatory changes in the small intestine.

Experimental infection of gnotobiotic piglets with human or porcine isolates of *C. jejuni* developed only a transient, moderate inflammation of the colonic mucosa two to three days post infection (Prescott and Munroe, 1982). Increased fluidity of intestinal contents accompanied by diarrhoea in five to six days post infection were also observed.

Taylor (1982) reported that pigs can be experimentally colonised by *C. jejuni*. Suckling piglets from herds with endemic campylobacter infections exhibited only mild clinical

signs of infection while colostrum deprived piglets developed creamy mucoid diarrhoea flecked with blood after 48 h post infection. The organism was also recovered from the lesions in the small intestine. Histologically the changes observed were similar to those seen in natural infections.

Munroe *et al*. (1983) screened 203 pigs and isolated 118 Campylobacter strains. Of the 118 isolates, 115 belonged to *C. coli* and three to *C. jejuni*.

Itaya (1984) isolated *C. jejuni*/*C. coli* from the intestinal contents of 48 out of the 51 pigs screened (94.1 per cent). In another study Sanguinetti and Vallisneri (1984) reported that 85.71 per cent of the pigs (54/63) carried campylobacters of which 29 per cent were *C. jejuni*.

Senk *et al*. (1984) could isolate 13 *C. jejuni* and 10 *C. sputorum* sub spp. *mucosalis* from 117 piglets in two large pig breeding centres where sporadic cases of haemorrhagic enteritis occurred.

Wang *et al*. (1984) isolated 74 strains of Campylobacters from swine. Twenty seven strains were isolated from fresh stools obtained from the stye and 47 strains from rectal swabs collected from individual piglets.

While studying the distribution of *C. jejuni* and *C. coli* in pigs, Banffer (1985) isolated two per cent *C. jejuni* biotype-I and 98 per cent *C. coli*. In another study Oosterom *et al*. (1985) examined 210 pigs at three abattoirs in Netherlands and reported a prevalence of 79 per cent for *C. jejuni* in the intestinal contents.

While examining one hundred and twelve freshly slaughtered pig carcasses from three packing plants before and after chilling for the presence of *C. jejuni*/*C. coli.*, Bracewell *et al*. (1985) reported an isolation rate of 12.5 per cent from fresh carcasses and none from chilled carcasses.

Isolation of campylobacters of zoonotic importance in Argentina was reported by Piazza and Lasta (1986). An isolation rate of 6.9 per cent was reported for *C. jejuni* from the intestinal contents of 58 pigs and the calculated projected rate was 1.9-16.8 per cent (binomial distribution). Intensive production of swine was considered as one of the factors that favour persistence of the agent in animal population.

In a study involving 631 rectal samples from mammals and birds in Southern Chile, Fernandez (1988) recovered *C. jejuni* biotype I and II from pigs. The recovery rate of biotype I was 10 per cent and biotype II five per cent.

Manser and Dalziel (1985) reported an isolation rate of 31 per cent (259/846) for Campylobacters from different animal species. Of the different animals screened, highest percentage of incidence (66 per cent) was recorded in pigs (117/178). In the same study the authors screened apparently healthy as well as diseased pigs to find out whether there was any significant difference in the isolation rate of _C. jejuni_ Seventy seven per cent of the diseased pigs were found to be positive as against 47 per cent among the healthy ones.

Yoon _et al_. (1989) examined 1395 seven week-old piglets of which 226 (16.2 per cent) showed diarrhoea. Campylobacters could be isolated from 105 diarrhoeic piglets. Of these 79 were found to be _C. coli_ (75%) and 26 _C. jejuni_ (25%).

Hariharan _et al_. (1990a) while screening caecal contents of 109 slaughtered pigs could isolate 69 Campylobacters of which 62 were _C. coli_ and seven were _C. jejuni_.

Kakkar and Dogra (1990) screened 95 pigs irrespective of clinical symptoms and diarrhoea. Out of 35 Campylobacters isolated, 34 (97 per cent) were from piglets of which 15 were asymptomatic. The species of Campylobacters isolated were _C. jejuni_, _C coli_ and _C. lardis_.

Kwaitek *et al*. (1990) screened 105 pig carcasses in Poland for Campylobacters and reported an incidence rate of 2.9 per cent (3/105). Of this two were *C. coli* and one was *C. jejuni*.

Three hundred and ninety seven faecal specimens from apparently healthy and diarrhoeic pigs, dogs, cats and cattle yielded 59 strains (15 per cent) of thermophilic Campylobacters (Rakotozandrindraini *et al*., 1990).

Furlanetto *et al*. (1991) reported no significant difference in the isolation rate of *C. jejuni* from pig carcass using enrichment procedure and direct selective plating.

While studying the incidence of *C. jejuni*/*C. coli* in Thesslonike area using 96 pig carcasses, Koides (1991) could isolate Campylobacter sp from 22 carcasses, the majority of which were from intestinal contents.

Fifty one out of 75 pigs suffering from intestinal disorder yielded bacterial pathogens of which 43 were Campylobacters. In 30 pigs, the only pathogen isolated was campylobacter (Scarcelli *et al*., 1991).

Faeces or rectal swabs from 689 diarrhoeic and non-diarrhoeic animals were cultured by for thermophilic Campylobacters (Adesiyan *et al*., 1992) who reported an

isolation rate of 45.7 per cent. Piglets showed a highest prevalence rate of 79.3 per cent (233/294). Frequency of isolation of Campylobacters (75.4 per cent) from semi-intensively managed animals was significantly higher than intensively managed animals.

Cabrita *et al*. (1992) studied the incidence of *C. jejuni* and *C. coli* in 188 wild and 681 domestic animals to correlate their importance as potential reservoirs of Campylobacter infection. Campylobacters were isolated from 65 pigs (59.1 per cent).

Incidence in man

King (1957) was perhaps the first to draw the attention of the scientific world towards the role of 'related vibrios' in human diarrhoeal disease.

A breakthrough in the procedures for the isolation of *C. jejuni* from human sources was made by Dekeyser *et al*. (1972). The authors applied conventional microbiological methods to isolate *C. jejuni* in which the suspected material was cultured in thioglycolate broth and incubated under microaerophilic atmosphere.

Later on, Butzler *et al*. (1973) isolated *C. jejuni* from five out of 100 cases of diarrhoea in Belgian children.

Similar observations were made by Skirrow (1974) from England. Since then, association of _C. jejuni_ with human diarrhoeal disease has been reported from many parts of the world (Lauwers _et al._, 1978; DeMol and Bosman (1978); Severin (1978); Lindquist _et al._, 1978 and Blaser _et al._, 1979).

Eventhough several workers reported the isolation of _C. jejuni_ from cases of enteritis/diarrhoea, it was Butzler and Skirrow (1979) who described the clinical disease and provided a well documented evidence of _C. jejuni_ infection in man. They reported that _C. jejuni_ rivalled Salmonella as a cause of enteric disease in man and described the disease as a mild to moderate, self limiting diarrhoea/enteritis of one week duration.

Karmali and Fleming (1979) reported that diarrhoea occurred in _C. jejuni_ infections within 24 hours of the onset of other symptoms. The faeces was loose to watery and contained mucus and frank blood. Nausea with vomiting was seen in one third of the patients. The severity of symptoms varied markedly from mild to severe, through moderate, mimicing salmonellosis or ulcerative colitis. In some cases the severity of symptoms necessitated exploratory laparotomy to rule out appendicitis, intestinal volvulus and intussusception. Occasionally life threatening cases of massive lower gastro-intestinal haemorrhage also occurred.

Blaser and Reller (1981) recognized *C. jejuni* as a leading cause of diarrhoea in human beings. In their study they could isolate *C. jejuni* from diarrhoeic patients as frequently as Shigella or Salmonella.

Kendall and Turner (1982) reported that one per cent of the population per year is affected by campylobacter enteritis. The same percentage of incidence was also noted by Griffiths and Park (1990).

C. jejuni was encountered as an opportunistic pathogen in a wide range of intercurrent infections, especially in debilitated patients or in cases of depressed immunocompetence (Prescott and Munroe, 1982).

Karmali *et al*. (1984) reported the occurrence of Campylobacter entrocolitis in four neonates in a nursery. Mothers were the source of infection and three of them were carriers of *C. jejuni* in stools. The clinical features exhibited by the infected neonates provided further evidence that neonatal Campylobacter entrocolitis is manifested typically as benign, self limiting, non-febrile diarrhoeal illness with bloody stools.

A total of 495 diarrhoeic and non-diarrhoeic patients aged between five to thirty years were examined for the

presence of C. jejuni, Salmonella and Shigella spp. About 20 per cent of the specimens from diarrhoeic patients were positive for C. jejuni as against six and ten per cent for salmonella and shigella respectively. Sixty two per cent of C. jejuni isolates were from diarrhoeic children below 10 years of age. The incidence was highest during the dry months (Olusanya et al., 1983).

In 1981 more than 50 residents in a village in Derbyshire, England had an acute gastro-intestinal illness. A second outbreak affected another 22 persons a month later. C. jejuni was isolated from 12 patients and no other gastro intestinal pathogen could be isolated from these two episodes (Wright et al., 1983).

Macaden et al. (1984) conducted a study in a rural area of Bangalore to assess the role of C. jejuni as an etiological agent in diarrhoeal diseases in children below five years of age. C. jejuni was isolated from eight (1.5 per cent) cases. They also reported the association of Shigella, Salmonella, enteropathogenic Escherichia coli or Vibrio cholerae in five cases.

C. jejuni was isolated from 7.7 per cent of 392 hospitalised patients with acute diarrhoea in Calcutta (Nair et al., 1984). The recovery rate of this organism from

healthy individual was 4.4 per cent. Concomitant isolation of other known bacterial pathogens was possible in 63.3 per cent of the cases. The incidence of *C. jejuni* was pronounced in the lower age groups with the highest isolation rate of 16.5 per cent among pre-school children.

During 1980-82, in USA 23 food borne Campylobacter outbreaks involving 748 persons were reported to the centres of Disease Control through National Food borne Surveillance programme (Finch and Blake, 1985).

In 1981, an outbreak of gastro-enteritis involving 250 persons who drank raw milk from a single local dairy occurred in Kansas. *C. jejuni* was recovered from 52 per cent of households that had one or more ailing family member. Seventy one per cent of individuals who drank raw milk became ill compared to 11 per cent (4/36) who did not. *C. jejuni* was recovered from 66 per cent raw milk drinkers but not from 26 individuals who did not drink raw milk (Kornblatt *et al.*, 1985).

Two hundred and fifty eight isolates of *C. jejuni* and *C. coli* were obtained over a period of one year from children with gastro-enteritis or bactermia at the Red-Cross Childrens Hospital, Cape Town, South Africa (Lastovica *et al.*, 1986). The authors averred that 95.4 per cent of the isolates were

C. jejuni biotype-I, 1.5 per cent C. jejuni biotype-II and 3.1 per cent C. coli.

De Guevara et al. (1989) screened 613 human diarrhoeal specimens for Campylobacter by direct plating, by storage at 4°C in campythioglycolate broth and by delayed plating after storage at 4°C for 24 h. By direct plating he could isolate 68 Campylobacters, 64 after storage in campythioglycollate broth and 57 after delayed plating.

The effects of temperature, holding time and holding atmosphere are important in the isolation of C. jejuni. Monfort et al. (1989) reported that the isolation rate remained at cent per cent level even when the specimens were cultured and transported at refrigeration temperature for three hours.

Lindblom (1990) in his study over a period of three years succeeded in isolating 372 human strains of C. jejuni/ C. coli from Sweden and 49 from Kuwait. He reported that C. jejuni was predominant in human beings compared to C. coli and that the relative frequency of C. jejuni and C. coli in humans differed from country to country.

A rise in the incidence of Campylobacter enteritis was attributed not only due to changes in the food habits and

growing trends towards consuming fast food, but also due to improved isolation techniques (Pearson *et al*., 1990).

Out of 186 strains of campylobacters isolated by Varga *et al*. (1990) 96.2 per cent was *C. jejuni*, 3.2 per cent *C. coli* and the rest *C. lardis*.

Prasad *et al*. (1991) screened 320 human patients with diarrhoea and 450 without diarrhoea for *C. jejuni* and *C. coli*. The organisms were detected in five per cent of subjects with diarrhoea and 0.7 per cent without diarrhoea and hence the difference was significant ($P \leq 0.01$). The isolation rate was higher in children below five years of age (8.3 per cent) in comparison to the older group (3.0 per cent). Out of the two strains of Campylobacters isolated in the above study, 89.5 per cent was *C. jejuni*.

Sengupta *et al*. (1991) observed 25 families consisting of 181 individuals longitudinally for the excretion of campylobacter species over a period of 2 years. The overall diarrhoeal bouts was 19 per 100 persons per year of which eight were Campylobacter associated. They also studied the stool samples from 1002 healthy individuals of which 32 (3.2 per cent) were positive for Campylobacter. Children below one year were more prone to Campylobacter enteritis (11.5 per cent).

Five of 30 (16.6 per cent) stools from animal handlers with diarrhoea and 19 of 90 (21.1 per cent) from healthy handlers were found positive for Campylobacters (Chattopadhyay *et al*., 1992). Out of the total 24 campylobacters isolated, 22 were identified as *C. jejuni*.

Public health

In a study spread over four large kitchens, Dawkins *et al*. (1984) observed that all the frozen meat, fresh poultry and hands of food handlers carried *Campylobacter jejuni* and considered this as a potential source of infection to man.

Prescott and Munroe (1982) reported that in countries with high standard of public health, lower animals are the major reservoirs of *C. jejuni* responsible for human campylobacteriosis. In several cases, diseases were acquired from cattle, dogs, pigs, lambs and poultry. Moreover the number of Campylobacter organisms require to produce enteritis (500 organisms) is far less than the number of Salmonella required to establish human enteritis (10,000 to 1,00,000).

Karmali *et al*. (1984) conducted a study involving neonates in a nursery affected with Campylobacter enterocolitis. They reported that the neonates acquired the infection during delivery from their respective mothers.

Seventy five per cent (3/4) of the mothers were found to harbour _C jejuni_ in their stools.

Sarkar _et al_. (1984) studied the prevalence and extent of contamination of _C. jejuni_ in an abattoir and at two retail outlets in central Calcutta. Meat examined from the carcasses in the abattoir and from the retailers showed no contamination eventhough 20.4 per cent and 23.4 per cent of the samples from the intestine and freshly voided faeces yielded _C. jejuni_. However _C. jejuni_ could be recovered from 86.7 per cent and 50 per cent of swabs obtained from the floors and walls of the abattoir respectively.

Bracewell _et al_. (1985) studied 112 freshly slaughtered pig carcasses before and after chilling for the presence of _C. jejuni_ and _C. coli_. He reported that chilling reduced _C. coli_ numbers to below detectable levels.

Finch and Blake (1985) studied 23 food borne outbreaks caused by Campylobacters in U.S.A. involving 748 ailing persons. Raw milk was implicated or suspected in 14 outbreaks. In four outbreaks food handling errors were identified and five outbreaks were attributed to meat.

Shane and Montrose (1985) reported a world wide distribution of campylobacteriosis with a high correlation between the presence of _C. jejuni_ in stools and clinical

enterocolitis. In developing countries, high recovery rates were encountered coterminous with deficiencies in hygiene and the opportunity for fecal contamination of food and water. The frequency of isolation of C. jejuni from surface water, faeces of domestic animals, meat and other food products suggests a significant potential for human infection. Environmental contamination through improper handling of food, animal wastes and processing plant effluents are epidemiologically significant.

Pork act as a source of C. jejuni infection in man. While studying the effect of freezing Bracewell et al. (1986) observed that freezing substantially decreased the recovery of C. jejuni indicating that consumption of frozen meat will reduce the incidence of human campylobacteriosis.

Walker et al. (1986) opined that the most common sources for establishment of campylobacter infection in man included unpasteurized milk, raw or partially cooked meat and contaminated water. But all the persons exposed to the above source did not develop the signs of campylobacteriosis.

Higher titres to C. jejuni antibodies were reported by Vaira et al. (1988) in a group of people who were in direct contact with the fresh carcass compared to the clerical

workers. Their studies indicated that Campylobacter of animal origin act as a source of infection to man.

Walton (1988) reported that campylobacters are the most important cause of food poisoning. The organisms are found in the intestinal tract of diseased and healthy animals especially, pig, cattle and poultry. A few strains of these organisms isolated from the above animals were implicated in food borne infections.

The prevalence of _C. jejuni_ in galliformes was reported to be 25.2 per cent (Yogasundaram _et al._, 1989). These workers also highlighted the public health aspect of its prevalence in relation to human campylobacteriosis.

Hariharan _et al_. (1990a) reported that swine carcasses are contaminated by Campylobacters more often than cattle carcasses due to intestinal spillage. Campylobacters could survive on tongue, heart and liver that are packed wet and sold unfrozen. These organisms under suitable conditions will survive in pork for several days and run the risk of Campylobacter infection.

Chattopadhyay _et al_. (1992) isolated 24 _C. jejuni_ from animal handlers. They reported that domestic animals like pigs, cattle, sheep and chicken act as reservoir for Campylobacter infection in human beings. The close

association between man and domestic animals observed in developing countries like India is bound to result in the persistence of infection due to recycling.

