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EPIDEMIOLOGY OF PORCINE ROTAVIRAL INFECTIONS

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OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY IN VETERINARY VIROLOGY AT MASSEY UNIVERSITY

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Abstract

The epidemiology of porcine group A rotavirus was studied in commercial herds of pigs in New Zealand.

A commercial enzyme-linked immunosorbent assay (ELISA) kit (Dakopatts, Copenhagen) was found to be highly sensitive (100%) and specific (96.8%) for the detection of group A rotavirus in pig faeces when compared to electron microscopy (EM). A highly sensitive and specific ELISA test for the measurement of antibody against group A rotavirus in pigs was also developed.

Of 521 faecal samples collected from Massey University Piggery in a cross-sectional survey, 47 (9%) were positive for group A rotavirus by the ELISA test. Only sucking (19.3%) and weaner (14.4%) pigs were shedding rotavirus. Rotavirus was not detected in the faeces of fatter pigs (over two months old) or adult pigs including sows and boars.

Three cohort studies revealed that all pigs became infected with group A rotavirus before they were 40 days of age and shed the virus for an average of eight days. Some of the piglets shed rotavirus a second time approximately 10 days after the first period of shedding. All piglets ceased to shed the virus by two months of age.

Rotaviral shedding was associated with the occurrence of milk scours in sucking piglets. Diarrhoea in weaner pigs (post-weaning diarrhoea, PWD) was more closely associated with the presence of haemolytic E.coli than with rotavirus in faeces.

Infection with group A rotavirus was transmitted from piglet to piglet and from litter to litter. In one cohort of 50 piglets from five litters, shedding of rotavirus was first detected in one litter, then in the second litter two days later, and finally in other litters of piglets. Over a period of 16 days, all piglets in the five litters were infected.

Group A rotavirus was also detected in dust, faeces and effluent collected from the farrowing and weaner houses, and from a weaner house which had not been used for three months.

Rotavirus was not detected in any of the sows (11) during the period of investigation. Neither was rotavirus detected in fatter pigs (from two months of age to the time of slaughter), nor in the environments where old pigs (fattener and sow houses) were housed. It was not therefore possible to confirm that adult pigs, especially sows, act as carriers for rotaviral infection of young piglets as has been suggested by other workers.

All the piglets acquired maternal antirotaviral antibody from their dams and the levels of antibody in piglets' sera were comparable to those in the colostrum of their dams. The maternally-derived antibody was also detected in piglets' faeces. Antibody in sera and in faeces declined rapidly after birth. Rotaviral shedding commenced in each of the cohorts when the geometric mean ELISA antibody titre fell below 1/1600 (equivalent to serum neutralizing antibody titre of 1/8 to 1/16). However, this correlation between antibody titres and protection was not observed in individual litters. In each of the cohorts studied, rotaviral shedding was usually detected initially in one or two piglets of a litter. The infection then spread to other piglets within the same litter and, finally, to piglets of other litters in the same group. Onset of rotaviral infection in particular litters was related to their location in the farrowing unit rather than to the levels of antibody.

The shedding pattern of group A rotavirus was studied further in another five New Zealand piggeries and was found to be similar to that observed at the Massey University Piggery.

Faecal samples from these piggeries were analyzed by polyacrylamide gel electrophoresis (PAGE) and no common electrophoretype of group A rotavirus was found in these piggeries. More than one electrophoretype of group A rotavirus was detected in three of these piggeries. Faecal samples collected from Massey University Piggery were also analyzed by PAGE and it was found that rotaviruses detected during the first two years of the present investigation had identical electrophoretotypes, but one isolate detected in the third year had a distinctively different pattern.

Non-group A rotaviruses were also detected for the first time in New Zealand. Nine samples had an electrophoretic pattern similar to that of group C rotavirus and one was similar to that of group B rotavirus. One of the samples containing group C rotavirus was from two litters of piglets with diarrhoea at two to four days of age. In one of

these piggeries, five electrophoretotypes of rotaviruses representing three groups were detected.

These observations indicate that rotaviral infections are important causes of milk scours in piglets, and are probably significant in exacerbating PWD. The epidemiology of rotaviral infections is complicated by a number of factors. These include the continuous transmission of virus from pig to pig and from litter to litter, the survival of the virus in the piggery environment, the incomplete protection afforded by maternally-derived antibody, and the simultaneous circulation of different strains and different groups of rotavirus in one piggery.

STATEMENT

This is to certify that the work on which this thesis is based has not been accepted in whole or in part for any other degree or diploma, and was carried out by the undersigned. Assistance received is specifically recorded in the Acknowledgments section bound with the thesis.

A handwritten signature in black ink, appearing to read 'Zhen Fang Fu' in a cursive style.

Zhen Fang Fu

(31 October 1988)

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Chapter One

LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

In the late 1960's, a new virus associated with neonatal calf diarrhoea was discovered by electron microscopy (EM) (Mebus et al. 1969). This virus was then called neonatal calf diarrhoea virus (NCDV). In 1973, a similar virus was observed under the EM in a biopsy of duodenal mucosa (Bishop et al. 1973) and in faeces (Flewett et al. 1973) from children with acute gastroenteritis. Subsequently, the virus was found associated with diarrhoea of the young in a variety of mammalian and avian species, including mice (Much and Zajac 1972), monkeys and sheep (Els and Lecatsas 1972), pigs (Rodger et al. 1975a), foals (Flewett et al. 1975), lambs (McNulty et al. 1976b), deer (Tzipori et al. 1976), rabbits (Bryden et al. 1976), pronghorn antelope (Reed et al. 1976), goats (Scott et al. 1978), apes (Ashley et al. 1978), impala, Thompson's gazelles, and addox (Eugster et al. 1978), dogs (Eugster and Sidwa 1979), kittens (Snodgrass et al. 1979), chickens (Jones et al. 1979), turkeys (McNulty et al. 1978), bison, ducks and parrots (Flewett and Woode 1978) and ferrets (Torres-Medina 1987).

Because these newly-discovered viruses morphologically resembled reovirus and orbivirus (Bishop et al. 1973, Fernelius et al. 1972, Flewett et al. 1973), they were at first frequently referred to as "reovirus-like agents" (Fernelius et al. 1972, Kapikian et al. 1976, Rodger et al. 1975a). Other terms such as neonatal (Nebraska) calf diarrhoea virus (NCDV) (Fernelius et al. 1972, White et al. 1970), infantile gastroenteritis virus (orbivirus group) (Petric et al. 1975), and duovirus (Davidson et al. 1975) were also used. Flewett et al. (1974a) were the first to propose the term rotavirus (from the Latin rota, a wheel) for these viruses. This proposal quickly gained support by other workers (Sharpee and Mebus 1975, Thouless et al. 1977, Woode et al. 1976a). Eventually in 1978, the International Committee on Taxonomy of Viruses adopted their proposal and classified these morphologically identical viruses into the genus Rotavirus under the family Reoviridae (Matthews 1979).

Rotaviral infections have important impact on human and animal

health and welfare. In man, it has been estimated that there are approximately 500 million episodes of diarrhoeal disease each year in the less developed countries of Africa, Asia and Latin America and that 1-4% of these are fatal, thus accounting for 5 to 18 million childhood deaths annually (Rohde and Northrup 1976). Studies in these countries have indicated that rotavirus is the leading cause of severe diarrhoeal disease in children. In the Philippines, Adkins et al. (1987) reported that diarrhoea in about 40% of patients under the age of five was associated with rotaviral infection. Black et al. (1980) also isolated rotavirus from 59% of patients less than two years of age in Bangladesh. It is thus likely that a portion of the mortality from severe diarrhoeal disease is accounted for by rotaviral infection. In Indonesia alone, Soenarto et al. (1981) estimated that 200,000 of 500,000 annual deaths due to diarrhoea in children less than five years old might be associated with rotaviral infection. In developed countries, mortality associated with rotaviral infection is infrequent (Carlson et al. 1978, Davidson et al. 1975), but morbidity is high. In the USA, it was estimated that one in 272 children between 0-12 months of age and one in 451 children between 13-24 months of age required hospitalization due to diarrhoeal illness associated with rotaviral infection (Rodriguez et al. 1980). This accounts for 220-370 hospitalizations per 100,000 children less than two years old. Based on this rate, an estimated 22,000 children under two years of age would require hospitalization in the USA for rotaviral diarrhoea. In animal populations, morbidity from rotavirus associated diarrhoea can reach 50% and mortality 10% in herds of pigs or calves (Bohl et al. 1978, Twiehaus et al. 1975).

Although rotaviral particles were first observed under EM in 1969 (Mebus et al. 1969), the implication of rotaviruses in infantile diarrhoea goes back to the early 1940's. Light and Hodes (1943) isolated a filtrable virus from stools of infants with acute gastroenteritis. When the filtrates were inoculated into newborn calves, diarrhoea occurred in these animals two days after inoculation. A faecal specimen from these early experiments that had been lyophilized and stored since 1943 was examined under the EM in 1974. Typical rotavirus particles with characteristic wheel-like morphology were observed in this 32 year old sample. However, this isolate was no longer infectious (Hodes 1977).

Also in the 1940's, epizootic diarrhoea of sucking mice (EDIM) was reported and cytoplasmic inclusion bodies were found in the intestinal epithelial cells of infected mice (Cheever and Mueller 1947,

Pappenheimer and Cheever 1948). It was latter established that EDIM was caused by a heat- and ether-resistant virus with a diameter of 65-75 nm (Kraft 1957, Adams and Kraft 1963). In 1958, a virus called simian agent 11 (SA11) was isolated from a rectal swab from a vervet monkey and was readily passaged in primary vervet monkey kidney cell culture with cytopathic effect (CPE) (Malherbe and Strickland-Cholmley 1967). Further characterization demonstrated that these viruses are also rotaviruses (Els and Lecatsas 1972, Lecatsas 1974, Much and Zajac 1972).

However, it was not until rotaviral particles were found in the faeces of children with diarrhoea (Bishop et al. 1973, Flewett et al. 1973) that extensive research into the characterization of rotaviruses, and investigation of the epidemiology and immune response of rotaviral infections in different species was conducted. That research has established that rotavirus comprise a group of viruses which are morphologically identical but antigenically diverse. On the basis of cross-reactivity of group antigen, genome profiles by polyacrylamide gel electrophoresis (PAGE) and one-dimensional terminal fingerprinting, most rotaviruses isolated to date can be classified into one of the following groups: A, B, C, D, E, F and possibly G (Bridger 1987, Pedley et al. 1983, 1986). Among these six groups, group A rotavirus has been the most studied and this review has an emphasis on this group.

1.2 CHARACTERISTICS OF GROUP A ROTAVIRUS

1.2.1 Rotaviral morphology

When samples are negatively-stained and examined under the EM, intact rotaviral particles show a distinct morphological appearance resembling a wheel with the core forming a hub, capsomeres radiating from the core forming spokes, and the precisely margined outer layer forming the rim (Flewett et al. 1974a). This distinguishes them morphologically from reoviruses and orbiviruses (Flewett et al. 1973, Kalica et al. 1978, Middleton et al. 1974). Like other members of the Reoviridae, the non-enveloped rotaviruses have a double-shelled structure composed of an outer and an inner capsid, and an icosahedral core. The double-shelled particles have a very definite edge ('smooth') whereas single-shelled particles could be described as having a rough, diffuse border ('rough') (Bridger and Woode 1976). Estimates of the size of the virion varies from 55-60 nm (rough particles) to about 70-75 nm (smooth particles) in diameter (Els and Lecatsas 1972, Martin et al.

1975, Saif et al. 1978). When measured against catalase crystal as the internal calibration standard, complete rotavirus is 67-68 nm in diameter (Palmer et al. 1977).

The inner capsid consists of capsomeres that radiate from an icosahedral core approximately 38 nm in diameter (Palmer et al. 1977, Saif et al. 1978). Many models of the arrangement of these capsomeres have been proposed (Kogasaka et al. 1979, Martin et al. 1975, Roseto et al. 1979, Stannard and Schoub 1977). It appears that the model proposed by Stannard and Schoub (1977) gained more support (Esparza and Gil 1978). The inner capsid of rotavirus has icosahedral symmetry and consists of 180 morphological units arranged in an open lattice formation with 12 spaces at the apices being surrounded by 5 capsomeres and the other 80 spaces by 6 capsomeres. The outer layer of rotavirus is composed of T-shaped capsomeres attached directly to the ends of the capsomeres of the inner capsid (Flewett et al. 1973, 1974b). These capsomeres of the outer capsid are similar in shape to "pushpins" and are covered by a thin layer of protein or glycoprotein (Palmer and Martin 1982).

1.2.2 Rotaviral genome

Early studies with rotaviruses from bovine, murine and human species established that these viruses contained double-stranded RNA (Much and Zajac 1972, Petric et al. 1975, Rodger et al. 1975b, Welch 1971). Subsequently, the genomes of rotaviruses isolated from other species were found to be the same (Todd and McNulty 1976, 1977). Analysis of rotaviral RNA from different animals by PAGE has clearly established that the double-stranded RNA is segmented and composed of 11 pieces (Fig. 1-1) (Rodger et al. 1975b, Newman et al. 1975, Todd and McNulty 1976, Kalica et al. 1976). These 11 RNA segments can be divided into four size-classes (groups I-IV) on the basis of their mobility (Kalica et al. 1976, Barnett et al. 1978).

Rotaviral RNA has a total molecular weight (MW) of $11 - 12 \times 10^6$ with the 11 segments ranging from 2.2×10^6 to 0.2×10^6 (Newman et al. 1975, Rodger et al. 1975b, Verly and Cohen 1977). The MW of the 11 segments varies slightly between isolates from different host species or different isolates from the same species (Rodger et al. 1975b, Verly and Cohen 1977). For the representative rotavirus (NCDV), the MW of its 11 segments is 2.18, 1.73, 1.64, 1.48, 0.94, 0.77, 0.50, 0.50, 0.50, 0.29 and 0.22×10^6 , respectively (Barnett et al. 1978).

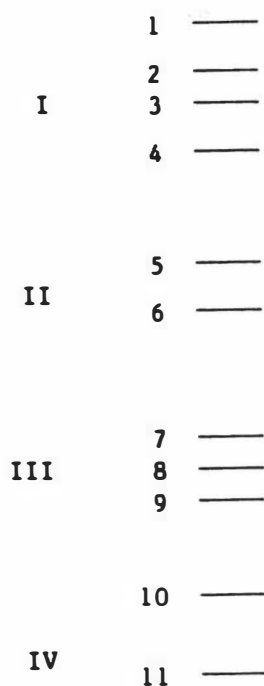


Fig. 1-1 Schematic RNA pattern of a typical group A rotavirus on PAGE.

The sequences, protein-coding assignments and functions of rotaviral RNA segments have been extensively studied (Arias et al. 1984, Both et al. 1983, Cohen et al. 1984, Dyall-Smith and Holmes 1981a, b, Espejo et al. 1981, Glass et al. 1985, Liu et al. 1988, Lopez et al. 1985, Mason et al. 1980, 1983, Richardson et al. 1984). Due to different methodologies, especially different PAGE systems employed, early studies have yielded discrepancies in regard to nomenclature, gene-coding assignment and numbers (5-13) of polypeptides. These discrepancies have been summarized in detail by Estes et al. (1983) and Holmes (1983). It is now believed that the 11 segments of rotaviral RNA code for 11 primary gene products. The 11 RNA segments of SA11, together with the numbers of base pairs, protein-coding assignments, and functions are summarized in table 1-1. In this table, the nomenclature of the 11 polypeptides is derived from the works of Mason et al. (1983) and Liu et al. (1988). Seven viral structural proteins are termed as VP followed by numbers, and the four non-structural proteins as NS followed by MW.

Table 1-1. The 11 segments of rotavirus, their functions and protein coding assignments

Segment No.	Base pair	Primary product (MW)	Final Viral polypeptide	Function	References ^a
1	ND ^b	125	VP1	Not known	
2	ND	94	VP2	Not known	
3	ND	88	VP3	Virion-associated polymerase	Liu et al. 1988
4	2359	88	VP4 ^c	Virulence, host-range specificity, neutralization, haemagglutination, protease-enhancement of plaque formation, trypsin cleavage for cultivation and infectivity, and restriction of human rotavirus in tissue culture	Espejo et al. 1981 Flores et al. 1984 Greenberg et al. 1981 Greenberg et al. 1984 Kalica et al. 1983 Offit and Blavat 1986 Offit et al. 1986a, b
5	ND	53	NS53	Not known	
6	1356	41	VP6	Common group and subgroup specificities	Thouless et al. 1977 Kalica et al. 1981b
7	ND	34	NS34	Not known	
8	1062	37	VP7	Major neutralization specificities	Greenberg et al. 1983a
9	ND	35	NS35	Not known	
10	ND	29	NS29	Not known	
11	ND	26	VP9	Not known	

^a References given in this table are related to the functions of these polypeptides. Other references in relation to the sequence, protein-coding assignment are given in the text.