Evans (1993) studied the seasonality of canine birth and human campylobacteriosis. He correlated the seasonal rise in human campylobacteriosis during the summer to the rise in the number of puppies being acquired as pets during that period.

From April 1982 to September 1983, 218 patients suffering from Campylobacter enteritis and 526 randomly selected controls were asked about their contact with animals during one week prior to illness or interview. 6.3 per cent of cases of Campylobacter enteritis were attributed to contact with animals with diarrhoea indicating a four-fold increase in risk (Saeed *et al.*, 1993).

Serology

Agglutination of soluble antigens by specific antisera necessitated the adsorption of relevant antigens on larger particles prior to reacting with antibody. Neter (1956) recommended erythrocytes for adsorption of soluble antigens bacterial polysaccharides for serological studies.

Development of antibodies to *C. jejuni* in human beings

by the fifth day of illness has been reported by Skirrow (1977). The author reported that such antibodies may complicate the serological differentiation of acute and convalescent stages of the disease.

A rapid, simple and economical method for the purification and analysis of immunoglobulins employing staphylococcal protein-A have been described by Goding (1978). Staphylococcal protein-A is covalently linked to the cell wall of most strains of Staphylococcal aureus and binds immunoglobulin (IgG) with high affinity. This property was used for enhancing the sensitivity of passive haemagglutination test.

Penner and Hennessy (1980) reported that somatic antigens of C. jejuni were capable of being adsorbed on to erythrocytes which made them agglutinable with specific antisera. The antigens obtained from different strains showed different immunologic specificities in rabbits. In the light of observable differences, the authors recommended passive haemagglutination as the test of choice for serotyping of C. jejuni.

An ascending titre of specific agglutinins to homologous strains of C. jejuni have been reported by Bokkenheuser and Sutter (1981).

In a serological study of diarrhoeic patients, Prescott and Munroe (1982) demonstrated specific antibodies to *C. jejuni*. From the serologically positive patients they could recover *C. jejuni*, employing selective medium.

Patton *et al*. (1983) recommended serotyping of *C. jejuni* using lipopolysaccharide antigens as an epidemiological tool in the serological diagnosis of *C. jejuni*. Penner and Hennessy (1980) also recommended haemagglutination as the best method for detecting heat stable antigens of *C. jejuni*.

Jagannath *et al*. (1984) described a method for enhancing the sensitivity of passive haemagglutination reaction (PHA) with the help of intact cells of *Staphylococcus aureus* (Cowan 1) strain bearing protein A (SAPA) which binds the Fc portion of mammalian IgG. The sensitivity of *Staphylococcus aureus* protein A antibody mediated haemagglutination assay (SAPA-AMHA) was found to be higher than that of PHA and the efficiency depended on protein A binding affinity.

Kornblatt *et al*. (1985) while correlating bacteriologic and serologic studies stated that 21 per cent (3/14) persons who were culture positive for *C. jejuni* did not exhibit a four fold or greater titre in paired serum samples

or a single titre of $\geq$ 1:64. Conversely, serologic studies have also identified two positive persons in a population of 17 (12 per cent) who were culture negative.

Blaser et al. (1985, 1986) monitored C. jejuni specific serum antibodies in children from Bangladesh, Thailand and USA employing PHA test. The authors reported that serum antibodies were present at significantly higher levels in children in developing countries than in United States.

Fricker et al. (1986) compared sheep, turkey and chicken erythrocytes for sensitization with C. jejuni antigen for performing PHA test. They reported a reduction in the PHA titre when avian erythrocytes were used compared to sheep erythrocytes.

Vaira et al. (1988) conducted a serologic study involving 98 abattoir workers for C. jejuni and C. pylori. They showed that workers who were in direct contact with freshly cut animal parts had significantly higher IgG antibody levels than those who were not.

Antimicrobial susceptibility pattern

In Sweden, Walder and Forsgren (1978) carried out antimicrobial susceptibility test to campylobacter by agar

plate dilution technique. They reported that majority of campylobacters were sensitive to erythromycin. However, approximately 10 per cent of the isolates were resistant to macrolide antibiotics including erythromycin. Cent per cent susceptibility was observed with regard to tetracycline, gentamicin and chloramphenicol.

Michel et al. (1983) from Israel tested the antimicrobial susceptibility of 103 human isolates of C. jejuni and reported erythromycin as the drug of choice in severe C. jejuni infections. Tetracycline was recommended as an alternative drug although 12.6 per cent resistance was recorded.

Corbel et al. (1984) made an attempt to identify an antibiotic among the 13 antibiotic preparations, which was universally active against pathogenic Campylobacters. Augmentin (clavulanate - potentiated amoxycillin) inhibited the growth of Campylobacter strains at therapeutically attainable concentrations.

Macaden et al. (1984) studied the antimicrobial susceptibility pattern of 10 C. jejuni isolates from children residing in a rural area of Bangalore. Cent per cent of the isolates were susceptible to ampicillin, chloramphenicol, erythromycin, gentamicin, streptomycin and tetracycline but

resistant to cephalothin. Seventy per cent of the isolates were resistant to penicillin.

Wang et al. (1984) subjected 104 human and 74 swine strains of campylobacters to antibiotics namely ampicillin, amoxycillin, clindamycin, chloramphenicol, erythromycin, furazolidone, norfloxacin, nalidixic acid, rosaxacin, rosaramycin, tetracycline, and Sch 32063. Nearly all human and swine strains were susceptible to furazolidone and nalidixic acid. Human isolates were significantly more susceptible to clindamycin, erythromycin, rosaramycin and Sch 32063. Campylobacter isolates from humans and swine showed different antibiogram patterns to certain antibiotics, such as clindamycin.

C. jejuni isolated from 73 primates where subjected to in vitro drugs sensitivity studies (Welshman, 1984). He could observe cent per cent sensitivity to Augmentin, 97 per cent to chloramphenicol, 91 per cent to erythromycin, 87 per cent to nalidixic acid, 79 per cent to tetracycline and 50 per cent to ampicillin. In the same study in vivo tests indicated that erythromycin at a dose rate of 40 mg/kg b.wt and Augmentin at a dose rate of 50 mg/kg b.wt were useful in the treatment of Campylobacter infections.

Bopp et al. (1985) isolated 31 strains of C. jejuni from 11 outbreaks involving human beings and conducted antimicrobial susceptibility studies. All the 31 strains were susceptible to erythromycin, clindamycin, chloramphenicol, kanamycin, cobramycin, streptomycin and gentamicin. Whereas five per cent of the strains were resistant to ampicillin and carbenicillin, six to metronidazole and 13 to tetracycline.

Antibiotic susceptibility study of 52 animal isolates of C. jejuni indicated that most of the isolates were susceptible to kanamycin, erythromycin, gentamicin, tetracycline and sulfonamide. Susceptibility to bacitracin was 19.5 per cent, ampicillin 55.8 per cent and streptomycin 51.9 per cent. None of the isolates were susceptible to penicillin (Bradbury and Munroe, 1985).

One hundred and five thermophilic Campylobacters isolated from human and swine faeces were tested for its sensitivity to eight macrolides and related compounds (Elharrif et al., 1985). Erythromycin, josamycin, clindamycin, pristinamycin and ASE136 BS (a new erythromycin derivative) were active against human strains. The swine strains were relatively resistant to all except prestinamycin. They concluded that the resistance pattern shown by the swine strain may be due to the addition of macrolides in the diet of these animals.

Gebhart *et al*. (1985) carried out antimicrobial sensitivity test involving 30 strains of Campylobacter spp. isolated from pigs employing 47 antimicrobial agents. Carbodox, furazolidone, nitrofurantoin, gentamicin and dimetridazole were found to be the most effective drugs.

Taylor *et al*. (1985) reported nalidixic acid resistant mutants of *C. jejuni*, *C. coli* as well *C. lardis*. Moreover they showed cross resistance to nalidixic acid and DNA gyrase subunit A inhibitor, enoxacin. All campylobacter species tested were resistant to novobiocin. Most strains were susceptible to another DNA gyrase subunit inhibitor, coumermycin A_1 and clorobiocin.

In an antibiogram study involving 688 isolates of *C. jejuni* and *C. coli* by disc diffusion technique, Tenover *et al*. (1985) reported that all the isolates were sensitive to chloramphenicol, while four per cent of the organisms were resistant to erythromycin, eight per cent to ampicillin, 10 per cent to streptomycin and 24 per cent to oxytetracycline.

Antibiotic sensitivity tests were carried out with 382 stool isolates of *C. jejuni* in Canada (Taylor *et al*., 1986). Forty six isolates of *C. jejuni* were resistant to ampicillin and 29 isolates to tetracycline. They concluded that tetracycline resistance was plasmid mediated.

The antimicrobial susceptibility of *C. jejuni* strains obtained from various species of livestock were investigated by standard disc diffusion technique (Diker *et al.*, 1987). All the fowl strains were sensitive to tetracycline, erythromycin, kanamycin, neomycin and streptomycin, but resistant to penicillin and ampicillin. The frequency of tetracycline and erythromycin resistance among the cattle and sheep isolates was 15 per cent and four per cent respectively.

Drug sensitivity of 116 Campylobacter spp. isolated from 226 piglets showed that more than 63.8 per cent of the isolates were susceptible to nalidixic acid, colistin, gentamicin and chloramphenicol (Yoon *et al.*, 1989).

Sixty-nine swine isolates of *C. jejuni/C. coli* was tested against erythromycin, tetracycline, kanamycin and ampicillin. Eleven isolates showed multiple resistance (Hariharan *et al.*, 1990a). Resistance to erythromycin was seen in 19 per cent and 28.6 per cent of *C. coli* and *C. jejuni* respectively. All the isolates were susceptible to nitrofurantoin, gentamicin, chloramphenicol and Nalidixic acid.

Hariharan *et al.* (1990b) studied the antimicrobial susceptibility pattern of 86 *C. jejuni/C. coli* of porcine origin from Prince Edward Island against 10 antibiotics.

Among the quinolones, enrofloxacin was the most active drug and pipemedic acid, the least. Thirty six per cent of C. jejuni were resistant to erythromycin, which is considered as the drug of choice against Campylobacter enteritis.

Monfort et al. (1990) treated 25 Campylobacter infected English Fox hounds. Nine were assigned to erythromycin treatment, nine to chloramphenicol and seven as controls. All the dogs that received erythromycin stearate ceased shedding of C. jejuni by fourth day of treatment while a reduction in shedding from 100 per cent to 57 per cent was noted in chloramphenicol treated group within a span of nine days. Within nine days of discontinuation of antibiotic treatment, C. jejuni was isolated from all chloramphenicol treated dogs and 89 per cent of erythromycin treated dogs.

Endtz et al. (1991) studied the antibiogram of 883 strains of Campylobacter sp. isolated between 1982 and 1989 from human faeces and poultry products, to fluroquinolones. In this period, the prevalence of resistant strain isolated from poultry products increased from zero to fourteen and human faeces from zero to eleven per cent.

Modolo et al. (1991) carried out antimicrobial susceptibility tests on 74 strains of C. jejuni. All 74 strains were sensitive to gentamicin, nitrofurantoin and

neomycin. All the isolates were resistant to oxacillin and benzylpenicillin. Seventy three per cent of C. jejuni were resistant to sulfonamides.

Two hundred and eleven rectal swabs from adult sheep and 278 rectal swabs from lambs yielded 45 C. jejuni (Valente et al., 1991). All the isolates were susceptible to gentamicin, nitrofurantoin and chloramphenicol. All were resistant to cephalothin and cefoxitin. Twenty-five (55.5 per cent) were resistant to ampicillin.

The antimicrobial susceptibility testing of C. jejuni/ C. coli isolated from domestic and wild animals showed that 5.5 per cent of the strains were resistant to ampicillin and tetracycline, 12.6 per cent to erythromycin and 23.5 per cent to streptomycin. Resistance to erythromycin (26.2 per cent) and streptomycin (58.4 per cent) was particularly high in pig isolates (Cabrita et al., 1992).

Adesiyan et al. (1992) screened 689 faeces/rectal swabs from animals and reported an isolation rate of 45.7 per cent for Campylobacter spp and they subjected the isolates to antimicrobial susceptibility test. A total of 245 (77.8 per cent) strains of Campylobacter spp exhibited resistance to one or more antibiotics and the highest resistance was to

streptomycin (76.5 per cent), followed by kanamycin (28.6 per cent) and neomycin (26.7 per cent).

Ahmed et al. (1992) investigated the susceptibility of 119 C. jejuni/C. coli to eight antimicrobial agents using standard disc diffusion technique. Cent per cent sensitivity was reported against enrofloxacin, chloramphenicol and nalidixic acid. Ninety eight per cent of the organisms were sensitive to tetracycline, 96 per cent to kanamycin, 93 per cent to erythromycin and 47 per cent to ampicillin. All the isolates were resistant to cephalothin.

Materials and Methods

MATERIALS AND METHODS

Preparation and sterilization procedures

Glassware

All the glassware used in this study were soaked in Extran-neutral (Merck) overnight. They were boiled for 1 h, washed several times in running tap water, rinsed in single glass distilled water and dried in hot air oven at 60°C. All glasswares were properly packed and sterilized in a hot air oven at 160°C for 1 h.

Syringes and needles

As far as possible sterile disposable syringes and needles were used. When glass syringes were used, they were washed, dried, wrapped in aluminium foil and autoclaved at 15 lbs for 15 minutes.

Blood collection apparatus

Flasks, glass beads/copper coils used for collection of sterile defibrinated sheep blood were cleaned in Extran neutral, washed in tap water and rinsed in single distilled water, dried and assembled for blood collection. The blood collection apparatus was wrapped in paper and sterilized in an autoclave at 15 lbs for 15 min.

Rubber corks and other rubber items

Rubber corks, plastic caps of vials and tubes were washed in Extran, followed by tap water, rinsed in distilled water, dried, packed and sterilized by autoclave at 15 lbs for 15 min.

Procedures for isolation of Campylobacter jejuni from field samples

Source and nature of materials

The prevalence of C. jejuni in piglets below two months of age and in children below five years of age was investigated.

Piglets

Piglets with diarrhoea/enteritis in the University Pig Breeding Farm were identified. Using sterile swabs rectal sambles from these piglets were collected with maximum aseptic precaution. Twenty six samples were collected, transported and processed in the laboratory for the isolation of C. jejuni within 30 min.

Human beings

Samples of faecal material from children admitted to diarrhoea treatment unit attached to Thrissur Medical College,

West Fort Hospital and Aswani Hospital were collected from the rectum using sterile swabs with maximum aseptic precautions. Thirty two samples were collected, transported and processed within 30 min for the isolation of C. jejuni.

Isolation of Campylobacter jejuni from rectal swabs

The procedure for isolation of C. jejuni described by Gracia et al. (1983) was followed. Rectal swabs transported to the laboratory were streaked on Brucella Blood Agar Plate supplemented with Difco campylobacter antimicrobial supplement (See Appendix-A-9). The inoculated plates were kept inside an anaerobic jar and candle jar system was employed. They were incubated at 42°C for 48 h. The plates were examined for non-haemolytic smooth flat tan or grey, wet glossy spreading type or non-haemolytic discrete, convex, entire, glistening colonies. The plates with colonies showing the typical characters were subjected to preliminary/tentative identification tests after subculturing. The plates with no typical discernable growth were further incubated upto 96 h before discarding.

Tests for identification of Campylobater jejuni

Preliminary tests

The colonies showing typical characters were subcultured on plain Brucella Agar plate (See Appendix-A-10)

and incubated at 42°C for 48 h under microaerophilic conditions. The growth obtained was used for preliminary tests.

1. Colony characters

The colony characters of the growth on subculturing was observed and confirmed whether it similated the typical colony character of *Campylobacter jejuni*.

2. Gram's staining

The organisms were stained with Grams stain and observed under oil immersion objective (1000x). The organism showed gram negative reaction with typical curved, ʃ-shape or seagull shape was considered as typical morphology.

3. Catalase reaction

A loopful of the organism from well isolated, distinct, surface colony was mixed with 2-3 drops of 3 per cent Hydrogen Peroxide on a clean glass slide (Appendix-A-11) and observed for the release of nascent oxygen which will develop frothness within one to two minutes in the case of positive reaction.

4. Oxidase reaction

Surface growth from well isolated typical colonies was

spread with a glass rod on to a wet filter paper strip impregnated with oxidase reagent (Tetramethyl paraphenylene diamine dihydrochloride) (Appendix-A-4). A positive reaction was indicated by the appearance of a dark purple colour within 30 seconds.

5. Motility

The motility of the culture was checked under dark field microscope and by hanging drop method. A cork-screw like darting type of motility was considered as typical of campylobacter.

Confirmatory tests

The culture which gave desired/typical results in preliminary tests were selected and further tests were performed to confirm the identification.

1. Growth at 42°C

Test cultures were inoculated into Brucella Agar plates and incubated at 42°C under microaerophilic conditions. A positive reaction was indicated by the development of surface colonies within 48 h.