^b Not done.

^c The product of gene 4 is still referred to as VP3 in the rest of the thesis to maintain consistency with the terminology used in most of the literature up to 1988.

1.2.3 Biophysical and biochemical properties of rotavirus

Caesium chloride density gradient centrifugation indicates that rotaviruses have buoyant densities of 1.36 g/ml and 1.38 g/ml, corresponding to the double- and single-shelled particles (Bridger and Woode 1976, Cohen et al. 1979, Rodger et al. 1977, Tam et al. 1976). The two types of particles have a sedimentation coefficient of 500-530S (Newman et al. 1975, Petric et al. 1975) and 380-400S (Tam et al. 1976), respectively. The icosahedral core (Palmer et al. 1977) has a buoyant density of 1.44 g/ml in CsCl and a sedimentation coefficient of 280S (Bican et al. 1982).

As enteric viruses, rotaviruses would be expected to be relatively resistant to a low pH and proteolytic enzymes. Indeed, the viruses were found to be stable at pH values between 3 and 11 (Meng et al. 1987, Palmer et al. 1977, Weiss and Clark 1985). Values below 3 and above 11 have resulted in inactivation of rotavirus infectivity, collapse of the outer shell and changes in capsomere morphology. The inactivation of rotavirus at extreme pH values is temperature-related. The half life of a bovine rotavirus is 5.2 min, 30 min and over 24 hours when tested at pH 3 at 37°C, 23°C and 4°C, respectively (Weiss and Clark 1985). Differences in resistance to acids between different strains of rotavirus have been reported by Meng et al. (1987). Human rotavirus was completely inactivated at pH 2 while SA11 was only partially inactivated. Proteolytic enzymes such as pancreatin, trypsin, elastase, pepsin or papain do not inactivate rotavirus. Conversely these enzymes are found to enhance the infectivity of rotavirus (Estes et al. 1979, Palmer et al. 1977, Steel and Torres-Medina 1984), and treatment with these enzymes has been required for successful cultivation of rotaviruses in cell culture (see section 1.2.5).

The resistance of rotaviruses has been tested to a range of disinfectants commonly used in hospitals (Kurtz et al. 1980, Meng et al. 1987, Sattar et al. 1983, Tan and Schnagle, 1981, 1983). Rotavirus was found to be inactivated by the following types of disinfectants: alcohol (70-90% alcohol and 70% isopropylalcohol (Sana Rinse)); aldehyde (8% formaldehyde and 2% acid glutaraldehyde (Cidex)); halogens (Proviiodine and 1% sodium hypochlorite (Milton)); phenolic compounds (2% phenol, 2% lysol) and surface active agents (quaternary ammonium (Zephiran) and amphoteric amino acid (Consept D)). Some of the synthetic phenolics, such as Biogram, Septisol, Hexol and Savlon, are effective in disinfecting rotavirus; while others, such as D.R.X.,

Germicidal, Hibitane, and Hibiclens, are not. Ether, chloroform and iodophor (Wescodyne) have no effect on the infectivity of rotaviruses.

Rotaviruses are inactivated by exposure to ultra violet (UV) light for 15 min, by heating at 56°C for 90 min or 80°C for one min (Meng et al. 1987), by Pasteurization (63°C for 30 min) (Woode and Bridger 1975) and by treatment with chlorine, chlorine dioxide, ozone and peracetic acid (Harakeh and Butler 1984). The latter authors found that human rotavirus was less sensitive than SA11 to inactivation by the agents investigated. Lyophilization, freezing and thawing, and sonication do not reduce rotavirus titres appreciably (Estes et al. 1979, Meng et al. 1987). Magnesium sulphate (2M) has been found to stabilize the infectivity of rotavirus when heated at 50°C, but $MgCl_2$ (2M) fails to do so (Estes et al. 1979). At pH values above 10, the infectivity of rotavirus is reduced by treatment with low concentrations (5mM) of the chelating agents ethylenediamine tetra-acetic acid (EDTA) or ethyleneglycobis (B-aminoethyl-ether)-N, N'-tetra-acetic acid (EGTA), or with high concentrations (2M) of $CaCl_2$. However, lower levels (0.15 - 1.5mM) of $CaCl_2$ have been found to stabilize rotavirus infectivity (Shirley et al. 1981) apparently by maintaining virion integrity. These authors found that the loss of infectivity when treated with EDTA or EGTA coincided with the removal of the outer capsid layer which is essential for infectivity (Bridger and Woode 1976, Elias 1977).

1.2.4 Cultivation of rotavirus in vitro

The simian rotavirus SA11 has been propagated in cell culture since the 1960s (Malherbe and Strickland-Cholmley 1967). In the 1970s, bovine rotaviruses (Fernelius et al. 1972, McNulty et al. 1976a, Mebus et al. 1971) and a porcine rotavirus (Woode et al. 1976a) were adapted with difficulty to grow on kidney cultures from cattle and pigs, respectively. Early attempts to culture human rotavirus in pig kidney cells (Banatvala et al. 1975) and in bovine foetal intestinal cells (Albrey and Murphy 1976) resulted in only limited success. The exception was the report by Wyatt et al. (1980) of the successful cultivation of a human rotavirus strain (Wa) in African green monkey kidney cells after the virus was serially passaged 10 times in newborn gnotobiotic piglets.

A major breakthrough for rotavirus cultivation in vitro came when proteolytic enzymes such as pancreatin and trypsin were used to treat virus samples before inoculation and/or their incorporation into the

maintenance medium (Babiuk et al. 1977, Theil et al. 1977). This led to the successful cultivation of rotaviruses from man (Schoub et al. 1979), pigs (Theil et al. 1977), cattle (Babiuk et al. 1977), horses (Hoshino et al. 1983) and avian species (McNulty et al. 1979). More success with cultivation of human rotaviruses was achieved by using cells in tubes incubated as rolling cultures, and with trypsin also being incorporated into maintenance medium (Sato et al. 1981, Urasawa et al. 1981). Rotaviral CPE consists of increased cytoplasmic granularity, rounding of cells, obscuring of cell boundaries and eventually detachment of cells from plastic walls.

Although proteolytic enzymes such as trypsin are required by many rotaviruses for their propagation in cell cultures, other rotaviruses seem to be less dependent. These include SA11 and some of the bovine rotavirus isolates described earlier in this section as well as feline and canine rotavirus (Hoshino et al. 1981, 1982) and some human rotaviruses (Agliano et al. 1985).

1.2.5 Detection of rotaviruses

Cultivation of rotaviruses was initially limited and could not be used as a diagnostic tool, but now, with the use of proteolytic enzymes such as trypsin, cultivation of these viruses has become possible. However, not all rotaviruses in faeces can be adapted to grow in cells, with or without trypsin (Agliano et al. 1985, Albert and Bishop 1984, Albert et al. 1987a). Even those which have been adapted usually required three to ten passages before CPE appeared (Agliano et al. 1985, Albert and Bishop 1984, Hoshino et al. 1983, Sato et al. 1981). Therefore cultivation of rotavirus in vitro still cannot be used for routine detection of the viruses, and other methods have therefore been developed for their detection. In this section only the four commonly used techniques are reviewed.

Electron microscopy (EM): Although a viral aetiology of gastroenteritis in humans and other animals was suspected as early as the 1940's (Light and Hodes 1943, Cheever and Mueller 1947), it was not until 1969 that rotavirus was discovered by EM in faecal filtrates from diarrhoeic calves (Mebus et al. 1969). Subsequently rotaviruses from other species including man (Bishop et al. 1973, Flewett et al. 1973) and pigs (Rodger et al. 1975a) were also discovered by EM. For many years thereafter, the EM technique, with or without use of specific antisera, was almost the only means for demonstration of rotavirus in

diarrhoeic faeces from different species (Bishop et al. 1974, Flewett et al. 1974b). The EM technique has also been widely used as a standard "bench mark" for the development of other systems for detection of rotavirus (Brandt et al. 1981, Cheung et al. 1982, Gerna et al. 1987b, Goyal et al. 1987, Grauballe et al. 1981). Even though other diagnostic systems have now been widely available for rotavirus detection (see below), EM remains one of the major diagnostic techniques throughout the world.

Polyacrylamide gel electrophoresis (PAGE): Rotaviral RNA consists of 11 double stranded segments which can be separated by electrophoresis on agarose or polyacrylamide gels (see Fig. 1-1). These techniques have been employed for taxonomic and epidemiological studies and have proved very useful for detecting variations in rotaviral RNA isolated from different animal species or from different individuals of a given species (Cash et al. 1982, Espejo et al. 1979, Kalica et al. 1976, 1978, Rodger et al. 1979, Smith and Tzipori 1979). This technique was not regularly used for diagnosis because the procedure, used to purify the virus from faeces or from tissue culture preparation, was tedious and time consuming. In 1982, Herring et al. (1982) developed a method for direct extraction of rotaviral RNA from faeces without further purification, and the use of silver nitrate to stain the RNA on gel. This procedure dramatically reduced the time and effort required to prepare rotaviral RNA. Since then the technique has become a standard procedure for detection and comparison of rotaviruses from faecal specimens and cell cultures (Estes et al. 1984). It can not only detect the presence and variation of group A rotavirus but also distinguish group A from non-group A rotaviruses.

Enzyme-linked immunosorbent assay (ELISA): The ELISA for detection of rotavirus in faeces from man and other animals was first developed by Yolken et al. (1977a, b, 1978a). This test is a sandwich ELISA in which goat anti-human rotaviral antibody was used as catching antibody. The test was found to be easier and more sensitive than EM, and more samples could be handled at one time. Later, however, it was found that this ELISA system gave many false-positive results since in one study (Brandt et al. 1981) 73% of 1834 samples which were presumptively positive for rotavirus by the ELISA were found to be false-positives on the basis of EM, IEM, blocking ELISA and confirmatory ELISA tests. These false-positive reactions were eliminated when this ELISA test was further modified by including a control of rotavirus-negative capture antibody (preimmunization) from the same animal in which anti-human

rotavirus antibody was prepared. Subsequently ELISA tests with similar procedures were developed and used all over the world and became the most commonly used test for the diagnosis of rotaviral infections (Askaa and Bloch 1981, Beards et al. 1984, Coulson and Holmes 1984, Cromien et al. 1987, Grauballe et al. 1981, Hammond et al. 1982). Some of the assays and the reagents used have become commercially available and have been found to be very sensitive and specific. Comparison between two or more of these ELISA tests revealed similar ranges of sensitivity and specificity (Cromien et al. 1987, Miotti et al. 1985, Yolken and Leister 1981).

Latex agglutination (LA) test: The LA test for the detection of rotavirus in faeces was first reported by Sanekata et al. (1981) and it was found to agree completely with the results from EM on 48 samples. Haikala et al. (1983) also developed a latex agglutination test which showed a sensitivity of 92% and a specificity of 95%. Subsequently many LA tests have also become commercially available. Investigations of different LA test kits have revealed that the specificity of all the tests was high (98-100%) whereas the sensitivity varied from 46-92% (Goyal et al. 1987, Haikala et al. 1983, Hammond et al. 1984, Knisley et al. 1986, Miotti et al. 1985, Pai et al. 1985). The wide range of sensitivity of these LA tests is due to the time of collection relative to the onset of symptoms. Nevertheless, the test is simple, rapid, and inexpensive and it is especially useful for clinical practitioners in small hospitals, emergency wards and small clinics for rapid diagnosis of rotaviral infections (Pai et al. 1985).

1.3 THE EPIDEMIOLOGY OF GROUP A ROTAVIRAL INFECTIONS

In this section the epidemiology of group A rotaviral infections is discussed in relation to three factors; the host, the virus and the environment.

1.3.1 Hosts

Group A rotaviruses have been detected in a number of species including man and domestic animals (see section 1.1). In this section only the infections in man and certain domestic animals are reviewed.

Man: Since Bishop et al. (1973) first identified rotavirus in faeces from children with diarrhoea, rotavirus has been established as

the leading cause of infantile gastroenteritis throughout the world (Anon 1975). Children of six to 24 months of age are most commonly affected (Adkins et al. 1987, Birch et al. 1977, Bryden et al. 1975). The rate of recovery of rotavirus from diarrhoeic children varies from 25% (Espejo et al. 1979) to over 60% (Konno et al. 1978, Nakagomi et al. 1985), but most investigators reported that nearly half of the children under five years of age that are hospitalized for gastroenteritis shed the virus (Birch et al. 1977, Black et al. 1980, Bryden et al. 1975, Holdaway et al. 1982, Kapikian et al. 1976, Persson et al. 1982, Stals et al. 1984).

Symptoms in patients infected with rotavirus may include diarrhoea and vomiting accompanied by fever and mild-to-moderate dehydration (Hieber et al. 1978, Konno et al. 1978, Lewis et al. 1979, Rodriguez, et al. 1979, 1987). Severe dehydration and deaths may also occur, especially in developing countries where the condition is exacerbated by malnutrition, unsanitary environments and inadequate medical care (Black et al. 1980, Soenarto et al. 1981). Usually, the diarrhoea is self limiting, lasting for two to eight days (Foster et al. 1980, Lewis et al. 1979, Shephard et al. 1975). Those who develop severe dehydration should be treated early by oral or intravenous methods of rehydration (Black et al. 1980). Oral rehydration with glucose or sucrose based electrolyte solutions, especially the formulae originally developed by WHO for patients with diarrhoea due to cholera, have been very successful (Black et al. 1980, Persson et al. 1982, Taylor et al. 1980).

Respiratory symptoms such as coryza, cough and otitis media have been observed in rotavirus infected patients (Hieber et al. 1978, Holdaway et al. 1982, Lewis et al. 1979) and the virus has also been detected in nasopharyngeal swabs and in tracheal aspirates from four patients with pneumonia (Santosham et al. (1983). However, the association of rotaviral infection with the respiratory symptoms is not clear (Goldwater et al. 1979, Persson et al. 1982).

Although children between six and 24 months old are most commonly infected with group A rotavirus, there have been reports that newborn babies can also be infected. However, the rates of detection in neonates varied from different nurseries. In some nurseries, nearly half or all of the newborn babies were found to shed rotavirus within the first week of life (Champsaur et al. 1984, Chrystie et al. 1975, Madeley et al. 1978, Murphy et al. 1975, Perez-Schael et al. 1984);

while in others, rotavirus was not detected at all or only sporadically (Appleton et al. 1978, Madeley et al. 1978, Perez-Schael et al. 1984, Steinhoff and Gerba 1978). The different rates of detection in different nurseries are likely to be due to different management systems such as cleaning and disinfecting procedures, isolation of infected patients and restrictions on visitors, especially children under five years of age. Babies do not normally shed rotavirus if the virus is not already present in a nursery, but once rotaviral infection is established in a nursery, neonates in that nursery are likely to become infected unless appropriate control measures, such as closing and fumigating the nursery, are taken to eliminate the infection.

Adult infections with group A rotaviruses have been reported on a number of occasions. These infections occurred either epidemically or endemically in communities. Epidemic infections by rotavirus in adults were associated with either the introduction of a rotavirus carrier into isolated communities (Foster et al. 1980, Linhares et al. 1981) or contamination of drinking water (Lycke et al. 1978). In these epidemics, all age groups were usually affected. Endemic rotaviral infections in adults occur sporadically in a proportion of a population, especially after contact with rotavirus infected children (Adkins et al. 1987, Grimwood et al. 1983, Rodriguez et al. 1979, Ryder et al. 1985, 1986). Rotaviral infection has also been reported to be associated with travellers' diarrhoea when people are exposed to different strains of virus in the new environment (Bolivar et al. 1978).