2. Growth at 25°C

Brucella Agar plates inoculated with test culture were incubated at 25°C under microaerophilic conditions. A positive reaction was indicated by the development of surface colonies within 48 h.

3. Growth on one per cent bile

The test culture was inoculated into the bile agar medium (Appendix-A-12) and incubated at 42°C under microaerophilic conditions for 48 h. A positive reaction was indicated by discernable growth.

4. Growth on one per cent glycine

The culture under test was inoculated into glycine agar medium (Appendix-A-13) and incubated at 42°C under microaerophilic conditions. A positive reaction was indicated by the development of discernable growth.

5. Growth on 3.5 per cent sodium chloride

The culture under test was inoculated into 3.5% sodium chloride agar medium (Appendix-A-15) and incubated at 42°C under microaerophilic conditions for 48 h. A positive reaction was indicated by visible growth.

6. Hydrogen sulphide production (Lead acetate paper)

A lead acetate impregnated filter paper strip (Appendix-A-2) was held between the glass and the cotton plug of a test tube containing cystine incorporated Brucella Agar (Appendix-A-14) inoculated with the test culture. The tube along with the filter paper was incubated at 42°C under microaerophilic conditions for a maximum of seven days and examined daily for a positive reaction. A positive reaction was indicated by blackening of the tip of the filter paper.

7. Hydrogen sulphide production (Triple sugar Iron Agar)

On Triple sugar Iron Agar (TSI) slants, (Appendix-A-16) the butt was stabbed and the slant was streaked with the test culture and was incubated at 42°C under microaerophilic conditions and observed daily for seven days. A positive reaction was indicated by blackening due to hydrogen sulphide production in the butt.

8. Sensitivity to Nalidixic acid

In vitro susceptibility of the test organism to Nalidixic acid was determined by disc diffusion test (Appendix-A-18). The surface of Brucella Plain agar was spread completely with saline suspension of the test culture and a nalidixic acid disc (Himedia 30 ug) was placed. The

inoculated plate was incubated at 42°C under microaerophilic conditions for 48 h. The presence of a clear zone of inhibition of growth above 19 mm diameter around the disc was taken as sensitive.

Production of *Campylobacter jejuni* antigen

Several Brucella blood agar plates, inoculated with the *C. jejuni* isolates (Strains SW 19/2B, SW 20/2D and HS 20/1A) were incubated at 42°C for 48h under microaerophilic conditions. The bacterial cells were harvested by gentle scoopping of the surface growth and suspended in five ml of 0.15 M tris hydrochloride buffer (pH - 7.0) (Appendix-A-8). The bacterial cell suspension was held in an ice bath and was disrupted by sonication for a total of 2 minutes with 8 x 15 sec bursts in Vibrionics-ultrasonic processor - P_2 (250 W). The sonication was interrupted for 30 seconds between each bursts for cooling. Then the protein content of the sample was estimated by Biuret method. Finally the protein content of the sample was adjusted to 2 mg/ml by diluting with tris hydrochloride buffer and stored in small quantities at -60°C. This sonicated antigen was used for raising hyper immune sera in rabbits and also for sensitization of stabilized sheep RBCs for monitoring antibody levels.

Production of Campylobater jejuni antibodies in rabbits

Strains SW 19/2B, SW 20/2D (swine strains) and strain No. HS 20/1A (human strain) were used for raising C. jejuni antibodies in rabbits. Two rabbits aged four months were used for the production of antisera against each isolate. Sonicated bacterial antigen with and without Freund's complete adjuant (FCA) was administered to rabbits as detailed below. FCA (Difco) was thoroughly mixed with the antigen before administration.

Week	Volume (ml)		Route
	Antigen	FCA	
1	0.5	0.5	I/M
2	0.5	--	I/V
3	0.5	--	I/M
4	1.0	--	I/M
5	1.0	--	I/M
6	1.0	--	I/M
7	1.0	--	I/M

The immunized rabbits were bled, ninth day after the last injection and serum was collected and stored in aliquots of 2 ml at -60°C.

Removal of non-specific antibodies by absorption of hyper immune sera with gluteraldehyde stabilized SRBC

Hundred microlitres of previously packed stabilized SRBC was suspended in 0.1 M glycine solution, centrifuged and packed.

To one ml of the hyperimmune serum, 100 ul of packed glycine washed, stabilized SRBC was added, incubated at 37°C for one hour, centrifuged, and supernatant serum was used in the test.

Stabilisation of Sheep Red Blood Cells (SRBC)

Source of sheep blood

Sheep blood was collected aseptically in Alsever's (Appendix-A-1) from the Municipal Slaughter house, Thrissur and stored at 4°C for 24 h.

Stabilisation procedure

Gluteraldehyde stabilization was followed in this study.

The sheep RBCs stored in Alsever's solution for 24 h at 4°C were washed thrice in sterile PBS (pH - 7.2) (Appendix-A-6) and 50 per cent suspension was prepared in PBS.

Gluteraldehyde stabilized sheep red blood cells (GAS-SRBC) mixture was prepared as described in Appendix-A-3, 7).

Gluteraldehyde SRBC mixture stored at 4°C for 24 h was washed with PBS five times, filtered through a gauze cloth and stored as 10 per cent suspension at 4°C.

Procedure for sensitization of stabilized SRBC with Campylobacter jejuni antigen

Suspension of SRBC stabilized with gluteraldehyde was washed thrice in PBS (pH - 7.2) and then centrifuged at 100 g for 10 minutes.

The cell deposits were further diluted with two volumes of acidic PBS (pH - 6.4) (Appendix-A-6) and gently mixed to provide a uniform suspension of cells.

Three ml each of gluteraldehyde stabilized SRBC preparations were mixed dropwise with 50, 100, 150, 200 and 250 ul of all the three strains of sonicated C. jejuni antigens separately.

The SRBC antigen mixture was incubated for 6 h at 37°C and washed in PBS (pH - 7.2).

After centrifugation at 800 g for 5 minutes, the cell deposits were resuspended in PBS to provide one per cent suspension.

Finally, 0.1 per cent sodium azide was added into the suspension, and stored at 4°C till further use.

Determination of optimum concentration of *C. jejuni* antigen for sensitization of SRBC by PHA

A Checker Board titration was carried out to determine the optimum concentration of the *C. jejuni* antigen for the use in PHA test.

Antiserum raised against the three strains of *C. jejuni* antigens were diluted serially in a two-fold manner to provide dilutions of 1:2 to 1:16384 with PBS in Laxbro microtitre plates.

Fifty microlitres of SRBC sensitized with different concentrations of *C. jejuni* antigen was individually added to each serum dilution.

The mixture was gently agitated and incubated at room temperature for 1½-2 h.

The lowest concentration of the antigen that gave maximum haemagglutination titre with the highest serum dilution was taken as optimum concentration of antigen for sensitization of stabilized SRBC.

Procedure for the preparation of *S. aureus* cultures producing protein A (SAPA)

SAPA cells were prepared by the procedure described by Kessler (1975).

Cowan-1 strain of *S. aureus* was obtained from the Department of Microbiology, Veterinary College, Thirupathi. They were cultivated on trypticase Soy agar in separate Roux flasks at 37°C for 24 h.

Cells collected by flooding the surface growth with 100 ml PBS (pH - 7.0) were deposited by centrifugation at 8000g for 10 min.

A ten per cent suspension was made in PBS-azide and the cells were stirred in the presence of 1.5 per cent formalin at 25°C for 1½ h.

The above suspension was transferred to a large Erlen Meyer flask and the cells were killed by rapid swirling in a water bath at 80°C for 5 minutes followed by rapid cooling in an ice bath.

After two more washes in PBS-azide solution the concentration of the cells were adjusted to ten per cent and were stored at -60°C till further use.

Determination of optimum concentration of SAPA cells for protein-A mediated haemagglutination assay (SAPA-AMHA)

A checker board titration was carried out to determine the optimum concentration of SAPA cells for the SAPA-AMHA test.

Rabbit antisera against the three strains of C. jejuni antigens were serially diluted to provide a serum dilution of 1:2 through 1:16384 using PBS in Laxbro microtitre plates.

Fifty microlitres each of the different concentrations of SAPA cells (0.01, 0.05, 0.1 and 0.2%) were added to individual serum dilutions. After thorough mixing the plates were incubated at 25°C for 2 h with periodic shaking at every 5-10 min.

Fifty microlitres of gluteraldehyde stabilized SRBC sensitized with 150 ul of C. jejuni antigen was individually added for each dilution.

The plates were kept at room temperature for 1½ to 2 h.

The lowest concentration of SAPA cells that gave maximum haemagglutination titre with the highest serum dilution was taken as the optimum concentration of SAPA cells for the test.

Determination of *C jejuni* antigen suitable for screening field serum samples

Swine isolates

Cross checker board titration assay was carried out by PHA employing hyper immune sera raised in rabbits against two campylobacter antigens prepared (150 ul antigen attached to one millilitre of packed GAS-SRBC). In all cases, a two fold serial dilution of hyper immune sera were carried out and the concentration of antigen was kept constant.

The reciprocal of the highest dilution of the serum at which a complete haemagglutination observed was taken as the end titre of the serum.

Human isolates

A checker board titration was carried out by passive haemagglutination test employing hyper immune sera raised in rabbits against one human campylobacter antigen prepared (150 ul antigen attached to 3 ml packed GAS-SRBC).

Procedure for bulk sensitization of GAS-SRBC with *Campylobacter jejuni* antigen

The *C. jejuni* antigen that gave high titres with homologous and heterologous hyper immune sera was used for

bulk sensitization with GAS-SRBC for screening field sera samples.

A suspension of SRBC stabilized with gluteraldehyde was washed twice in PBS and then centrifuged at 1000x g for 10 min.

The cell deposit was diluted further with two volumes of acidic PBS (6.4).

One fifty microlitres of sonicated _C. jejuni_ antigen (SW 20/2D, swine strain and HS 20/1A, human strain) were used for every three millilitre of packed GAS-SRBC for bulk sensitization.

The SRBC antigen mixture was incubated for six hours at 37°C and washed in PBS.

After centrifugation at 800 g for five minutes the cell deposit was resuspended in PBS to provide one per cent suspension and 0.1 per cent sodium azide was added and stored at 4°C.

Source and nature of field serum samples

Fifty swine serum samples were collected from pigs slaughtered at Meat products of India, Koothattukulam. The

samples were representative of different age groups and managed under intensive, semi-intensive conditions.

Fifty human serum samples obtained from Medical College, Thrissur and Sudharma Laboratories, Thrissur collected from diarrhoeic patients were used in the study.

Protocol for PHA test

Test serum samples adsorbed by mixing 100 ul of serum with an equal quantity of glycine washed GAS-SRBC (unsesnsitized) were used in PHA to know whether adsorption has any effect on the titre. After two hour of incubation of the serum-SRBC mixture at room temperature, 50 ul of supernatant, was taken for further titration by PHA. The same sera samples without adsorption were also subjected to PHA.

A two-fold serial dilution of adsorbed serum (50 ul) and unadsorbed serum was prepared in PBS to provide 1:2 through 1:16384 dilutions in a microtitre plate.

Fifty microlitre of antigen sensitized GAS SRBC was added to all the dilutions of sera and mixed thoroughly.

Readings were taken after one and half to two hours of incubation at room temperature. The reciprocal of the highest

dilution of the serum at which complete haemagglutination occurred was taken as the titre of serum.

Protocol for SAPA-AMHA test

A two-fold serial dilution of the unadsorbed serum was prepared in PBS to provide 1:2 through 1:32768 dilutions in a microtitre plate.

To each well, 50 ul of SAPA cells were added and incubated for 1½-2 h with periodic shaking at an interval of 10-15 min.

To each dilution 50 ul of antigen sensitized GAS-SRBC was added and mixed thoroughly.

Readings were taken after 1½-2 h of incubation at room temperature. The reciprocal of the highest dilution of the serum at which complete haemagglutination occurred was taken as the SAPA-AMHA titre.

Antibiotic sensitivity

The antibiotic sensitivity patterns of the twelve *Campylobacter jejuni* isolates were studied.

The method employed for sensitivity study was standard disc diffusion technique (Bauer *et al.*, 1966), using commercially available discs (Hi-media) (Appendix-A-18). The

antimicrobial agents employed were ampicillin, chloramphenicol, erythromycin, furazolidone, gentamicin, nalidixic acid, oxytetracycline, penicillin, streptomycin and sulphadiazine.

Medium

Brucella plain agar (Difco) was used for growing the organisms for sensitivity study.

Preparation of inoculum

The organisms to be tested were grown on Brucella plain agar for a period of 24 h. A few colonies were removed from the Brucella agar and inoculated into sterile peptone water so as to have a moderate cloudiness equal to the standard described by Blair et al. (1970).

Inoculation of plates

Brucella agar plates were inoculated with the culture suspension by smearing the entire surface by sterile cotton swab dipped in the inoculum prepared as above. The plates were then allowed to dry in the inverted position at room temperature for five minutes.

Application of Discs

The discs containing different antibiotics mentioned above were placed on the medium suitably spaced and the plates were incubated at 42°C under microaerophilic conditions for 48 h.

Reading of plates

The plates were read after 48 h of incubation under microaerophilic conditions. The diameter of the inhibition zone around each disc was measured with a scale. The diameter of the disc also being included in the measurement. The findings were recorded and interpreted adopting the guidelines suggested by the manufacturer.

Results

RESULTS

Isolation of *Campylobacter jejuni* from pigs

Twenty six rectal swabs collected from piglets with diarrhoea/enteritis when processed for the isolation of the etiological agent yielded 11 *Campylobacter jejuni*.

Isolation of *C. jejuni* from man

Thirty two rectal swabs collected from children with diarrhoea/enteritis when cultured on Campylobacter selective media, resulted in the recovery of one *C. jejuni*.

Characterisation of *C. jejuni* isolates

Preliminary tests

C. jejuni after 48 h of incubation under microaerophilic conditions, showed two distinct type of colonies. Some colonies were non-haemolytic, smooth, flat tan or grey, wet, glossy spreading type (Plate I). Some others appeared as non-haemolytic, discrete, convex, entire glistening (Plate II). On continued incubation (96 h) majority of the colonies were irregular, thickened, and were tan in colour.

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Plate 1 Smooth flat wet glossy spreading type colonies

Plate 2 Discrete convex entire glistening type colonies

Gram stained preparations of 48 h old culture of *C. jejuni* showed negative reaction. The organisms were typically curved, ' ' shaped or seagull shaped with tapering ends (Plate III). Occasionally spindle shaped bacilli were also demonstrated. Older cultures invariably showed coccoid forms.

All the isolates gave positive catalase and oxidase reactions.

Mortility of the isolates were studied under dark field microscope and by hanging drop method. Cent per cent of the isolates showed darting type of motility, typical of *C. jejuni*.

Reactions of *C. jejuni* isolates are presented in Table 1.

Confirmatory tests

The isolates which gave typical/desired results in the preliminary tests were subjected to confirmatory tests.

None of the *C. jejuni* isolates showed growth at 25° in 48 h whereas confluent growth was obtained under microaerophilic conditions at 42°C in 48 h.

Plate 3 Gram stained 48 h old primary C. jejuni culture showing typical spiral, curved and sea-gull wing shaped organisms

Table 1. Reactions/tests employed for the identification of *C. jejuni* isolated from swine and man

Tests	SW1	SW2	SW4	SW5	SW6	SW7	SW8	SW17/ B	SW19/ 2B	SW20/ 2A	SW20/ 2D	HS20/ 1A
PRELIMINARY TESTS												
Colony characters	T	T	T	T	T	T	T	T	T	T	T	T
Grams stain reaction	-	-	-	-	-	-	-	-	-	-	-	-
Catalase reaction	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase reaction	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+
CONFIRMATORY TESTS												
Growth at 42°C	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 25°C	-	-	-	-	-	-	-	-	-	-	-	-
Growth on 1% bile	+	+	+	+	+	+	+	+	+	+	+	+
Growth on 1% glycine	+	+	+	+	+	+	+	+	+	+	+	+

Table 1 (Contd.)

Tests	SW1	SW2	SW4	SW5	SW6	SW7	SW8	SW17/ B	SW19/ 2B	SW20/ 2A	SW20/ 2D	HS20/ 1A
Growth on 3.5% sodium chloride	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production on cystine medium (lead acetate strips)	+	+	+	+	+	+	+	+	+	+	+	+
H_2S production on TSI medium	-	-	-	-	-	-	-	-	-	-	-	-
Sensitivity to Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+

\+ - positive reaction

\- - negative reaction

T - typical colony characters of C. jejuni

All the cultures tested showed growth on one per cent bile and one per cent glycine slants at 42°C under microaerophilic conditions but no growth was seen on 3.5 per cent sodium chloride slants. All the isolates were found sensitive to nalidixic acid by disc diffusion test.

Hydrogen sulphide production in medium containing cystine was positive as evidenced by blackening of the filter paper strips but was negative on TSI.

C. jejuni antigen

Sonicated C. jejuni antigen prepared from the three strains (SW 19/2B, SW 20/2D and HS 20/1A) were found suitable for sensitisation of stabilized sheep RBC for serum monitoring. The sonicated antigen retained its affinity to sheep RBC, even after six months of storage at -60°C.