Pigs: Rotaviral infection in pigs was first suggested when sera obtained from pigs were found to contain antibodies against calf rotavirus (Woode and Bridger 1975). This was followed by the discovery of rotaviral particles in faeces of diarrhoeic pigs by EM (Rodger et al. 1975a). Since then, rotaviral infection and associated diarrhoea has been recorded in pigs throughout the world. Experimental infections with the virus have been carried out in gnotobiotic, colostrum-deprived and conventionally reared pigs (Bohl et al. 1978, Crouch and Woode 1978, Lecce et al. 1976, McNulty et al. 1976c, Tzipori and Williams 1978, Woode et al. 1976a). In gnotobiotic and colostrum-deprived piglets, signs invariably occurred 12 to 30 hours after oral inoculation. Anorexia and vomiting were initially observed in infected piglets, followed by profuse, watery, yellowish diarrhoea. The piglets then became dehydrated and some, especially very young piglets, died three to seven days after inoculation. Surviving piglets recovered from diarrhoea rapidly and lost weight was gradually regained.

Conventionally reared piglets developed only mild diarrhoea after experimental infection (Tzipori and Williams 1978).

Under natural circumstances, rotavirus has been associated with two common diarrhoeal syndromes in pigs. The first is the so called "white scours", "milk scours" or "three-week-old scours" (Bohl 1979) which occurs in two to four week old sucking pigs and is characterized by white or yellow diarrhoea (Mouwen 1971). The development of this syndrome coincides with the shedding of group A rotavirus in the faeces (Askaa and Bloch 1981, Bohl et al. 1978, Utrera et al. 1984, Woode et al. 1976a). Affected animals usually develop mild diarrhoea lasting for one to three days. Dehydration is more evident in pigs less than 10 days old and in pigs with prolonged diarrhoea. Herd mortality rates due to rotaviral diarrhoea vary from 0 to 10% in pigs (Bohl et al. 1978, Tzipori 1985, Woode and Bohl 1981).

The second diarrhoeal syndrome (postweaning diarrhoea, PWD) occurs four to seven days after weaning (Tzipori 1985). Pigs weaned at an early age (1 to 3 days old) are more severely affected (Barrow et al. 1979, Lecce and King 1978, Svensmark 1983). Rotavirus has also often been detected in diarrhoeal faeces of weaned pigs (Barrow et al. 1979, Bohl et al. 1978, Lecce et al. 1982, Tzipori et al. 1980a). However, the role of rotavirus in PWD is not clear since dietary factors and enterotoxigenic *Escherichia coli* (ETEC) are also involved. Lecce et al. (1983) reported that diarrhoea in weaner pigs with high nutrient intake lasted longer than that in pigs with low nutrient intake. ETEC has long been incriminated as a causative agent in PWD (Tzipori 1985). A shift to nearly pure haemolytic ETEC in the aerobic faecal flora in pigs four to seven days after weaning coincides with the development of PWD (Lecce et al. 1982, Tzipori et al. 1980b). When piglets were inoculated with rotavirus, or ETEC or both, at the point of weaning (Tzipori et al. 1980a), it was found that those infected with rotavirus alone developed mild diarrhoea, those infected with ETEC developed severe diarrhoea whereas those infected four days apart with both agents developed the most severe disease. Under natural conditions, weaned pigs with rotaviral infection only developed mild diarrhoea while those infected with both rotavirus and ETEC developed severe diarrhoea (Lecce et al. 1982). Furthermore, severe diarrhoea could not be induced in pigs by artificial inoculation of ETEC unless the pigs were inoculated with ETEC during a rotavirus-associated diarrhoea. Pigs infected with ETEC alone developed only mild diarrhoea. From these results, the authors (Lecce et al. 1982) concluded that rotavirus damaged the epithelium of the

small intestines, thus altering the luminal environment to one that favoured colonization by ETEC. Therefore both rotavirus and ETEC were believed to play a role in the development of PWD.

Cattle: Rotavirus was implicated as an important cause of neonatal calf diarrhoea in the late 1960's when Mebus et al. (1969) discovered the virus by EM in diarrhoeic faeces of calves and successfully transmitted the disease to other calves by oral inoculation with faecal filtrates. Affected animals develop watery, yellow or yellowish-green diarrhoea (White et al. 1970, Woode and Bridger 1975). Experimental infection of gnotobiotic, colostrum-deprived and conventionally reared calves with bovine rotavirus resulted in the development of diarrhoea 18 to 72 hours after inoculation (Bridger and Woode 1975, Mebus et al. 1969, Tzipori et al. 1981, Woode 1978, Woode et al. 1974). Dehydration and subsequent death occurred more frequently in gnotobiotic and colostrum-deprived calves. Under natural circumstances, calves become infected with group A rotavirus before four weeks of age, usually between three and 15 days, and develop mild to moderate diarrhoea (De Leeuw et al. 1980, McNulty and Logan 1983, Woode and Bridger 1975). Most of the affected animals recovered with or without fluid therapy and mortality rates were low.

Pattern of rotaviral infection: As described above, rotaviral infection is rare in infants less than six months of age. If it does occur, it is usually asymptomatic (Chrystie et al. 1975, Perez-Schael et al. 1984). The most susceptible period for rotaviral infection in children is between six to 24 months of age. Most children become infected during this period (Bryden et al. 1975, Holdaway et al. 1982, Mata et al. 1983). Rotaviral infection in people older than five years of age is much less frequent (Adkins et al. 1987). Repeated infections with rotavirus have been reported frequently, especially in children during the period of greatest susceptibility (Bishop et al. 1983, Chiba et al. 1986, Konno et al. 1978, Yolken et al. 1978c). In a cohort of 45 children followed for three years from birth, Mata et al. (1983) found that 44 of these children had at least two and some as many as seven episodes of rotaviral infection.

The pattern of infection by group A rotavirus has also been studied in pigs. Pigs less than one week of age are rarely infected (Bohl et al. 1978, Utrera et al. 1984). Piglets become infected at the beginning of the second week and the prevalence then increases with age to reach a peak at five to six weeks of age (Askaa et al. 1983,

Svensmark 1983, Utrera et al. 1984). By this time most of the piglets have shed the virus (Debouck and Penseart 1983). The duration of shedding is generally five to eight days in both experimentally and naturally infected piglets (Crouch and Woode 1978, Debouck and Pensaert 1983, Tzipori and Williams 1978). Piglets born of gilts usually become infected with rotavirus earlier (5 to 14 days) than those born of sows (21 to 35 days) (Askaa et al. 1983). Svensmark (1983) observed that piglets weaned at two weeks of age developed rotaviral infection earlier and suffered more severe diarrhoea than those weaned at 4 to 5 weeks of age. Shedding of rotavirus in fattener pigs has also been studied. Debouck and Pensaert (1983) reported that shedding of rotavirus by pigs two weeks after weaning was an exception, while Grom et al. (1984) detected rotavirus from 15% of fattener pigs. The differences in these studies were either due to different methods used for detection, or due to differences in virulence of different strains of rotavirus. Rotavirus has also been detected occasionally in faeces of old pigs, especially in gilts (McNulty et al. 1976c) and sows (Benfield et al. 1982, Corthier et al. 1980).

Similar patterns of rotaviral infection have also been observed in other animals such as cattle (De Leeuw et al. 1980, Fernelius et al. 1972, McNulty and Logan 1983, Woode and Bridger 1975) and mice (Eydelloth et al. 1984, Sheridan et al. 1983, Wolf et al. 1981).

The apparent lower susceptibility of neonatal animals to rotaviral infection may be due to the lower activity of proteolytic enzymes and the presence of maternal antibody in the gut. Proteolytic enzymes have been shown to increase rotavirus infectivity both in vitro and in vivo (Estes et al. 1981, Steel and Torres-Medina 1984). The lower concentration of proteolytic enzymes in the gut (Wolf et al. 1981) and the presence of enzyme inhibitors in colostrum and milk (Steel and Torres-Medina 1984) may serve as natural defence mechanisms against rotavirus. The protective effect of maternally-derived antibodies in neonates will be discussed in detail in section 1.5.3.

The apparent lower susceptibility of adult animals to rotaviral infection has been thought to be due to the maturation of intestinal cells and the acquisition of "age resistance". When mice born of sero-negative dams were inoculated with EDIM virus at different ages, it was found that rotavirus replication peaked in the intestinal cells of seven to 14 day old mice. Only minimal replication was observed in mice older than 21 days of age (Eydelloth et al. 1984, Sheridan et al.

1983, Wolf et al. 1981). By treating eight day old mice with cortisone to induce partial intestinal maturation, Wolf et al. (1981) observed that the susceptibility to rotaviral infection was reduced to 60% in treated mice compared with 94% susceptibility in age-matched controls.

The immune status of individuals and the virulence of virus may also influence the susceptibility of older animals. Also more than one serotype of rotavirus has been identified in many species of animals (see section 1.3.2). Usually, immunity developed after infection with one serotype does not protect against infection with another serotype (Chiba et al. 1986, Yolken et al. 1978c). Therefore adult animals may become infected when they are exposed to a serotype which is different from that to which they were previously infected. This could be supported by those reports on rotaviral epidemics which occurred after the introduction of a rotaviral carrier into isolated communities (Foster et al. 1980, Linhares et al. 1981). In these epidemics, people of all age groups were affected.

Differences in virulence have been demonstrated between bovine rotavirus strains. Caprio et al. (1981) studied four bovine rotavirus isolates in the ligated intestinal loop model and consistently found that three of the four strains induced significant villous atrophy in the jejunum while the other strain failed to do so. The latter isolate had a different RNA pattern from those of the other three more virulent viruses. Bridger and Pocock (1986) studied three bovine isolates in gnotobiotic calves. Two of these caused only subclinical infection in calves less than 16 days of age, while the third isolate caused severe diarrhoea in calves up to at least 116 days of age. On the basis of these observations, Bridger and Pocock (1986) concluded that some strains caused disease in all ages, some infected all ages but caused disease only in the young, while others infected the young without causing disease.

Cross infection of rotaviruses between species: The question of whether or not rotaviruses can cross species barriers has considerable importance in the epidemiology of rotaviral infection and public health. Experimental cross-species transmissions have revealed that human rotavirus could infect gnotobiotic pigs (Bridger et al. 1975, Davidson et al. 1977), calves (Light and Hode 1943, Wyatt et al. 1983b), lambs (Snodgrass et al. 1977), monkeys (Wyatt et al. 1976), mice (Gouvea et al. 1986) and dogs (Tzipori 1976) as judged by the development of diarrhoea, the shedding of virus and a serological response.

Cross species infections with rotaviruses of other animals have also been described. Gnotobiotic piglets are susceptible to bovine, equine, simian and ovine rotaviruses (Bridger and Brown 1984, Woode and Bohl 1981, Woode and Bridger 1975, Woode et al. 1976b). However, calves appear to be less susceptible to porcine viruses (Bridger and Brown 1984, Woode et al. 1976b, Zissis et al. 1983). Sucking mice have also been reported to be susceptible to monkey rotavirus (SA11 and RRV) (Greenberg et al. 1986, Offit and Clark 1985). Generally, the symptoms in the animals infected with rotavirus from other species are milder than those in animals infected with virus from the same species.

Natural transmission of rotaviruses between species has not been recorded. Ryder et al. (1986) found no correlation between rotaviral infection in cattle and cattle farmers in Panama. However, indirect evidence suggests that natural transmission of rotaviruses between species may occur. Rotavirus serotype 3 embraces rotaviruses from a wide range of species including human, simian, porcine, murine, equine, lapine, canine and feline species (Hoshino et al. 1987a). Heath et al. (1986) suggested that natural transmission between cats and man might have occurred since two feline rotavirus strains could not be distinguished using monoclonal antibodies (MAbs) from seven strains of human rotavirus isolated in the same laboratory. Moreover, the virus, belonging to subgroup I but with a long RNA pattern (see section 1.3.2), isolated from a man in Japan (Nakagomi et al. 1985) might be an animal rotavirus strain that infected man. The virus had homology only of its VP7 with human rotavirus serotype 3 but had weak homology with all the RNA segments of NCDV and RRV (a strain of monkey rotavirus) as revealed by RNA-RNA hybridization (Nakagomi et al. 1987).

From the above findings, the statement made by Holmes (1983) still holds true that "rotaviruses are moderately, though certainly not absolutely, host-specific in nature".

1.3.2 The viruses

The characterization of rotaviruses has been described in detail in section 1.2. In this section, the classification of group A rotavirus into subgroup, serotype and electrophoretype is presented in relation to the epidemiology of rotaviral infection.

Subgroups: Early classification of rotaviruses isolated from different species was confusing since rotaviruses with different

antigenic composition revealed by various serological methods such as complement fixation (CF), ELISA, immune electron microscopy (IEM) and serum neutralization (SN) were called different serotypes or types (Thouless et al. 1977, 1978, Yolken et al. 1978c, Zissis and Lambert 1978). Later Kapikian et al. (1981) found that the antigen recognized by ELISA and immune adherence haemagglutination (IAHA) systems was different from that recognized by neutralization. The authors proposed that the term 'subgroup' be employed for antigen recognized by ELISA and IAHA and the term 'serotype' for that recognized by neutralization. This proposal was further supported by biological evidence that these two antigens segregated with different gene segments (Kalica et al. 1981b, Greenberg et al. 1983a, Smith et al. 1980). Subgroup specificity is associated with VP6, coded by RNA segment 6 while serotype specificity is defined by VP7, coded by segment 8 or 9 depending on the particular strains of rotavirus.

The subgroup antigen could be readily detected by using CF, ELISA, IEM and IAHA and up to now, two subgroups have been well defined (WHO 1984, Greenberg et al. 1983b). ELISA tests using MAbs against the two subgroups have also been developed to detect subgroup antigen in viruses already adapted to cell culture or present in faecal samples (Greenberg et al. 1983b, Singh et al. 1983). By employing one or more of these assays, most rotaviruses isolated throughout the world, even the 'super-short' strains, could be classified into one of the two subgroups (Albert et al. 1987b, Bohl et al. 1984, Hoshino et al. 1984, 1985b, Matsuno et al. 1985, Nagesha and Holmes 1988, Nakagomi et al. 1985, Urasawa et al. 1987, White et al. 1984, Wyatt et al. 1983a). Most human rotaviruses belong to subgroup II except for those short and 'super-short' strains which are subgroup I. In contrast, most animal rotaviruses belong to subgroup I except for two strains of porcine rotaviruses (G and SB-1A) which are subgroup II specific. In addition, some rotaviruses are not subgroupable by these tests. These include all avian group A rotaviruses (Greenberg et al. 1983b, Hoshino et al. 1984), equine rotavirus strain H-2 (Hoshino et al. 1984), murine rotavirus strain EDIM (Greenberg et al. 1983b), human rotavirus strain G402 (Urasawa et al. 1987) and some human strains which have not yet been adapted to cell culture (Lambert et al. 1984, Tufvesson 1983, White et al. 1984).

The classification of two subgroups of rotavirus has been supported by biological evidence. Rotaviruses belonging to the same subgroup have higher degrees of homology in sequence of gene 6 and in predicted amino

acid sequences than those belonging to different subgroups. Thus, Cohen et al. (1984) observed 87% homology in sequence of gene 6 and 97% homology in that of predicted amino acid between bovine rotavirus RF and simian rotavirus SA11. Both of the strains belong to subgroup I. When subgroup I (SA11 and RF) and subgroup II (Wa) rotaviruses were compared, only 78% of their gene 6 sequences and 91% of their deduced amino acid sequences were conserved (Both et al. 1984).

However, the fact that many rotaviruses isolated to date can not be classified into either of the two subgroups (see above) indicates the existence of more than two subgroups of group A rotaviruses. This hypothesis could be further supported. Hoshino et al. (1987a) found that an equine rotavirus (F1-14) (Gillespie et al. 1984) bore both subgroup I and II specificities on its VP6. Six groups of MABs against VP6 of this isolate were produced and two of these reacted specifically with subgroup I and subgroup II specificity, respectively. Others reacted either with a specific strain, or certain strains or all of the mammalian rotaviruses when tested against a panel of 49 rotaviral isolates from nine species including man. Similar results were also achieved by Lambert et al. (1984) in which two of their four MABs reacted specifically with subgroup I and II respectively. One of the other two reacted only with those strains which the authors previously claimed to be a new subgroup (subgroup III) (Lambert et al. 1983) and the other reacted with some strains in both subgroups I and II.