C. jejuni antibodies in rabbits

All the three strains of C. jejuni (SW 19/2B, SW 20/2D, HS 20/1A) of both swine and human origin elicited immunological response. After the fifth injection, practically there was no increase in the antibody titres by PHA upto the seventh injection. Antibody titres obtained by PHA employing homologous and heterologous strain of C. jejuni are presented in Table 2.

Table 2. PHA titres of rabbit hyperimmune sera to three chosen strains of C. jejuni

Test serum	Strains of C jejuni employed for sensitization of stabilized SRBC		
	SW 19/2B	SW 20/2D	HS 20/1A
Hyperimmune serum against 19/2B antigen	64	64	0
Hyperimmune serum against 20/2D antigen	1024	1024	128
Hyperimmune serum against HS 20/1A antigen	2048	2048	4096

Stabilization of SRBC

Gluteraldehyde stabilization was found to be a suitable method for the preservation of SRBC. Gluteraldehyde stabilized SRBC stored at 4°C were used upto a period of three months. The sensitisation property of gluteraldehyde stabilized SRBC to *C. jejuni* antigen was evidenced from the seromonitoring studies using PHA.

Optimum concentration of *C. jejuni* antigens for sensitization of SRBC

Eventhough different concentrations of *C. jejuni* antigens were employed for sensitisation of stabilized SRBC, the cells sensitized with 150 ul and 200 ul gave the same PHA titre when titrated with the hyperimmune sera raised in rabbits. Identical results were shown by all the three strains of *C. jejuni* irrespective of their origin. One hundred and fifty microlitre of sonicated *C. jejuni* antigen containing 2 mg/ml of protein was found sufficient to sensitise three millilitre of stabilized SRBC.

Optimum concentration of SAPA cells

Although varying concentrations (0.01, 0.05, 0.1, 0.2%) of Cowan-I strain of SAPA cells were employed for the SAPA-AMHA test to titrate the *C. jejuni* specific antibodies in

the hyperimmune sera, 0.1 per cent was found to be the optimum concentration to bring about an enhancement in the titre. SAPA cells gave similar titres at 0.1 and 0.2 per cent concentration under similar experimental conditions. _S. aureus_ Woods 46 strain did not enhance the PHA titres despite the addition of 0.2 per cent cells indicating the fact that protein-A is needed for enhancing the PHA titre.

Cross titration assay

The results of cross titration assay employing the hyper immune sera raised in rabbits are presented in Table 2. From the table it is evident that SW 19/2B is least immunogenic. The PHA titre obtained was 1:64 with homologous and heterologous antigen. Whereas SW 20/2D was highly immunogenic as evidenced from a PHA titre of 1:1024 with both strains of _C. jejuni_ obtained from pigs. But a PHA titre of 1:128 only was obtained when a heterologous _C. jejuni_ antigen obtained from human was used. The hyperimmune sera raised against human strain of _C. jejuni_ gave a PHA titre of 1:4096 with homologous antigen, whereas one well reduction in titre was noted with heterologous antigen.

Banking on the superiority of the antigen prepared from SW 20/2D and HS 20/1A strains of _C. jejuni_, they were used for screening the field serum samples.

Assay of C. *jejuni* antibodies in field serum samples

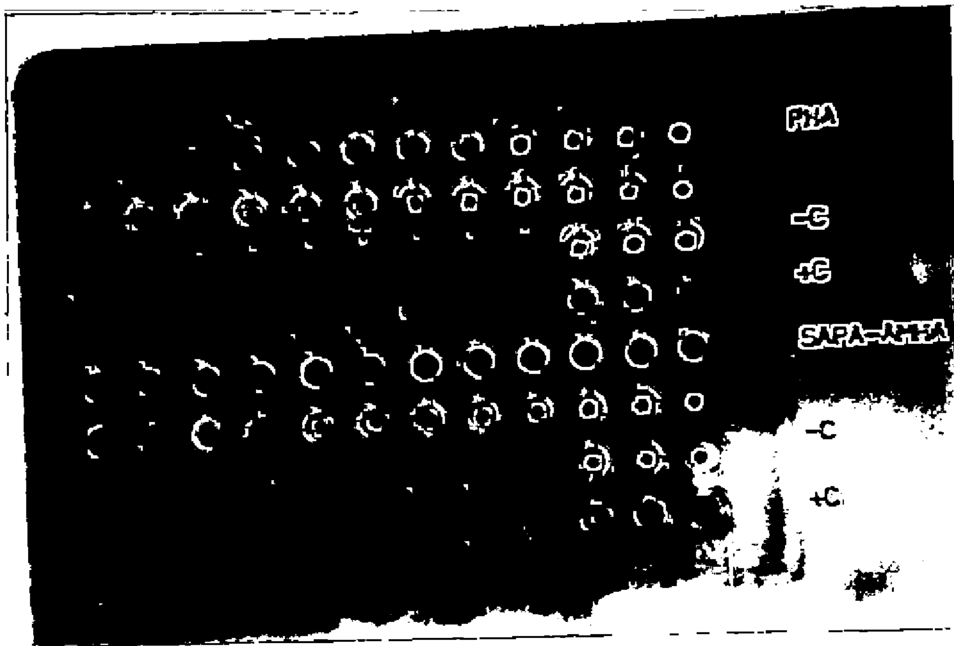
Results of fifty swine sera sample monitored for C. *jejuni* antibodies by PHA and SAPA-AMHA (Plate IV), employing homologous and heterologous strains are presented in Table 3. The samples were also titrated for C. *jejuni* antibodies by PHA after removal of non-specific antibodies (i.e. after adsorption).

Human serum samples obtained from 50 diarrhoeic/enteric patients were also titrated for C. *jejuni* antibodies by PHA and SAPA-AMHA. The samples were also titrated for specific antibodies before and after adsorption. The results are presented in Table 4.

When swine serum samples were monitored for antibodies against C. *jejuni*, by PHA using, swine antigen sensitized SRBC the mean value obtained was 201.8. However when human antigen sensitized SRBC were used, the mean value was 69.8. Analysis of the data by students t test indicate that the results were statistically significant ($P < 0.05$) (Table 5a and Fig.1a).

Adsorption of swine sera samples with SRBC had no significant difference in PHA titre compared to unadsorbed sera sample while monitoring for C. *jejuni* antibodies using

Plate 4 A microtitre plate showing the result of PHA and SAPA-AMHA test on a field serum sample



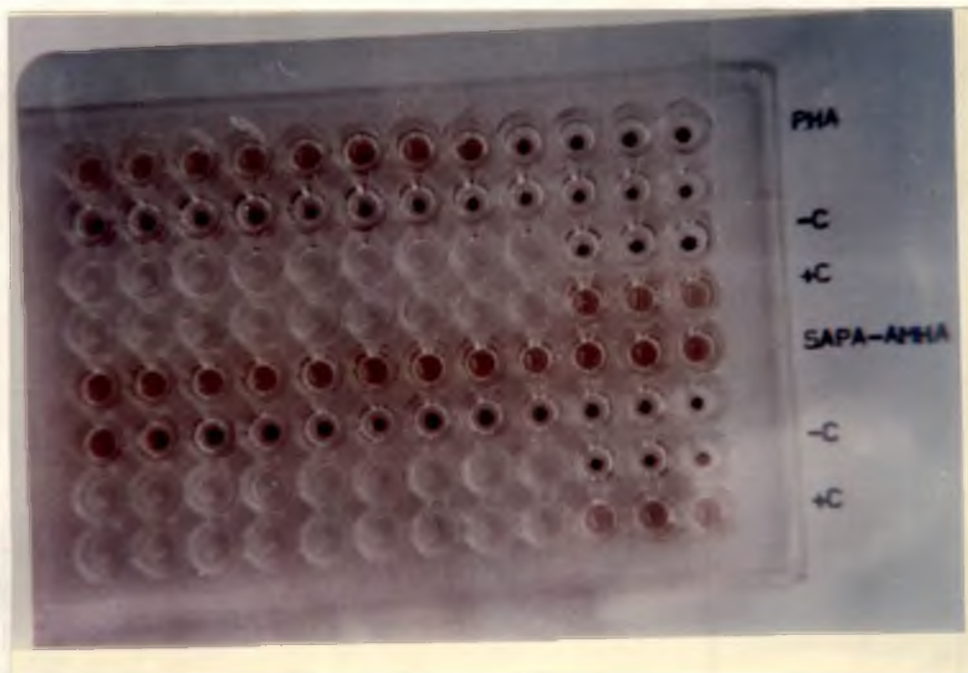
PHA titre 1:256

SAPA-AMHA titre 1:8192

-c = Negative control

+c = Positive control

Plate 4 A microtitre plate showing the result of PHA and SAPA-AMHA test on a field serum sample



PHA titre 1:256

SAPA-AMHA titre 1:8192

-c = Negative control

+c = Positive control

Table 3. PHA and SAPA-AMHA titres of swine serum samples

Sl. No.	Sample No.	PHA titres before adsorption		PHA titres after adsorption of serum samples		SAPA-AMHA titres without adsorption
		With SW 20/2D antigen	With HS 20/1A antigen	With SW 20/2D antigen	With HS 20/1A antigen	With SW 20/2D antigen
1.	S_1	16	8	8	4	128
2.	S_2	128	64	64	4	4096
3.	S_3	128	64	64	32	8192
4.	S_4	16	8	8	64	64
5.	S_5	8	4	4	8	64
6.	S_6	64	64	32	4	512
7.	S_7	8	2	4	16	16
8.	S_8	4	4	2	0	4
9.	S_9	64	128	128	0	1024
10.	S_{10}	128	512	128	128	512
11.	S_{11}	128	512	256	128	2048
12.	S_{12}	16	16	16	8	64

Contd.

Table 3 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
13.	S_{13}	64	64	32	32	1024
14.	S_{14}	128	64	64	16	1024
15.	S_{15}	128	512	64	64	8192
16.	S_{16}	64	8	32	4	1024
17.	S_{17}	256	512	64	64	8192
18.	S_{18}	512	8	128	16	4096
19.	S_{19}	32	64	64	32	256
20.	S_{20}	64	16	32	8	256
21.	S_{21}	64	16	4	4	512
22.	S_{22}	64	16	2	0	1024
23.	S_{23}	256	128	256	8	4096
24.	S_{24}	256	16	512	32	2048
25.	S_{25}	64	64	32	32	256
26.	S_{26}	32	32	16	32	256
27.	S_{27}	256	0	256	8	2048
28.	S_{28}	32	16	64	32	512

Table 3 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
29.	S_{29}	64	32	32	16	1024
30.	S_{30}	128	32	128	16	4096
31.	S_{31}	256	64	128	32	8192
32.	S_{32}	16	0	4	0	128
33.	S_{33}	1024	64	1024	64	32768
34.	S_{34}	64	16	32	16	512
35.	S_{35}	1024	32	1024	32	16384
36.	S_{36}	128	64	256	256	512
37.	S_{37}	4096	128	256	256	5536
38.	S_{38}	512	64	128	64	16384
39.	S_{39}	128	32	64	32	1024
40.	S_{40}	1024	256	1024	512	32768
41.	S_{41}	64	64	64	16	512
42.	S_{42}	64	64	128	32	512
43.	S_{43}	128	128	128	28	1024
44.	S_{44}	64	64	128	32	512

Contd.

Table 3 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
45.	S_{45}	128	128	256	16	1024
46.	S_{46}	256	64	256	8	8192
47.	S_{47}	32	32	64	64	128
48.	S_{48}	32	64	64	8	128
49.	S_{49}	128	128	128	64	1024
50.	S_{50}	128	64	64	32	1024

Cut-off value for PHA - $\geq$ 1:128

Cut-off value for SAPA-AMHA - $\geq$ 1:1024

Table 4. PHA and SAPA-AMHA titres of human serum samples

Sl. No.	Sample No.	PHA titres before adsorption		PHA titres after adsorption of serum samples		SAPA-AMHA titres without adsorption
		With HS 20/1A antigen	With SW 20/2D antigen	With HS 20/1A antigen	With SW 20/2D antigen	With HS 20/1A antigen
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1.	H_1	64	64	32	32	2048
2.	H_2	512	256	512	256	4096
3.	H_3	4	2	4	0	4
4.	H_4	8	8	8	4	16
5.	H_5	8	4	4	4	8
6.	H_6	32	32	16	16	512
7.	H_7	4	2	4	2	8
8.	H_8	1024	256	512	256	2048
9.	H_9	32	32	16	16	1024
10.	H_{10}	64	32	64	32	1024
11.	H_{11}	16	8	2	0	64
12.	H_{12}	2	2	2	0	4

Table 4 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
13.	H_{13}	32	16	16	16	2048
14.	H_{14}	2	2	2	0	64
15.	H_{15}	64	32	4	2	512
16.	H_{16}	8	8	4	2	256
17.	H_{17}	8	4	8	4	128
18.	H_{18}	8	8	4	4	512
19.	H_{19}	4	4	4	2	256
20.	H_{20}	8	4	4	4	8
21.	H_{21}	32	16	16	16	128
22.	H_{22}	64	32	64	64	512
23.	H_{23}	8	8	4	2	2048
24.	H_{24}	128	64	64	16	4096
25.	H_{25}	4	4	4	0	512
26.	H_{26}	4	2	4	4	512
27.	H_{27}	16	8	8	16	64
28.	H_{28}	32	16	16	16	64

Table 4 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
29.	H_{29}	32	16	32	16	512
30.	H_{30}	16	8	8	8	128
31.	H_{31}	32	16	16	8	256
32.	H_{32}	2	4	2	0	16
33.	H_{33}	32	16	16	8	64
34.	H_{34}	64	32	32	32	8192
35.	H_{35}	8	16	8	8	1024
36.	H_{36}	16	8	8	2	256
37.	H_{37}	32	32	32	16	256
38.	H_{38}	2	4	2	2	64
39.	H_{39}	4	4	2	2	64
40.	H_{40}	4	4	4	2	32
41.	H_{41}	16	16	16	16	64
42.	H_{42}	0	0	0	0	4
43.	H_{43}	32	32	16	8	128
44.	H_{44}	8	16	0	0	512

Contd.

Table 4 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
45.	H_{45}	4	0	0	0	32
46.	H_{46}	8	16	16	4	1024
47.	H_{47}	2	2	0	0	32
48.	H_{48}	8	0	4	0	128
49.	H_{49}	4	0	4	0	16
50.	H_{50}	128	64	64	32	8192

Cut-off value for PHA - $\geq$ 1:64

Cut-off value for SAPA-AMHA - $\geq$ 1:512

antigens derived from swine (homologous species)(Table 5b and Fig.1b).

However when swine sera samples were monitored for C. jejuni antibodies using antigens derived from human strain, adsorption for non-specific antibodies using SRBC had significant difference (P <0.05) (Table 5c and Fig.1c).

When swine serum samples after adsorption with sheep RBC were subjected to PHA test using homologous antigen, the mean titre was 154.3 whereas mean titre for heterologous antigen was 49.5. The titres obtained while using the two antigens after adsorption with SRBC were found to be significant (P <0.05) by students 't' test (Table 5d and Fig.1d).

When SAPA-AMHA test was performed using swine serum samples, a substantial increase in the PHA titre value was noticed. The mean titre value for SAPA-AMHA test was 4899 when compared to PHA 249.4. Statistical analysis of the data of students 't' test (after logarithmic transformation) indicated that the mean value differed significantly (P <0.05) (Table 5e and Fig.1e).

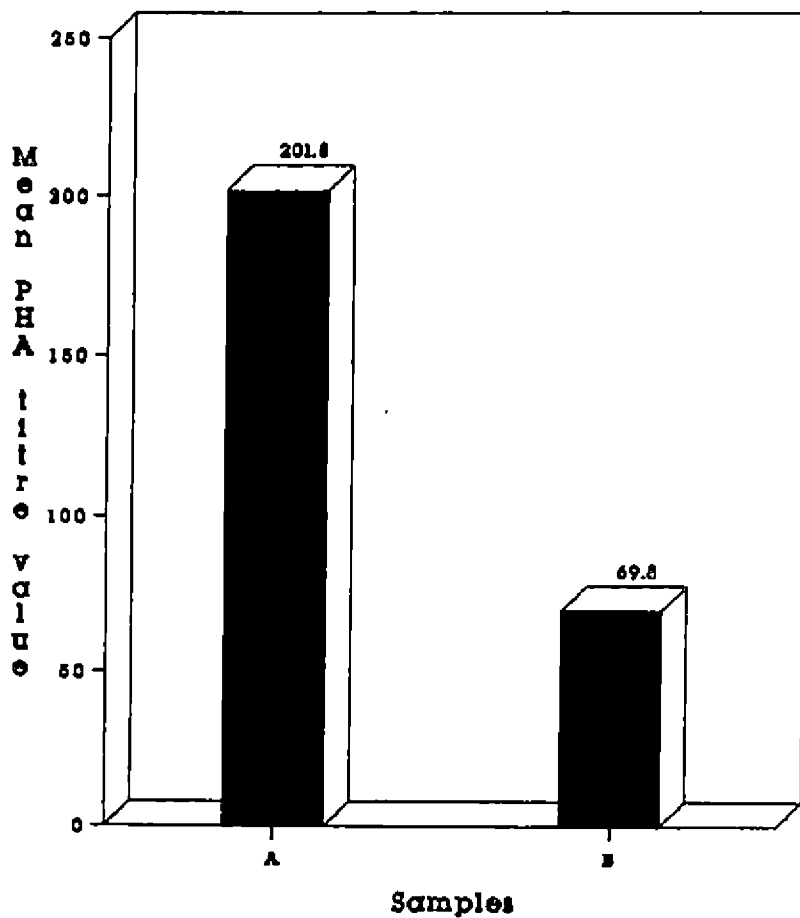
Monitoring human serum samples for C. jejuni antibodies using homologous and heterologous antigens,

Table 5a. Results of students 't' analysis of swine serum sample PHA titres, employing SW 20/2D and HS 20/1A antigens

Swine serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Serum samples screened with SW 20/2D antigen	201.8	2069.6335	45.4932	99	2.9020	Significant t value
B. Serum samples screened with HS 20/1A antigen	69.8					

($P < 0.05$)

Fig. 1A MEAN PHA TITRE OF SWINE SERUM SAMPLES SCREENED WITH SW 20/2D AND HS 20/1A ANTIGENS



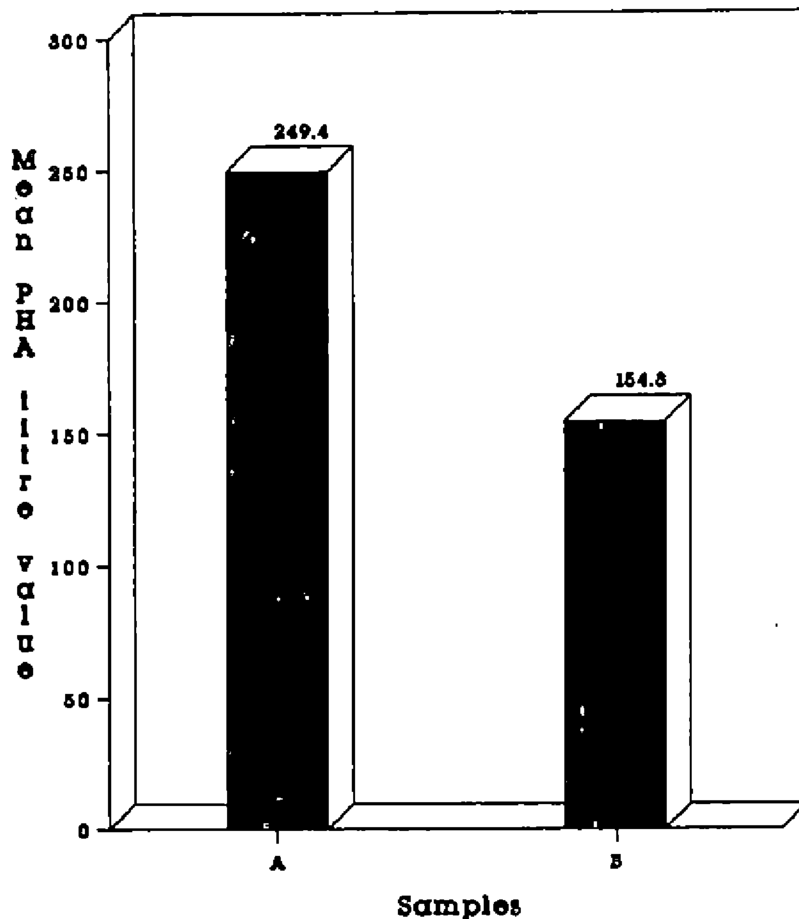
A - Mean PHA titre of swine serum samples screened with SW 20/2D antigen

B - Mean PHA titre of swine serum samples screened with HS 20/1A antigen

Table 5b. Results of students 't' analysis of swine serum sample PHA titres, with and without adsorption employing SW 20/2D antigen

Swine serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Unadsorbed serum samples screened with SW 20/2D antigen	249.4	6046.1036	77.7567	49	1.2223	Non-significant t value
B. Adsorbed serum samples screened with SW 20/2D antigen	154.3					

Fig.1B MEAN PHA TITRE OF SWINE SERUM SAMPLES SCREENED WITH SW 20/2D ANTIGEN WITH AND WITHOUT ADSORPTION



A - Mean PHA titre of unadsorbed swine serum samples screened with SW 20/2D antigen

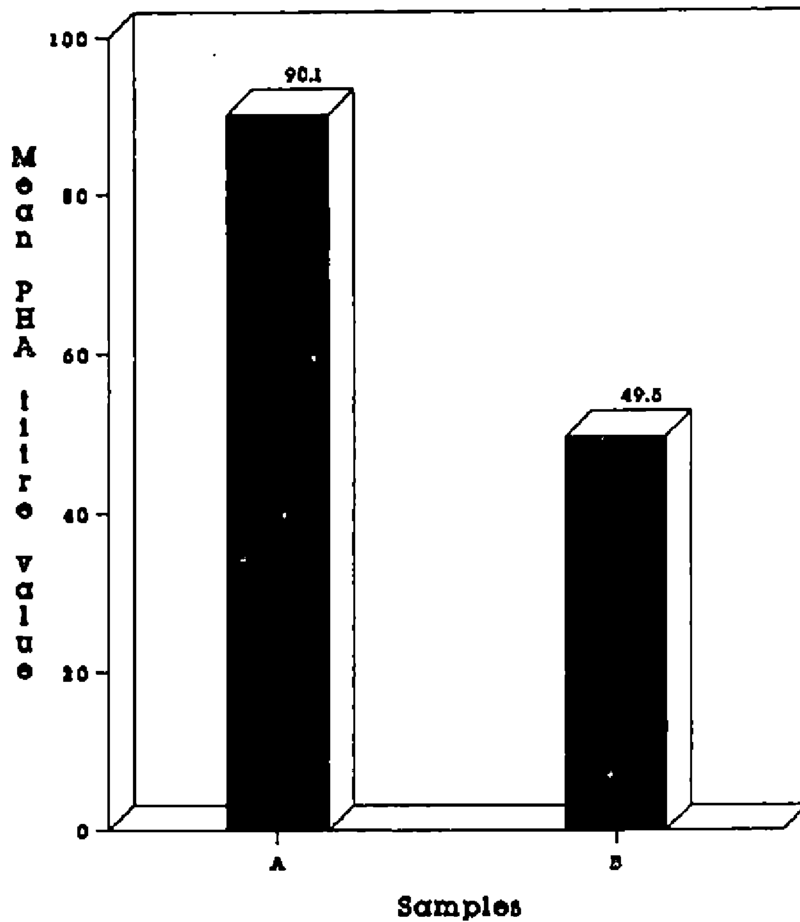
B - Mean PHA titre of adsorbed swine serum samples screened with SW 20/2D antigen

Table 5c. Results of students 't' analysis of swine serum sample PHA titres with and without adsorption employing HS 20/1A antigen

Swine serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Unadsorbed serum samples screened with HS 20/1A antigen	90.1	331.6253	18.2106	49	2.2295	Significant t value
B. Adsorbed serum samples screened with HS 20/1A antigen	49.5					

($P < 0.05$)

Fig. 1C MEAN PHA TITRE OF SWINE SERUM SAMPLES SCREENED WITH HS 20/1A ANTIGEN WITH AND WITHOUT ADSORPTION



A - Mean PHA titre of unadsorbed swine serum samples screened with HS 20/1A antigen

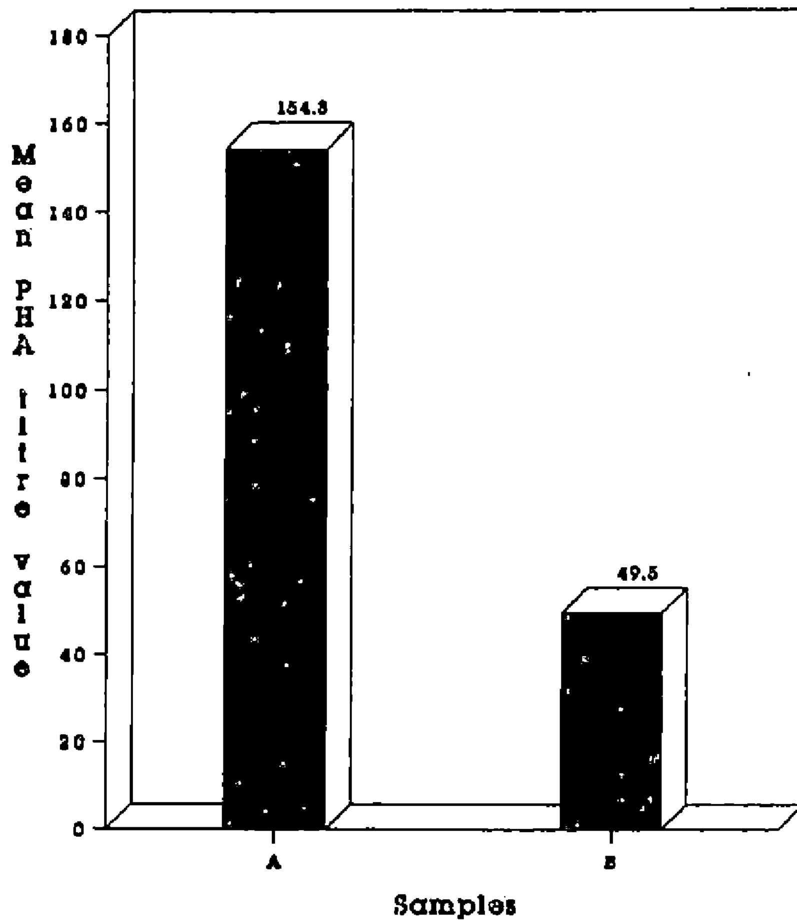
B - Mean PHA titre of adsorbed swine serum samples screened with HS 20/1A

Table 5d. Results of students 't' analysis of swine serum sample PHA titres after adsorption employing SW 20/2D and HS 20/1A antigens

Swine serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Adsorbed serum sample screened with SW 20/2D antigen	154.3	901.0776	30.0180	49	3.4912	Significant t value
B. Adsorbed serum sample screened with HS 20/1A antigen	49.5					

($P < 0.05$)

Fig.1D MEAN PHA TITRE OF ADSORBED SWINE SERUM SAMPLES SCREENED WITH SW 20/2D AND HS 20/1A ANTIGENS



A - Mean PHA titre of adsorbed swine serum samples screened with SW 20/2D antigen

B - Mean PHA titre of adsorbed swine serum samples screened with HS 20/1A antigen

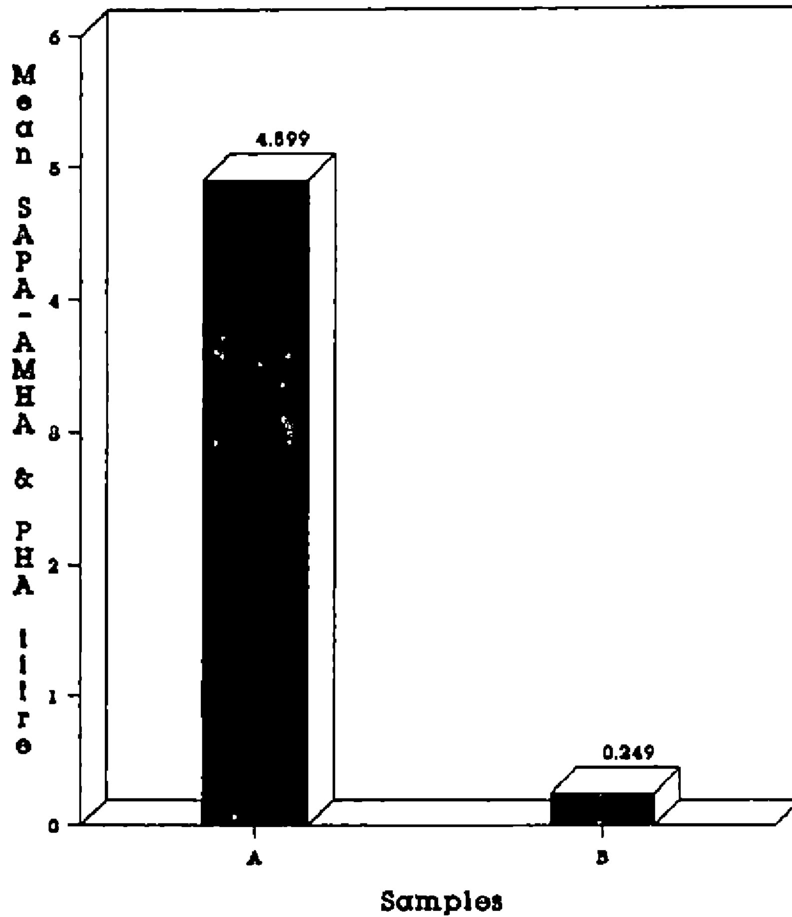
Table 5e. Results of students 't' analysis of swine serum sample SAPA-AMHA and employing SW 20/2D antigen

Swine serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value
A. Unadsorbed serum sample screened with SW 20/2D antigen (SAPA-AMHA)	2.9820*				
		0.0027*	0.05211*	49	19.3399
B. Unadsorbed serum sample screened with HS 20/2D antigen (PHA)	1.9680*				

* Logarithmic values

($P < 0.05$)

Fig. 1E MEAN SAPA - AMHA AND PHA TITRE OF SWINE SERUM SAMPLES SCREENED WITH SW 20/2D ANTIGEN



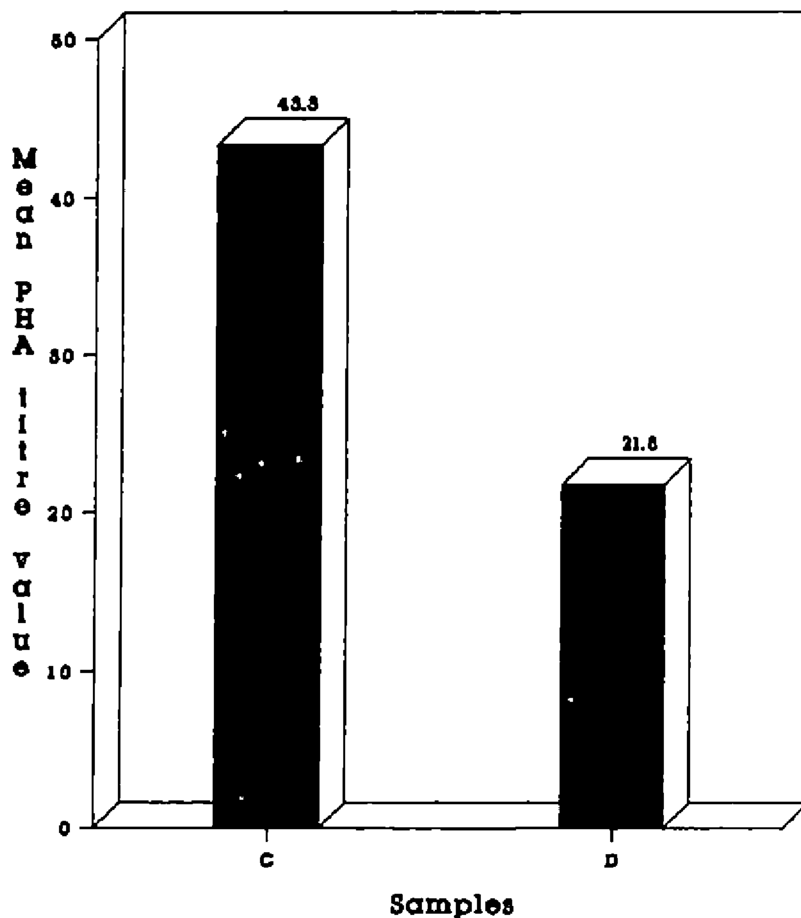
A - Mean SAPA-AMHA titre of unadsorbed swine serum samples screened with SW 20/2D antigen

B - Mean PHA titre of unadsorbed swine serum samples screened with SW 20/2D antigen

Table 6a. Results of students 't' analysis of human serum sample PHA titres, employing HS 20/1A antigen and SW 20/2D antigen

Human serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Screened with HS 20/1A antigen	43.3					
		76.7843	8.7627	99	2.4513	Significant t value
B. Screened with SW 20/2D antigen	21.8					

($P < 0.05$)