Serotypes: Since Kapikian et al. (1981) defined rotavirus serotypes as detected by neutralization of infectivity and Sato et al. (1981) successfully adapted human rotavirus in cell culture by employing the roller culture system in the presence of trypsin, many rotavirus strains from different species have been serotyped. Tests used for serotyping include plaque reduction neutralization (PRN) (Wyatt et al. 1982, 1983a, Hoshino et al. 1984), fluorescent focus assay (FFN) (Coulson et al. 1985), and serum neutralization (SN) (Gaul et al. 1982). A virus is defined as belonging to a new serotype if titration using hyperimmune sera in a PRN and SN assay showed at least a 20-fold reciprocal difference in titre between homologous and heterologous reactions. If convalescent sera are used in PRN, or if FFN is used, then it might not be possible to demonstrate more than an 8-fold difference between homologous and heterologous strains (WHO 1984).

Up to 1984, at least four serotypes of human rotaviruses (Wyatt et al. 1983a), two serotypes each of porcine (Bohl et al. 1984) and

equine rotaviruses (Hoshino et al. 1983), and three bovine rotavirus serotypes (Bridger and Brown 1984, Ihara et al. 1983, Woode et al. 1983) had been described. Hoshino et al. (1984) unified the classification system for rotavirus serotypes by comparing 16 different strains of rotaviruses from seven mammalian and two avian species. Among these strains, seven serotypes were found and the authors classified the four human serotypes as serotypes 1 to 4, respectively. Animal rotaviruses were tentatively classified as serotypes 5 (one each of porcine and equine rotavirus strains), 6 (bovine rotavirus NCDV and UK), and 7 (avian rotaviruses), respectively. In that study, it was found that simian rotavirus, canine rotavirus, and one equine rotavirus strain shared serotype specificity with human rotavirus serotype 3; two porcine rotavirus strains shared serotype specificity with human serotype 4. One of these porcine strains also shared serotype specificity with rotavirus serotype 5 (OSU porcine rotavirus) and therefore bridged between serotypes 4 and 5.

The classification system described has been supported by studies of the molecular biology of the virus. By RNA-RNA hybridization, Midthun et al. (1987a) showed a high degree of homology between the genes (gene 8 or 9) within each of the four human serotypes. Hybrid bands could not be demonstrated in the genes between the representatives of the four human serotypes (D, DS-1, P, ST3) and the corresponding genes of human rotaviruses belonging to a different serotype. By comparing the nucleotide sequence of the gene coding for the neutralization protein VP7, high degrees of homology (95-98%) were observed between three bovine rotavirus strains (NCDV, UK and RF), which belonged to the same serotype (Charpilienne et al. 1986). Only 74-77% homology was demonstrated between different serotypes (such as RF and SA11, Wa and RF, UK and SA11, Wa and UK, Wa and SA11) (Arias et al. 1984, Charpilienne et al. 1986, Dyall-Simth and Holmes 1984, Richardson et al. 1984). Along the predicted amino acid chain of VP7, significant divergences were observed in the regions of 39-50, 87-101, 120-130, 143-152, 208-221, 233-242 and were designated as A, B, C, D, E and F, respectively by Glass et al. (1985). These regions may be involved in serotype specificity. Further investigation of four human serotypes (serotypes 1, 2, 3 and 4) and two animal serotypes (serotypes 5 and 6) by Green et al. (1987) confirmed the finding of Glass et al. (1985). Rotaviruses of different serotypes exhibited significant sequence divergence (15-29%) in regions A to F whereas marked amino acid sequence conservation (91-99%) was found in these regions among members of a given serotype independent of place of origin or year of isolation.

Two more serotypes of human group A rotaviruses, which do not belong to any of the existing four human or any other animal serotypes, have been identified recently. One was the virus detected in Indonesia (Albert 1985, Hasegawa et al. 1984). The virus possesses a unique genomic pattern as revealed by PAGE and is called the 'super-short' strain of rotavirus. Three isolates (B37, B38, 69M) have been adapted to MA-104 cells (Albert et al. 1987b, Hasegawa et al. 1984, Matsuno et al. 1985) and further characterization by PRN and FFN has revealed that they cross-react with each other but not with any of the other known human or animal serotypes. The virus was designated tentatively as human rotavirus serotype 5. Another is the virus strain WI61 isolated in Philadelphia, USA from an 18 month old girl with a history of chronic failure to thrive (Clark et al. 1987). PRN assay has shown the isolate to be distinct from the other five human serotypes and three animal isolates which were serotypes 5, 6 and 7 as defined by Hoshino et al. (1984). This virus was designated tentatively as human rotavirus serotype 6.

In pigs, two or possibly three more serotypes have also been identified recently. One was isolated in Australia (Nagesha and Holmes 1988). This virus (MDR-13) had similar serotype specificity as human serotype 3 and showed a two-way cross-reaction with other serotype 3 viruses. The other two was isolated in the USA (Paul et al. 1988). These two viruses did not show any relationship to porcine serotypes 4 (G strain), 5 (OSU strain) or to each other. But their relationship to other known serotypes from other species have not yet been studied.

The recognition that both VP7 and VP3 are involved in eliciting neutralizing antibodies (Hoshino et al. 1985a, Offit and Blavat 1986) explains the observation made by Hoshino et al. (1984) that a porcine rotavirus strain SB-1A bridged between serotypes 4 and 5. This was due to that the virus derived its VP3 from one serotype and VP7 from another. Now it is known that SB-1A shares antigenic specificity of VP3 with OSU strain (serotype 5) and VP7 with G strain (serotype 4) (Hoshino et al. 1987b). More rotaviruses showing dual serotype specificities have been detected since 1984. Thus Hoshino et al. (1985b) found that a neonatal strain (M37) from Venezuela belonged to both serotypes 1 and 4. This strain shares VP3 with serotype 4 and VP7 with serotype 1 (Midthun et al. 1987b). Another human rotavirus (F153) with dual serotype specificities between serotypes 1 and 4 was isolated in Kenya and was also found to possess serotype 4 specific VP3 and serotype 1 specific VP7 (Urasawa et al. 1987). At the same time, these authors

(Urasawa et al. 1987) isolated two other strains (F247 and G402) that showed cross-reactivity with both serotypes 3 and 4. A porcine rotavirus strain (CRW-8) was found to bridge serotypes 3 and 5 and this strain may share VP3 with serotype 5 and VP7 with serotype 3 (Nagesha and Holmes 1988). Other intertypic rotaviruses may include human strains RV-3 (Bishop et al. 1986) and WI61 (Clark et al. 1987), bovine strain PP1 (Bridger and Brown 1984), and canine strain CU-1 (Hoshino et al. 1984, 1987b). Since both VP3 and VP7 are responsible for neutralization and their neutralization specificities are segregated independently in nature, Hoshino et al. (1985a) suggested that a binary system is needed for classification and nomenclature similar to that used for influenza A viruses (Webster and Laver 1975) to indicate the neutralization specificity of both VP3 and VP7. This proposal has been supported by many other workers (Midthun et al. 1987b, Urasawa et al. 1987, Nagesha and Holmes 1988).

Electrophoretotypes: Analysis of rotaviral genomes on PAGE has shown marked differences between viruses isolated from different hosts throughout the world (Estes et al. 1984). Viruses with different RNA patterns are referred to as different electrophoretotypes.

A classification system for rotavirus electrophoretotypes was proposed by Lourenco et al. (1981) based on the relative migration of RNA bands in each of the four size-classes (see Fig. 1-1). In this system, different RNA patterns in each of the four size-classes are denoted by a lower case letter. A particular electrophoretotype is expressed as a combination of its patterns in each of the four size-classes, e.g. one electrophoretotype may have the pattern Ia, IIa, IIIb, IVa and another Ib, IIa, IIIa, IVb, etc. This classification scheme has been adopted by some authors (Dimitrov et al. 1984, Gomez et al. 1986, Nicolas et al. 1984) and a number of electrophoretotypes within each size-class have been demonstrated. However, this classification system has limitations. Due to the large number of electrophoretotypes detected, comparison of electrophoretotypes from one place with another is usually not possible. Thus, others simply designated their own rotavirus electrophoretotypes alphabetically (Chiba et al. 1984, Gerna et al. 1987a, Liprandi et al. 1987, Rodger, et al. 1981, Spencer et al. 1983a, b).

The most noticeable variation from the typical group A rotaviral RNA pattern has been with segments 10 and 11. Smith and Tzipori (1979) reported a murine rotavirus with segment 11 running close to segment 10.