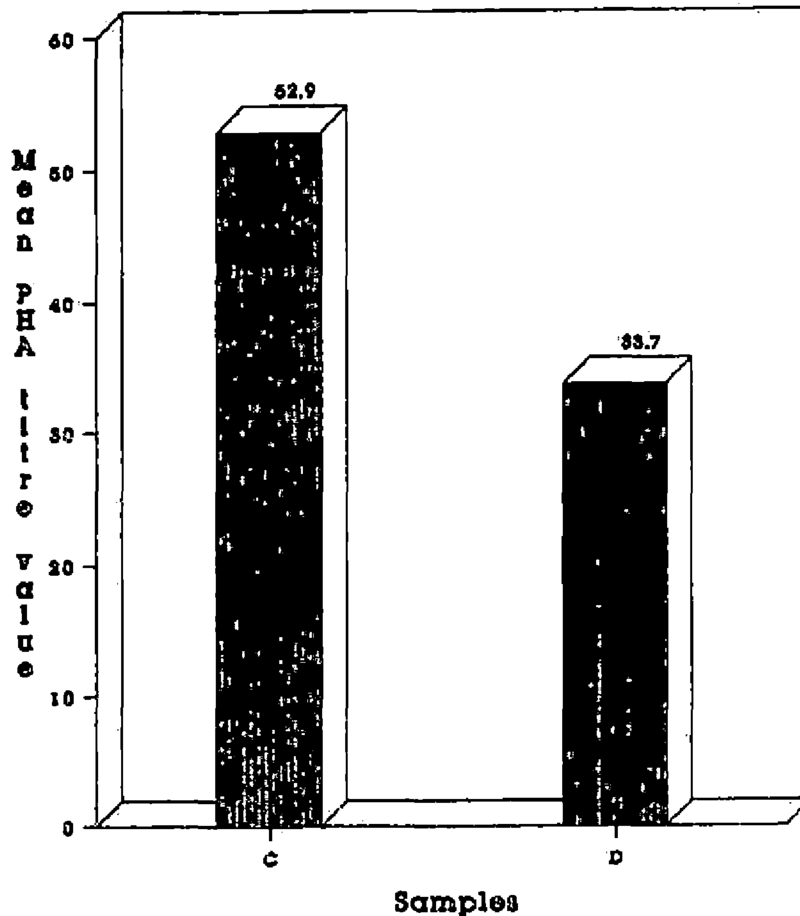
C - Mean PHA titre of human serum samples screened with HS 20/1A antigen

D - Mean PHA titre of swine serum samples screened with SW 20/2D antigen

Table 6b. Results of students 't' analysis of human serum sample PHA titres, with and without adsorption employing HS 20/1A antigen

Human serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Unadsorbed serum sample screened with HS 20/1A antigen	52.9	106.1874	10.3047	49	1.8671	Non-significant t value
B. Adsorbed serum sample screened with HS 20/1A antigen	33.7					

Fig. 2B MEAN PHA TITRE OF HUMAN SERUM SAMPLES SCREENED WITH HS 20/1A ANTIGEN WITH AND WITHOUT ADSORPTION



C - Mean PHA titre of unadsorbed human serum samples screened with HS 20 20/1A antigen

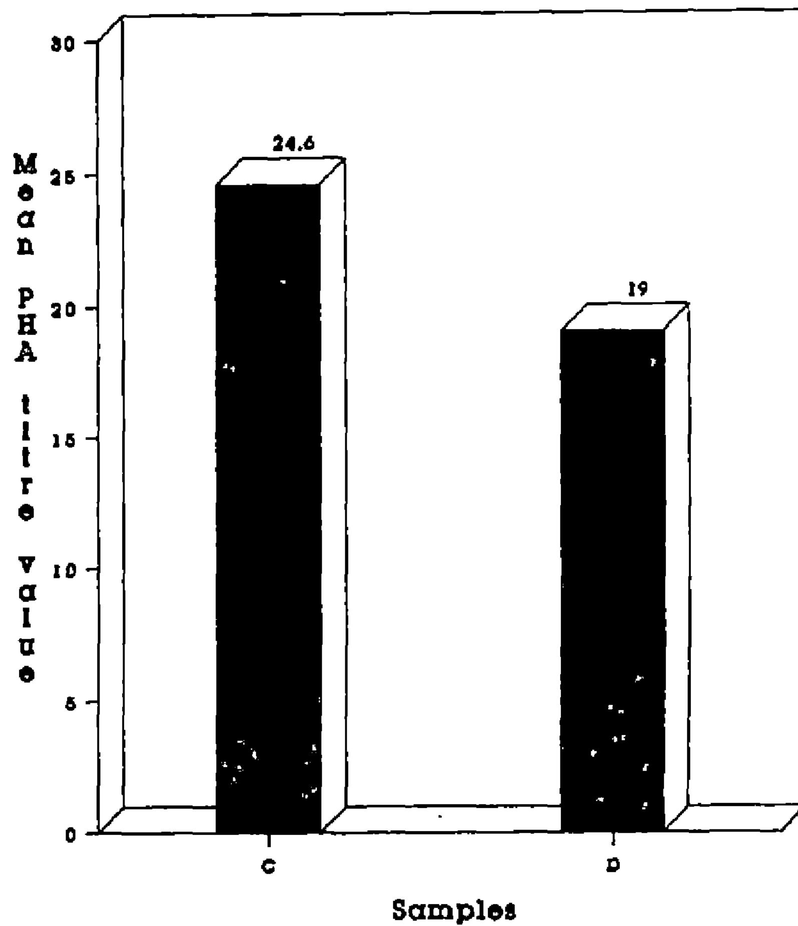
D - Mean PHA titre of adsorbed human serum samples screened with HS 20/1A antigen

Table 6c. Results of students 't' analysis of human serum sample PHA titres, with and without adsorption employing SW 20/2D antigen

Human serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Unadsorbed serum sample screened with SW 20/2D antigen	24.6	2.8398	1.6852	49	3.3468	Significant t value
B. Adsorbed serum sample screened with SW 20/2D antigen	19.0					

($P < 0.05$)

Fig. 2C MEAN PHA TITRE OF HUMAN SERUM SAMPLES SCREENED WITH SW 20/2D ANTIGEN WITH AND WITHOUT ADSORPTION



C - Mean PHA titre of unadsorbed human serum samples screened with SW 20/2D antigen

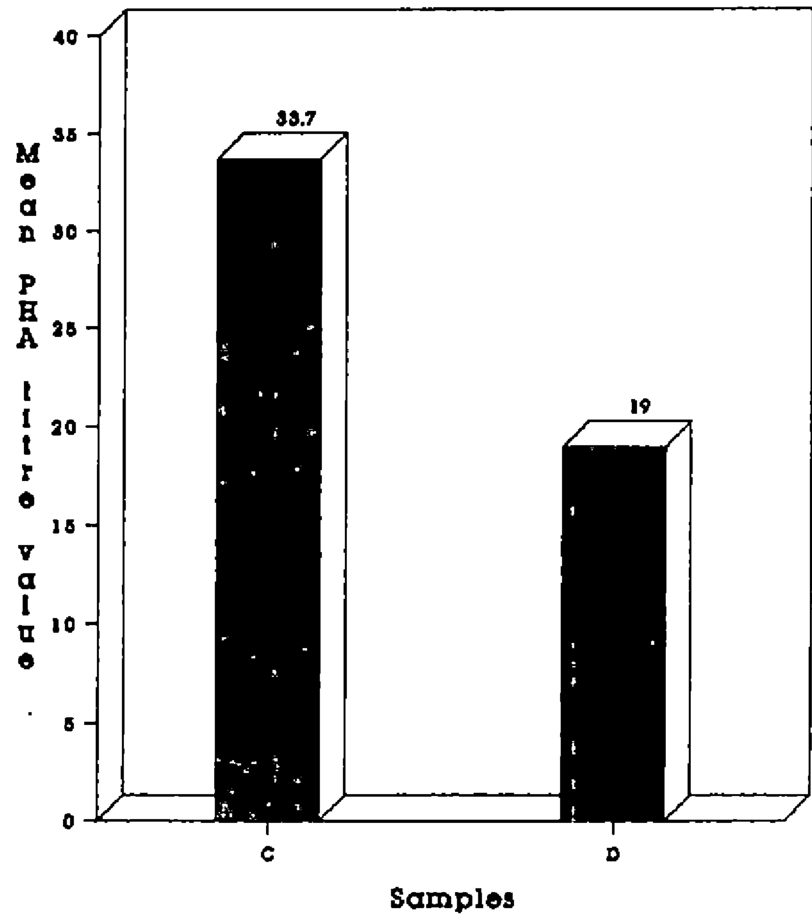
D - Mean PHA titre of adsorbed human serum samples screened with SW 20/2D antigen

Table 6d. Results of students 't' analysis of human serum sample PHA titres, after adsorption employing HS 20/1A and SW 20/2D antigens

Human serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Adsorbed serum sample screened with HS 20/1A antigen	33.7	51.3195	7.1638	49	2.0492	Significant t value
B. Adsorbed serum sample screened with SW 20/2D antigen	19.0					

$(P < 0.05)$

Fig. 2D MEAN PHA TITRE OF ADSORBED HUMAN SERUM SAMPLES SCREENED WITH HS 20/1A AND SW 20/2D ANTIGENS



C - Mean PHA titre of adsorbed human serum samples screened with HS 20/1A antigen

D - Mean PHA titre of adsorbed human serum samples screened with SW 20/2D antigen

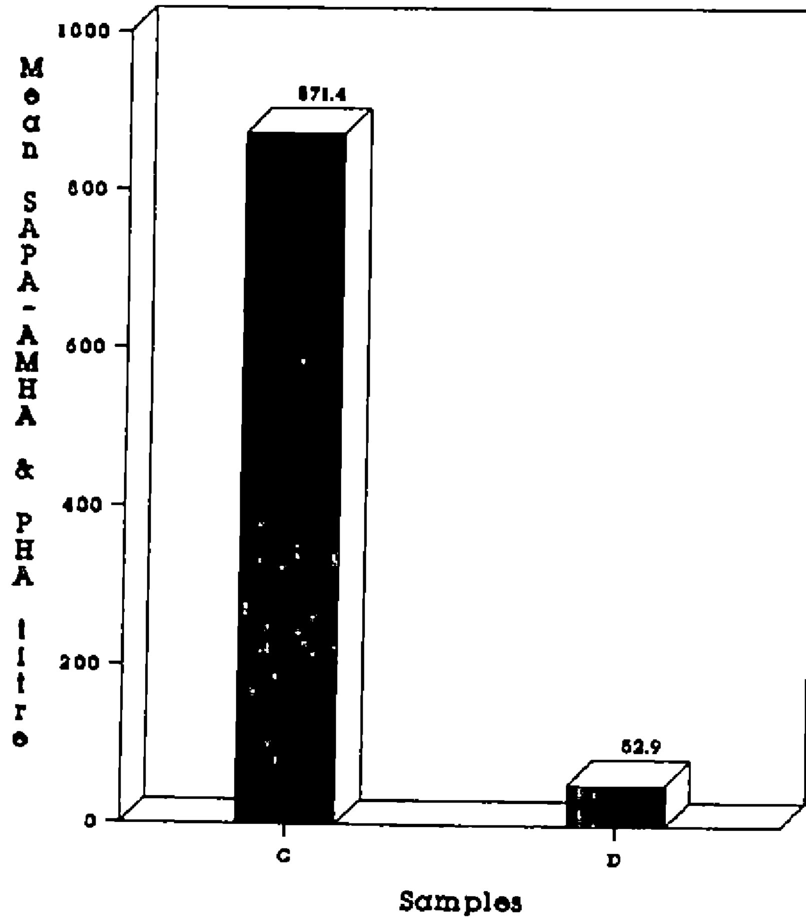
Table 6e. Results of students 't' analysis of human serum sample SAPA-AMHA and PHA titres employing HS 20/1A antigen

Human serum sample	Mean	Variance of the difference between means	Standard deviation of the difference	Degrees of freedom	t value	Results
A. Unadsorbed serum sample screened with HS 20/1A antigen (SAPA-AMHA)	2.2403*					
		0.0079*	0.0886*	49	12.1955	Significant t value
B. Unadsorbed serum sample screened with HS 20/1A antigen (PHA)	1.1596*					

* Logarithmic values

(P <0.05)

Fig. 2E MEAN SAPA - AMHA AND PHA TITRE OF HUMAN SERUM SAMPLES SCREENED WITH HS 20/1A ANTIGEN



C - Mean SAPA-AMHA titres of unadsorbed human serum samples screened with HS 20/1A antigen

D - Mean PHA titres of unadsorbed human serum samples screened with HS 20/1A antigen

indicated a significant difference ($P < 0.05$) in PHA titre (Table 6a and Fig.2a).

Adsorption of human serum samples with SRBC before PHA had no significant effect when homologous antigen (*C. jejuni* derived from man) was used (Table 6b and Fig.2b).

When heterologous antigen was used (*C. jejuni* derived from swine) to screen human serum samples, adsorption with SRBC prior to PHA had significant ($P < 0.05$) effect (Table 6c and Fig.2c).

Human serum samples after adsorption with sheep RBC, when subjected to PHA test, indicated significant difference in titre when homologous and heterologous antigen were used (Table 6d and Fig.2d).

SAPA-AMHA tested human serum samples showed a mean titre value of 871.4 in contrast to 52.9 showed by PHA. Statistical analysis by students 't' test (after logarithmic transformation) indicated significant difference ($P < 0.05$) (Table 6e and Fig.2e).

Antimicrobial susceptibility tests

Antimicrobial susceptibility pattern of 12 strains of *C. jejuni* isolated from swine and man are presented in Table 7.

Table 7. Antimicrobial susceptibility of 12 strains of *C. jejuni* isolated from swine and human

	Ampicillin	Chloramphenicol	Erythromycin	Furazolidone	Gentamicin	Nalidixic acid	Oxytetracycline	Penicillin G	Streptomycin	Sulphadiazine
Active ingredient/disc	10 µg	30 µg	15 µg	50 µg	30 µg	30 µg	30 µg	10 IU	10 µg	300 µg
Diameter of sensitive zone in mm	≥ 14	≥ 18	≥ 18	≥ 17	≥ 15	≥ 19	≥ 19	≥ 20	≥ 15	≥ 17
Sample No.										
SW 1	S	S	S	S	S	S	S	S	S	R
SW 2	R	S	S	R	S	S	S	S	S	S
SW 4	S	S	S	S	S	S	S	R	S	R
SW 5	S	S	S	S	S	S	R	R	R	R
SW 6	R	S	S	S	S	S	R	R	R	R
SW 7	S	S	I	S	S	S	R	R	S	R
SW 8	S	S	I	S	S	S	R	R	S	R
SW 17B	R	S	S	R	S	S	S	R	R	R

Contd.

Table 7 (Contd.)

	Ampicillin	Chloramphenicol	Erythromycin	Furazolidone	Gentamicin	Nalidixic acid	Oxytetracycline	Pencillin G	Streptomycin	Sulphadiazine
SW 19/2B	S	S	S	S	S	S	R	R	S	R
SW 20/2A	S	S	S	S	S	S	R	R	S	R
SW 20/2D	R	S	S	S	S	S	S	R	S	R
HS 20/1A	R	S	S	S	S	S	S	R	S	R
No. of sensitive strains	7/12	12/12	10/12	10/12	12/12	12/12	6/12	2/12	9/12	1/12
Percentage sensitivity	58.3%	100%	83.3%	83.3%	100%	100%	50%	16.6%	75%	8.3%

S - Sensitive I - Intermediate R - Resistant

C. jejuni showed cent per cent sensitivity to chloramphenicol, gentamicin and nalidixic acid. Sensitivity of the organisms to erythromycin was 83.3 per cent (10/12) while 16.7 per cent (2/12) gave an intermediary zone of inhibition. None of the organisms were resistant to erythromycin. 83.3 per cent (10/12) of the organisms were sensitive to furazolidone, 75 per cent (9/12) to streptomycin and 58.3 per cent (7/12) to ampicillin. 50 per cent (6/12) of the isolates showed resistance to oxytetracycline. Of the 10 antibiotics tested, 16.6 per cent (2/12) organisms were sensitive to penicillin. *C. jejuni* recorded highest resistance to sulphadiazine as only 8.3 per cent (1/12) of the organisms were sensitive to sulphadiazine.

Discussion

DISCUSSION

Isolation of C. jejuni from pigs

ı this study out of 26 rectal swabs screened, 11 yielded C. jejuni i.e., an isolation rate of 42.3 per cent.

Various workers from abroad had reported different rates of isolation of C. jejuni from pigs ranging from 2.9 per cent to 79.3 per cent. The reported incidence of C. jejuni varied from place to place. The highest percentage of isolation of C. jejuni was reported by Adesiyan et al. (1992) is 79.3 per cent in piglets. Cabrita et al. (1992) reported an isolation rate of 59.1 per cent. Scarcelli et al (1991) recorded 43 isolations of campylobacters from 75 pigs suffering from intestinal disorders. From 30 pigs the only pathogen isolated was campylobacter, indicating the potential power of C. jejuni to produce intestinal infections. In the present study also the samples were collected from piglets manifesting enteritis/diarrhoea. Since only selective media were employed, intercurrent infection with other pathogens could not be elucidated.

In India Kakkar and Dogra (1990) screened 95 pigs from Haryana and reported the isolation of C. jejuni from 35 animals. Of the 35 isolates, 97 per cent were from piglets.

In the present study 42.3 per cent of isolations from piglets, indicated that, in this part of the country also *C. jejuni* is one of the important pathogen responsible for causing diarrhoea/enteritis. Adesiyan *et al*. (1992) reported a higher frequency of isolation of *C. jejuni* in the case of piglets managed under semi-intensive conditions. In this study the rectal swabs were collected from University Pig breeding farm where piglets were maintained in semi-intensive conditions. So *C. jejuni* can even cause diarrhcea/enteritis in animals maintained under good managemental conditions.

Isolation of *C. jejuni* from man

Out of the 32 rectal swabs screened for the isolation of *C. jejuni* only one yielded the organism with an isolation rate of 3.12 per cent. In this study, isolation of other enteropathogenic organisms was not attempted. But there are reports by Wright *et al*. (1983) from England with regard to the association of *C. jejuni* in the causation of acute gastro intestinal disease outbreaks. In two episodes no other gastro intestinal pathogen was isolated except *C. jejuni*. Report from India (Calcutta and Bangalore) also indicated the association of *C. jejuni* in gastro-intestinal disturbance. From Calcutta, Nair *et al*. (1984) isolated *C. jejuni* from 7.7 per cent of patients with diarrhoea whereas Macaden *et al*., 1984 could obtain an isolation rate of 1.5 per cent only from

Bangalore. Prasad *et al*. (1991) from Lucknow reported an isolation rate of five per cent for *C jejuni*. Majority of the workers from developed countries indicated that the incidence of *C. jejuni* was pronounced in the lower age groups. In the present study age wise incidence could not be obtained because all the samples were collected from children below five years of age. Prasad *et al*. (1991) also reported that children below five years of age had a higher incidence rate of *C. jejuni* enteritis (8.3%) as against three per cent in older subjects. Sengupta *et al*. (1991) after continuous observation for two years reported that children below one year were more prone to campylobacter enteritis.

Characterisation of *Campylobacter jejuni* isolates

Standard procedures recommended for the isolation of *C. jejuni* were adopted in this study. Although different culture media were recommended for the primary isolation of *C. jejuni*, Brucella agar (Difco) enriched with 10 per cent sterile defibrinated sheep blood (BBA) and supplemented with Butzler's antimicrobial supplement-B (Difco) gave consistently satisfactory results in the isolation of *C. jejuni*. The defibrinated blood enriched the media and antimicrobial supplement eliminated the over growth of fecal contaminants, enabling easy detection and appreciation of the colonies. The superiority of the above culture media for the isolation of

C. jejuni were reported by Chattopadhyay et al. (1992). Subculturing was done on Plain Brucella agar without blood and antimicrobial supplement.

The gaseous atmosphere used in this study for the isolation and subculturing of C. jejuni was produced by Candle jar technique. The isolation rate of C. jejuni using Candle jar and by recommended gas mixtures were studied by different scientists and reported no significant difference, if the plates are incubated at 42°C for 48 h (Goossens et al., 1984; Griffiths and Park, 1990).

Incubation temperature of 42°C was found to be superior to 37°C for isolation of C. jejuni especially when the candle jar system was practiced. Goossens et al. (1984) proved that for the isolation of C. jejuni, candle jar incubated at 42°C for 48 h did not differ significantly from other superior, newer expensive methods. Therefore, the temperature of incubation and gaseous atmosphere employed in the study were found to be adequate for the isolation.

Two distinct colony types of C. jejuni recognised in the study have been encountered by other workers also (Skirrow and Benjamin, 1980; Macaden et al., 1984). Glossy, spreading type of colonies were predominantly seen on fresh culture plates with high amount of moisture, whereas, smooth convex

colonies were seen on plates kept in refrigerator for more than seven days were used for the isolation. Inoculation of the organism on freshly prepared moist media produced swarming glossy colonies in contrast to circular smooth convex colonies on older plates. This fact was reported by Buck and Kelly (1981). Whereas Skirrow and Benjamin (1980) reported simultaneous occurrence of both colonial forms together in one and the same plate. Concomitant occurrence of both type of colonial forms were not observed in this study.

Typical seagull-wing shaped to spiral or coma shaped Gram negative organism with tapering ends were demonstrated in 48 h old cultures. Coccoid forms were seen in cultures aged over five days and after eighth day the organisms did not revert back to original shape. Similar observations on the morphologic features of C. jejuni have been reported by Pillai (1989); whenever coccoid forms are encountered, subculturing should be done immediately to revive the culture.

The thermophilic nature of C. jejuni was clearly evidenced from its ability to grow at 42°C and its inability to grow at 25°C. This thermophilic nature of the organism was exploited in the primary isolation and subculturing of C. jejuni organisms. At 42°C, the overgrowth of contaminants were also inhibited to some extent.

In the present study all the 12 *C. jejuni* isolates showed similar type of biochemical reactions, as reported by Skirrow and Benjamin (1980).

C. jejuni antigen

Sonicated antigens prepared from three strains of *C. jejuni* were found to be suitable for sensitization of SRBC. The affinity of sonicated antigen to SRBC was retained even after six months of storage at -60°C. Since the affinity and specificity is retained after a lapse of six months, sonicated *C. jejuni* antigen sensitized RBC can be used for seromonitoring of *C. jejuni* specific antibodies under field conditions.

C. jejuni antibodies in rabbits

From Table 2 it is evident that all the three strains of *C. jejuni* did not elicit similar immunological response. All the strains gave maximum PHA titre after the fifth injection. For raising hyperimmune sera against *C. jejuni* antigen it is advisable to limit to five injections because in this study no increase in the PHA titre of hyperimmune serum was noticed after fifth injection. Among the two strains of *C. jejuni* of porcine origin, 19/2B gave a PHA titre of 1/64 only with the hyper immune sera raised against the *C. jejuni* from swine. The same antigen gave no titre with hyperimmune

sera raised against C. jejuni of human origin. Whereas 20/2D gave a PHA titre of 1:1024 with sera raised against porcine origin but gave a titre of only 1:128 with sera raised against human origin. Maximum PHA titre of 1:4096 was given by HS 20/1A strain of human origin. When this hyper immune serum was titrated with the antigens derived from swine, the PHA titre was 1:2048. The above results point to the probable prevalence of different antigenic components in varying proportions in different strains from various sources.

Stabilization of SRBC

In this study, gluteraldehyde stabilized SRBC used for sensitisation with C. jejuni antigen for carrying out PHA test. Gluteraldehyde stabilized SRBC could be stored upto three months without any change in the sensitization property to C. jejuni antigen. Eventhough different (stabilization materials) aldehydes were used for stabilization of SRBC, Pillai (1986) reported that gluteraldehyde stabilization is superior to pyruvicaldehyde and formaldehyde for sensitization with C. jejuni. Gluteraldehyde stabilized SRBC were used for sensitization with other antigens like mycobacterial antigen for monitoring serum antibodies by Jagannath and Sengupta (1983).

Optimum concentration of *C. jejuni* antigens for sensitization of SRBC

Previous studies in this field have indicated that the concentration of antigen used to sensitize erythrocytes affected the titre obtained in passive haemagglutination assays (Neter *et al*., 1952). The original description of Penner serotyping method did not standardize the antigen concentration used (Penner and Hennessy, 1980).

Fricker *et al*. (1986) standardized the concentration of bacteria used to prepare antigens in the serotyping of *C. jejuni*/*C. coli* by passive haemagglutination. They showed that a wide range of antigen concentrations had no effect on the serum titre obtained. However, high dilution of antigen (over 1:80) reduced the titre.

In this study, the protein level of the sonicated antigen was adjusted to 2 mg/ml (Biuret method) and 50, 100, 150, 200 ul of this protein adjusted sonicated antigen was used to sensitize 3 ml of packed RBC. By cross checker titration assay, it was found that 150 ul and 200 ul gave the same PHA titre values. So the lowest concentration i.e. 150 ul was selected and practiced throughout in the study. Pillai (1989) also reported similar observation while attaching

C. jejuni antigens derived from chicken to SRBC for conducting PHA studies.

Optimum concentration of SAPA cells

Although different concentrations of SAPA cells (0.01, 0.05, 0.1 and 0.2 per cent) were used in the present study to enhance the sensitivity of PHA test, 0.1 per cent concentration was found to be optimum. Similar studies carried out by Pillai (1989) also indicated 0.1 per cent as optimum for SAPA-AMHA.

Cross titration assay

Hyperimmune sera were raised against three campylobacter antigens (SW 19/2B, SW 20/2D, HS 20/1A). The sera were titrated against homologous and heterologous antigens to select the suitable antigens.

The hyperimmune serum raised against SW 19/2B antigen was titrated against all the three antigens. The serum gave a titre of 1:64 to both the antigens of porcine origin and did not show any PHA titre with HS 20/1A (heterologous antigen). But the hyperimmune sera raised against SW 20/2D gave satisfactory PHA titres with homologous and heterologous antigen. With antigens of porcine origin, it gave a PHA titre of 1:1024 and with heterologous antigen, the titre was 1:128.

From the titre results it is inferred that, the hyper immune sera raised against SW 19/2B is inferior to SW 20/2D. This may be due to the poor immunogenicity of SW 19/2B antigen in the biological system that might have elicited a lesser immune response or may be due to the poor immune response of that particular animal.

SW 20/2D and SW 19/2B antigens gave similar results when titrated against hyperimmune sera raised against SW 20/2D antigen. This clearly indicates that SW 19/2B antigen may also be used for PHA titration. But the superior one SW 20/2D was selected for screening field sera samples as it gave comparable PHA titres with both homologous and heterologous hyperimmune sera.

The hyperimmune serum raised against HS 20/1A showed the highest titre when PHA was carried out (1:4096). So this antigen was also selected for screening field sera samples.

Table 2 indicated that *C. jejuni* antigen of porcine origin can give satisfactory titre with sera raised against *C. jejuni* antigen of human origin and vice versa.

The PHA titre values from Table 2 indicated that there is considerable degree of antigenic relationship between porcine and human strain. But the reduction in titre with heterologous antigen/serum indicated that, different antigenic

components in varying proportions may be present in different strains from varying sources.

Assay of *C. jejuni* antibodies in field serum samples

In the present investigation, 50 sera sample from pigs were monitored for the presence of *C. jejuni* antibodies by PHA and SAPA-AMHA. PHA test detected *C. jejuni* antibodies in 48 per cent compared to 54 per cent by SAPA-AMHA test (Table 3). The PHA titres ranged from 1:4 to 1:4096 compared to 1:4 to 1:32768 by SAPA-AMHA. The cut-off value was taken as the base line for repeated culture negative control pigs sera. The baseline was taken as ≥ 1:128 by PHA and ≥ 1:1024 by SAPA-AMHA.

An attempt was also made in this study to titrate 50 human serum samples for *C. jejuni* antibodies by PHA and SAPA-AMHA test. PHA test detected *C. jejuni* antibodies in 18 per cent when compared to 38 per cent by SAPA-AMHA (Table 4). The PHA titres ranged from no reaction to 1:1024 compared to 1:4 to 1:8192 by SAPA-AMHA. The cut off value was taken as the base line for repeated culture negative control sera from children. The baseline was taken as ≥ 1:64 by PHA and ≥ 1:512 by SAPA-AMHA test.

The result of the present study indicated that SAPA-AMHA test could advantageously replace the conventional

PHA. But PHA have been used for the serological diagnosis of animal and human campylobacteriosis because of its simplicity and reliability (Fricker et al., 1986).

Statistical analysis by students 't' test (after logarithmic transformation) revealed a significant difference (P <0.05) between PHA and SAPA-AMHA indicating that the latter detected more number of seropositives and the titre values were also invariably higher.

According to Hebert (1982) PHA are mainly used for quantitating the IGM antibodies. But in the case of SAPA-AMHA test the *S. aureus* bind to the FC terminal portion of IgG molecule leaving the antigen combining Fab region free. So this test also detect the presence of IgG. This may also be a contributing factor for observance of high titres in SAPA-AMHA compared to PHA.

The SAPA-AMHA test is simple and employ the antigen coated SRBCs and the SAPA cells which have higher shelf life for routine screening of serum samples. The test is of particular interest because Jagannath et al. (1984) observed that SAPA cells were found to bind the mycobacterial antibodies and enhanced the sensitivity of the test approximately to that of ELISA. In this study also eventhough SAPA-AMHA has not been compared with ELISA, this test detected

more number of seropositives and the titres were invariably higher to PHA. So SAPA-AMHA test may have great potential for application in other animal and human diseases of bacterial origin.

Swine sera samples when monitored to *C. jejuni* antibodies, by PHA using homologous and heterologous antigen sensitized SRBC, indicated a difference in the mean titre value (Table 5a). Eventhough the mean titre value differs, some antigenic relationship exist between *C. jejuni* of porcine and human origin. Analysis of data by students 't' test indicated that the results were statistically significant ($P<0.05$). Therefore, while monitoring antibodies against *C. jejuni* in pigs, it is always recommended to use SRBC sensitizied with antigens derived from the homologous species.

Efforts were also made in this study to find out whether adsorption of swine serum samples with SRBC prior to PHA had any effect on PHA titre compared to unadsorbed sera sample. From Table 5b it is evident that adsorption had no effect on the PHA titre while monitoring for *C. jejuni* antibodies employing antigens derived from same species.

However, when swine sera samples were monitored for *C. jejuni* antibodies using heterologous antigen, adsorption for non-specific antibodies using SRBC had significant effect

compared to unadsorbed sera (Table 5c). From the table it is clear that when heterologous antigen is used for monitoring *C. jejuni* antibodies, adsorption of sera should be done.

Attempts were also made to analyse the data in such way that absorbed sera only had been titrated with homologous and heterologous antigen. The titres obtained while using the two antigens were found to be significant by students 't' test (Table 5d) indicating that if PHA have to be done after adsorption with SRBC, homologous antigen should be used.

Efforts were made to demonstrate the efficiency of SAPA-AMHA test over PHA test in screening swine serum samples for *C. jejuni* antibodies. The statistical analysis showed that mean SAPA-AMHA titre values was 4899 compared to 249.4 for PHA. This clearly indicated that SAPA-AMHA is superior to PHA in screening specific *C. jejuni* antibodies. Eventhough the cut off value for SAPA-AMHA was fixed at $\geq$ 1:1024 compared to $\geq$ 1:128 for PHA, SAPA-AHMA could detect more seropositives. By SAPA-AMHA test it was shown that 54 per cent serum samples contained *C. jejuni* antibodies, PHA could detect only 48 per cent. In this study the superiority of SAPA-AMHA test in serum monitoring is clearly demonstrated.

Monitoring human sera for *C. jejuni* antibodies using homologous and heterologous antigen showed a difference in PHA

titre. Statistical analysis of the data by students 't' test indicated that the difference was significant (P <0.05). From the Table 6a it could be further inferred that for monitoring *C. jejuni* antibodies in human sera, homologous *C. jejuni* antigen should be used.

Attempts to find out whether adsorption of human sera sample with SRBC had any effect on PHA titre compared to unabsorbed sera sample showed that such a process had no effect on the PHA titre while monitoring for *C. jejuni* antibodies using homologous antigen.

Human sera samples when monitored for *C. jejuni* antibodies by PHA using heterologous antigen, adsorption for non-specific antibodies using SRBC had significant effect (Table 6c). From the table it is evident that when heterologous antigen is used for monitoring *C. jejuni* antibodies adsorption of sera is emphasised.

Efforts were also made in this study to find out the effect on PHA titre when adsorbed sera were titrated with homologous and heterologous antigen (Table 6d) statistical analysis indicated that the difference in PHA titre was significant (P<0.05). From the table it is evident that for performing PHA after adsorption with SRBC homologous antigen is preferred.

In this study efforts were made to demonstrate the sensitivity of SAPA-AMHA test in detecting C. jejuni specific antibodies in human serum samples. The mean SAPA-AMHA titre value was 871.4 compared to 52.9 for PHA. By SAPA-AMHA test it was detected that 38 per cent of the serum screened contained C. jejuni specific antibodies. But by PHA C. jejuni specific antibodies could be demonstrated only in 18 per cent of the individuals. Pillai (1989) also reported the superiority of SAPA-AMHA test over conventional PHA test in screening C. jejuni specific antibodies in human and chicken serum samples. In this study for screening human serum samples also SAPA-AMHA test was found superior to PHA to monitor C. jejuni specific antibodies.

Antimicrobial susceptibility test

Antibiogram patterns of the 11 strains of Campylobacter jejuni isolated from swine and one strain from man are presented in Table 7. From the table it could be observed that all the 12 strains tested showed cent per cent sensitivity to chloramphenicol, gentamicin and nalidixic acid irrespective of their source of origin. Yoon et al. (1989), Hariharan et al. (1990b), MoDolo et al. (1991) and Valente et al. (1991) from different corners of the world also reported that cent per cent of the C. jejuni organisms tested were sensitive to chloramphenicol, gentamicin and nalidixic

acid. From this study and from various literature screened it can be emphasised that irrespective of the source of origin and geographical barrier, for treating clinical cases of campylobacteriosis due to *C. jejuni*, chloramphenicol, gentamicin or nalidixic acid should be the drug of choice. Eventhough all the three antibiotics showed cent per cent sensitivity, nalidixic acid gave the maximum diameter of zone of inhibition (37 mm) compared to chloramphenicol (31 mm) and gentamicin (28 mm).

In the order of sensitivity, erythromycin can be placed in the fourth position because 83.3 per cent of the isolates showed a sensitive zone of inhibition whereas 16.7 per cent showed only an intermediary zone of inhibition. None of the *C. jejuni* isolated in this study was resistant to erythromycin. Resistance to erythromycin has been reported by other workers. Hariharan *et al*. (1990a) reported that 28.6 per cent of the isolates from swine were resistant to erythromycin. Cabrita *et al*. (1992) also recorded 12.6 per cent resistance to erythromycin and he concluded that resistance to erythromycin was particularly high in pig isolates. Resistance to erythromycin could not be observed in this study but two strains of *C. jejuni* isolated from swine showed only an intermediary zone of inhibition.

C. jejuni isolates recorded 83.3 per cent sensitivity to furazolidone, 75 per cent to streptomycin and 58.3 per cent to ampicillin. Adesiyan et al. (1992) and Cabrita et al. (1992) also recorded similar sensitivity percentages to the above antibiotics in the case of C. jejuni.

Fifty per cent of the C. jejuni studied were resistant to oxytetracycline. Many workers in this field also recorded tetracycline resistance. But it was Tenover et al. (1985) and Cabrita et al. (1992) who reported that tetracycline resistance in C. jejuni was plasmid mediated.

Of the ten antibiotics tested only 16.6 per cent of C. jejuni isolates were sensitive to penicillin. Highest resistance was recorded against sulphadiazine as only 8.3 per cent of the organisms were susceptible. Relative resistance of C. jejuni to sulphadiazine and penicillin have been reported from different parts of the world (Diker et al., 1987, Modolo, 1991; Bradbury and Munroe, 1985).

Summary

SUMMARY

In the present investigation to assess the association of *C. jejuni* with swine and human enteritis/diarrhoea, piglets below two months and children below five years of age were selected. Twenty six rectal swabs from piglets and thirty two rectal swabs from children having diarrhoea/enteritis were culturally screened for *C. jejuni*.

For the isolation of *C. jejuni*, Brucella blood agar plates supplemented with Difco Campylobacter supplements were used. The inoculated plates were incubated at 42°C under microaerophilic conditions(Candle Jar system) for 48 h. The plates were examined for typical wet, glossy, spreading or discrete convex colonies. The colonies which showed the typical characters were subjected to final identification methods described by Gracia *et al*. (1983) and yielded 12 *C. jejuni*, 11 from swine and one from human origin.

Gluteraldehyde stabilisation was found to be a suitable method for the preservation of Sheep RBC. Gluteraldehyde stabilised SRBC were stored at 4°C for a period of three months. The sensitization property of gluteraldehyde stabilised SRBC to *C. jejuni* antigen was not reduced by

storage, as evidenced from the seromonitoring studies using passive haemagglutination test.

Sonicated *C. jejuni* antigen prepared from the three selected isolates (SW 19/2B, SW 20/2D and HS 20/1A) were found suitable for sensitization of stabilized SRBC for serum antibody monitoring. The sonicated antigen retained its affinity to sheep RBC, even after six months of storage at -60°C. One hundred and fifty microlitres of *C. jejuni* antigen whose protein concentration was adjusted to 2 mg/ml was found to be the optimum level for sensitization of a suspension made from three millilitres of packed SRBC.

Two strains of *C. jejuni*, one from swine and the other of human origin elicited good immunological response. The hyperimmune sera raised in rabbits were employed for standardization of PHA and SAPA-AMHA.

Two *C. jejuni* antigens, one from swine (SW 20/2D)and the other from human origin (HS 20/1A), were selected for bulk sensitization of SRBC for screening field serum samples.

PHA titre values obtained by cross titration clearly indicated that there was antigenic relationship between porcine and human strains. This study also point to the probable prevalence of different antigenic components in varying proportions in different strains from various sources.

The optimum level of SAPA cells required in the seromonitoring of *C. jejuni* specific antibodies by SAPA-AMHA test was found to be 0.1 per cent.

In the present investigation, 50 serum samples from pigs were monitored for the presence of *C. jejuni* antibodies by PHA and SAPA-AMHA. PHA test detected 48 per cent as seropositives, compared to 54 per cent by SAPA-AMHA. The PHA titres ranged from 1:4 to 4094, compared to 1:4 to 1:32768 by SAPA-AMHA.

An attempt was also made in this study to titrate 50 human serum samples for *C. jejuni* antibodies by PHA and SAPA-AMHA. PHA could detect 18 per cent cases as positives compared to 38 per cent by SAPA-AMHA.

The result of the present study indicated that SAPA-AMHA could advantageously replace the conventional PHA for detecting *C. jejuni* antibodies.

Efforts were also made in this study to find out the change in PHA titre with homologous and heterologous antigens employing sera before and after adsorption with unsensitized SRBC to remove the non specific antibodies.

Statistical analysis of the data indicated that when titrating for *C. jejuni* antibodies by PHA, homologous antigen

is preferred over heterologous antigen. When homologous antigens were used, adsorption of serum samples with stabilized SRBC for removal of non specific antibodies had no significant effect on the end titre. On the other hand, the heterologous antigen when used for monitoring _C. jejuni_ antibodies adsorption with SRBC had significant effect.

Attempts were also made to study the _in vitro_ antimicrobial sensitivity patterns of the 12 _C. jejuni_ isolates by standard disc diffusion technique. The results indicated all the _C. jejuni_ isolates were sensitive to chloramphenicol, gentamicin and nalidixic acid, irrespective of their source/origin. Eventhough none of the _C. jejuni_ isolated in this study was resistant to erythromycin, only 83.3 per cent of the isolates showed a sensitive zone of inhibition,while, 16.7 per cent showed an intermediary zone of inhibition. _C. jejuni_ isolates recorded 83.3 per cent sensitivity to furazolidone, 75 per cent to streptomycin and 58.3 per cent to ampicillin. Fifty per cent of the isolates showed resistance to oxytetracycline. Of the ten antibiotics tested, 16.6 per cent of the organism were sensitive to penicillin. _C. jejuni_ recorded highest resistance to sulphadiazine as only 8.3 per cent of the organisms were sensitive to sulphadiazine.

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* Originals not consulted

Appendix

Appendix

Buffers reagents and solutions

A1 Alsever's solution

Sodium chloride	- 4.20 g
Trisodium citrate	- 8.00 g
Citric acid	- 0.55 g
Glucose	- 20.50 g
Distilled water	- 1000 ml

The above ingredients were weighed and transferred to a one litre flask. Five hundred ml of sterile double distilled water was added and stirred well to dissolve the contents. Then again 500 ml of sterile distilled water was added to make up the volume to 1000 ml. The final solution was transferred to small flasks (150 ml), filling upto a 1/3 level, closed and sterilized by autoclaving at 10 lbs for 45 min. The solution was freshly prepared before use.

A2. Lead acetate strips

Lead acetate	- 10.0 g
Distilled water	- 10.0 ml

A saturated solution of lead acetate was prepared by heating the ingredients in water. The hot saturated aqueous

solution was absorbed onto filter paper strips (5-10 mm wide and 50-60 mm length), dried at 50-60°C and stored at 4°C.

A3. Neutral gluteraldehyde solution

Gluteraldehyde (25%)	-	10 ml

Sodium carbonate 10% solution was added dropwise till the pH of gluteraldehyde reached 7.0. The solution was stored at 4°C till further use.

Normal saline solution (NSS pH 7)

Sodium chloride	-	8.5 g
Distilled water	-	1000 ml

The solution was sterilized at 15 lbs for 15 minutes and stored at 4°C till further use.

A4. Oxidase reagent strips

Tetramethyl - Paraphenylene diamine dihydrochloride	-	100 mg
Sterile distilled water	-	10 ml

Dissolved the ingredients in distilled water and absorbed onto filter paper strips (5-10 mm x 50-60 mm), dried and stored in amber coloured wide mouth bottles at 4°C.

A5. Phosphate buffer pH - 8

Solution A

Monobasic sodium phosphate $2H_2O$	- 31.2 g
Distilled water	- 1000 ml

Solution B

Dibasic sodium phosphate - $12H_2O$)	- 35.8 g
Distilled water	- 1000 ml

Working solution

Solution A	- 5.0 ml
Solution B	- 94.7 ml
Distilled water	- 100.3 ml

The working solution was sterilized at 15 lbs for 15 min and stored at 4°C till further use.

A6. Phosphate buffer saline - PBS

Solution A

Monobasic sodium phosphate $2H_2O$	- 31.2 g
Distilled water	- 1000 ml

Solution B

Dibasic sodium phosphate - $12H_2O$	- 35.8 g
Distilled water	- 1000 ml

Solution C

Sodium chloride	- 8.0 g
Distilled water	- 1000 ml

Working solution - PH - 6.4

Solution A	- 73.5 ml
Solution B	- 26.5 ml
Solution C	- 100 ml

Working solution - PH 7.0

Solution A	- 39.0
Solution B	- 61.0 ml
Solution C	- 100 ml

Working solution pH 7.2

Solution A	- 28.0 ml
Solution B	- 72.0 ml
Solution C	- 100 ml

The working solutions were sterilized at 15 lbs for 15 min and stored at 4°C till further use.

A7. Reaction mixture - for stabilization of sheep RBC with gluteraldehyde

Neutral Gluteraldehyde*	- 1.5 ml
Normal saline	- 5.0 ml
Phosphate buffer (pH 8)	- 1.0 ml
Sheep RBC - 50% Suspension	- 1.0 ml

The reaction mixture was stored at 4°C for 24 hours with occasional stirring.

* pH Gluteraldehyde was adjusted to seven using 10% sodium carbonate solution.

A8. Tris hydrochloride buffer

Solution A

Tris	- 24.2 g
Distilled water	- 1000 ml

Solution B

Hydrochloric acid	- 7.2 ml
Distilled water	- 1000 ml

Working solution

Solution A	- 50 ml
Solution B	- 44.2 ml
Distilled water	- 105.8 ml

The solution was freshly prepared before use.

Bacteriological culture and biochemical test media

A9. Brucella Blood agar with antibiotics (for primary isolation)

Basal medium

Brucelle agar (Difco) - 43.0 g

Distilled water - 1000 ml

Brucella agar was dissolved in distilled water by boiling and the pH was adjusted in 7.2 and sterilized at 15 lbs for 15 min.

B. Sheep blood

Sterile defibrinated sheep blood - 100 ml

C. Antibiotic supplement

1. Campylobacter antimicrobial supplement - B (Difco)

" one vial was diluted with 5 ml of sterile distilled water and added to 500 ml of the prepared Brucella Blood Agar at 50°C.

Complete media

The sterilized molten basal medium (1000 ml) was held at 50°C in a water bath. When the temperature was reduced to 50°C, sterile defebrinated blood (100 ml) and two vials of Difco Campylobacter antimicrobial supplement were added.

After thorough mixing, the medium was poured into sterile petri dishes and checked for sterility by overnight incubation at 37°C. The prepared medium was stored at 4°C and used within seven days.

A10. Brucella Blood Agar (BBA)

Preparation of Brucella Blood Agar was similar to the selective media used in the primary isolation of C. jejuni exept for the antibiotic supplement.

A11. Hydrogen peroxide (3%)

Hydrogen peroxide (20 vol.) 6%	- 5 ml
Steriled distilled water	- 5 ml

Hydrogen peroxide stored at 0°C was thawed and 5 ml was mixed with equal quantity of sterile distilled water. The solution was freshly prepared before use.

A12. Bile Agar

Brucella Broth (Hi-media)	- 2.9 g
Agar (Difco)	- 1.0 g
Bile (Bacto ox gall)	- 1.0 g
Distilled water	- 100 ml

The ingredients were dissolved and sterilized at 15 lb for 15 min and distributed in five ml quantities in sterile

test tubes and slants were made. Sterility was checked and stored at 4°C.

A13. Glycine Agar

Brucella Broth (Hi-media)	-	2.9 g
Agar (Difco)	-	1.0 g
Glycine	-	1.0 g
Distilled water	-	100 ml

The medium was sterilized at 10 lb for 45 min. and distributed in five ml quantities in sterile test tubes and slants were made. After sterility check, stored at 4°C.

A14. Media for H_2S production - By Lead acetate strips

Brucella broth (Hi-media)	-	2.9 g
Agar (BDH)	-	1.0 g
L-cystine	-	20 mg
Distilled water	-	100 ml

The medium was sterilized at 10 lb for 45 minutes and distributed is five ml quantities in sterile test tubes and stored at 4°C till use.

A15. Sodium chloride agar

Brucella broth	-	2.9 g
Agar	-	1.0 g
Sodium chloride	-	3.5 g
Distilled water	-	100 ml

The medium was sterilized at 15 lb for 15 minutes and distributed is five ml quantities is sterile test tubes and stored at 4°C.

A16. Triple sugar iron agar (TSI)

Dehydrated media from M/s Hi-media was used as per manufacturers recommendation.

A17. Soyabean casein digest agar (TSA Hi-media)

Dehydrated media from M/s Hi-media Laboratory Ltd., Bombay was purchased and used.

Tryptone	-	15 g
Soyabean protein	-	5 g
Sodium chloride	-	5 g
Agar	-	15 g

The media was rehydrated by dissolving 40 g/1000 ml, boiled to dissolve completely, sterilized by autoclaving at 15 lbs pressure for 15 min and poured onto sterile petri-dishes.

A18. Antibiotic Discs

Antibiotic discs were purchased from M/s Himedia Laborataries Ltd., Bombay. Ampicillin, chloramphenicol, erythromycin, furazolidone, gentamycin, nalidixic acid, oxytetracycline, penicillin G, streptomycin, sulphadiazene were the antibiotic discs used in this study.

CHARACTERIZATION OF *Campylobacter jejuni* ISOLATED FROM PIGS AND MAN

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

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ABSTRACT

Twenty six rectal swabs collected from piglets below two months of age with diarrhoea/enteritis when culturally screened yielded 11 isolates of *Campylobacter jejuni*.

Thirty two rectal swabs from children below five years of age with diarrhoea/enteritis when processed for the isolation of etiological agent, resulted in the recovery of one strain of *C. jejuni*.

Sonicated *C. jejuni* antigen prepared from the selected strains of *C. jejuni* from porcine and human origin were found suitable for sensitization of gluteraldehyde stabilized sheep RBC for serum antibody monitoring. The sonicated antigen retained its affinity to sheep RBC even after six months of storage at -60°C. Gluteraldehyde stabilization was found to be a suitable method for the preservation of SRBC. Gluteraldehyde stabilized SRBC stored at 4°C upto a period of three months did not show any reduction in the sensitization property, as evidenced from the PHA titre.

One hundred and fifty microlitres of *C. jejuni* antigen whose protein concentration adjusted to 2 mg/ml was found to be the optimum level for sensitisation of a suspension made from three millilitres of packed SRBC.

The optimum level of SAPA cells required in the seromonitoring of _C. jejuni_ specific antibodies by SAPA-AMHA test was found to be 0.1 per cent.

Cross titration assay employing homologous and heterologous antigens of _C. jejuni_ and the hyperimmune sera indicated that there was antigenic relationship between porcine and human strains. The results also point to the probable prevalence of different antigenic components in varying proportions in different strains from various sources.

In the present investigation, 50 serum samples from pigs were monitored for the presence of _C. jejuni_ specific antibodies by PHA and SAPA-AMHA. PHA detected 48 per cent cases as positives compared to 54 per cent by SAPA-AMHA.

An attempt was also made in this study to titrate 50 human serum samples for _C. jejuni_ specific antibodies by PHA and SAPA-AMHA. PHA detected _C. jejuni_ antibodies in 18 per cent compared to 38 per cent of SAPA-AMHA.

Statistical analysis clearly indicated that SAPA-AMHA is superior to PHA in screening _C. jejuni_ specific antibodies. The result of the present study indicated that SAPA-AMHA test could advantageously replace the conventional PHA for serological diagnosis of animal and human Campylobacteriosis.

Efforts were also made in this study to find out the

change in PHA titre with homologous and heterologous antigens employing sera before and after adsorption with unsensitized SRBC to remove the non specific antibodies.

Statistical analysis of the results showed that for performing PHA, homologous antigen should be preferred over heterologous antigen and when homologous antigen were used, adsorption of sera with stabilized unsensitized SRBC had no significant effect of PHA titre.

Attempts were also made to study the _in vitro_ antimicrobial sensitivity patterns of the 12 _C. jejuni_ isolates by standard disc diffusion technique. The results indicated all the _C. jejuni_ isolates were sensitive to chloramphenicol, gentamicin and nalidixic acid, irrespective of their source/origin. Eventhough none of the _C. jejuni_ isolated in this study was resistant to erythromycin, only 83.3 per cent of the isolates showed a sensitive zone of inhibition,while, 16.7 per cent showed an intermediary zone of inhibition. _C. jejuni_ isolates recorded 83.3 per cent sensitivity to furazolidone, 75 per cent to streptomycin and 58.3 per cent to ampicillin. Fifty per cent of the isolates showed resistance to oxytetracycline. Of the ten antibiotics tested, 16.6 per cent of the organism were sensitive to penicillin. _C. jejuni_ recorded highest resistance to sulphadiazine as only 8.3 per cent of the organisms were sensitive to sulphadiazine.