Segments 10 and 11 with identical or similar electrophoretic mobilities were shown in the avian group A rotaviruses (Todd et al. 1980). This appears to be unique to the avian group A rotaviral genome. The 'abnormal' electrophoretic patterns of segments 10 and 11 were also demonstrated in human group A rotaviruses with segments 10 and 11 running close or very close to the triplet (Albert 1985, Espejo et al. 1977, 1979, Rodger et al. 1981). These were called short or super-short strains, respectively. By gene coding assignment, Dyall-Smith and Holmes (1981a) showed that the short electrophoretype was due to an increase in the size of segment 11. This might also explain the super-short pattern reported.

Recently, an unusual RNA pattern of group A rotavirus has been reported in man (Beselaar et al. 1986), calves (Pocock, 1987), rabbits (Thouless et al. 1986) and pigs (Bellinzoni et al. 1987a) in which segment 11 is absent and an additional segment between segments 6 and 7 appears. Surprisingly, analysis of the bovine rotavirus with the unusual pattern showed that this was also due to the increase in the size of segment 11 since the polypeptide produced after translation of the extra RNA transcript is equivalent to the product of segment 11 (Pocock 1987).

Numerous electrophoretotypes with other segment changes have also been detected throughout the world. In longitudinal investigations which lasted from four months to four years, up to 36 electrophoretotypes have been detected. One or two electrophoretotypes were almost always found to predominate throughout, while others appeared and disappeared in a sequential manner during the period of investigation (Chiba et al. 1984, Dimitrov et al. 1984, Gomez et al. 1986, Rodger et al. 1981, Schnagl et al. 1981, Spencer et al. 1983a, Todd et al. 1984). In investigations involving outbreaks of rotaviral infection, one single electrophoretype is normally found initially. This type has usually persisted throughout the outbreak, but in the later stages, a variety of electrophoretotypes have been identified (Buesa et al. 1987, Konno et al. 1984). Many electrophoretotypes have also been found to circulate in both human and animal populations at any one time (Albert et al. 1982, 1983, de San Juan et al. 1987, Gerna et al. 1987a, Liprandi et al. 1987).

When human rotaviruses detected in different geographical locations were compared, in some cases, no common electrophoretype was found, whereas the same types have been identified in different areas during other investigations. This may be due to the movements of people and

the remoteness of the locations studied. Common electrophoretotypes were found in different locations in France (Lourenco et al. 1981, Nicolas et al. 1984). The same electrophoretotype was also recorded in Mexico City and in Texas (Dimitrov et al. 1984). In the study conducted by Chiba et al. (1984) in two districts of Kenya, which are 480 km apart, only one common electrophoretotype was detected. A common electrophoretotype was also detected in two locations, 200 Km from each other, in Hungary (Szucs et al. 1987). However, no common electrophoretotypes were found in Central Australia and South Australia (Schnagl et al. 1981), in Chile and Mexico (Espejo et al. 1980), and in the USA, Colombia and Argentina (Dimitrov et al. 1984).

The mechanisms leading to the heterogeneity of rotaviruses may be due to point mutation, gene reassortment or a combination of both. In studies of outbreaks, both Buesa et al. (1987) and Konno et al. (1984) found that a single electrophoretotype was identified during the first few weeks and thereafter many electrophoretotypes appeared. Some of the latter electrophoretotypes might have originated from the initial one by point mutations. When SA11 viruses from five independent laboratories were compared on PAGE, four electrophoretotypes were recognized (Pereira et al. 1986). These five viruses came from the same origin (Malherbe and Strickland-Cholmley 1967). The likely explanation for these observations is that point mutations had occurred during repeated passages in the laboratory.

Due to the segmented nature of rotavirus genomes, reassortment frequently occurs both in vitro and in vivo during mixed infection by exchange of genome segments (Gombold and Ramig 1986, Greenberg et al. 1981, 1982, Kalica et al. 1981b, Matsuno et al. 1980). The point mutation in one strain of virus paves the way for reassortment between the original and the new mutant strains. This is one explanation for the phenomenon reported by Buesa et al. (1987) and Konno et al. (1984) that various electrophoretotypes appear in the later stages of outbreaks of rotaviral infection.

Mixed infections, between two or more electrophoretotypes of group A rotavirus, in individuals are very common (Bellinzoni et al. 1987a, Buesa et al. 1987, Lourenco et al. 1981, Todd et al. 1980, Uhnöo and Svensson 1986, White et al. 1984). This provides the opportunity for reassortment by genome exchange, thus giving rise to the many different electrophoretotypes detected in different investigations.

Although group A rotaviruses can be extremely varied, as described above, some workers have failed to detect variations in particular groups of isolates. Herring et al. (1982) reported that all rotavirus strains from a single outbreak of infection in cattle showed the same electrophoretype. Similar observations were also reported by Todd et al. (1984) in both cattle and pigs. Furthermore Todd et al. (1984) detected only a single electrophoretype over a period of 16 months in a closed dairy herd. In human populations, Albert et al. (1983) found that during a four month period 33 samples, from Kundiawa, Papua New Guinea, showed identical electrophoretotypes. These results may have been due to the relative isolation of the animal and human populations studied and may also indicate that mutation of rotaviral RNA may not occur in nature as often as expected.

Relationships between subgroups, serotypes and electrophoretotypes:
It has been found that the electrophoretotypes of the 'short' and the 'super-short' strains belong to subgroup I while those of long strains belong to subgroup II (Albert 1985, Kalika et al. 1981a, Thouless et al. 1982). Analysis of field samples or cell-adapted rotaviruses revealed an almost complete correlation between the electrophoretotypes of long or short patterns and subgroup II or I, respectively (Albert et al. 1987b, Greenberg et al. 1983b, Hoshino et al. 1984, 1985b). However, this correlation is not found among animal rotaviruses since most animal rotaviruses belong to subgroup I, yet they have long electrophoretotypes. Since subgroup-specificity is located on VP6, coded by segment 6, and the difference between the long and short patterns lies in mobility variations of segment 11, there is no genetic basis for correlation between the subgroup I specificity and the short electrophoretype. Indeed, a rotavirus (AU-1) from man was serologically classified as subgroup I but showed a long pattern by PAGE (Nakagomi et al. 1985). The authors suggested that the virus may in fact be an animal rotavirus infecting man.

No correlation has been found between serotypes and electrophoretotypes. Strains within the same serotype might show heterogeneity in electrophoretotypes, and identical electrophoretotypes have also been observed in rotaviruses of different serotypes (Beards 1982, Gerna et al. 1987a). Furthermore, Clarke and McCrae (1982) noted that identical electrophoretotypes did not necessarily have identical nucleotide sequences. By analysing rotavirus strains with MAbs, Coulson et al. (1985) also found that antigenic drift occurred in two strains isolated three months apart although their electrophoretotypes were

identical and both belonged to the same serotype.

Relationships between subgroups and serotypes have also been noted. Subgroup I specificity is associated with serotype 2 (Hoshino et al. 1984) and the newly discovered human serotype 5 (Albert et al. 1987b), and subgroup II is associated with other serotypes of both human and animal rotaviruses.

From the preceding discussion, it can be seen that serotype 2 rotavirus has subgroup I specificity and possesses a short pattern, and human rotavirus serotype 5 has subgroup I specificity and possesses a super-short pattern.

1.3.3 Maintenance of rotavirus in the environment

Rotaviruses have been detected in treated drinking water, well water, raw and treated sewage, and sewage-contaminated seawater (Deetz et al. 1984, Goyal and Gerba 1983, Murphy et al. 1983, Smith and Gerba 1982, Steinmann 1981).

After seeding rotavirus into water samples, its reduction in infectivity was monitored over a period of time. Pancorbe et al. (1987) found that the titre was reduced by $2 \log_{10}$ in tap water in two to three days, in lake water in 17 days, in creek water in 8 days and in ground water and secondary effluent in 14 to 16 days. A longer survival time has been reported by Raphael et al. (1985) in different types of waters. The titre was reduced by $2 \log_{10}$ in raw water after 10 and 32 days at 20°C and 4°C , respectively and in tap water after 64 days at 20°C . No significant drop in titre was found during a period of 64 days in tap water kept at 4°C and in filtered water.

Rotavirus has also been detected on surfaces at child day care centres including sinks, refrigerator handles and children's hands (Keswick et al. 1983). In a programme run by Black et al. (1981), hand washing by staff and children before eating and after passing stools dramatically reduced the incidence of diarrhoea in children in a day care centre compared with a control group. The incidence of diarrhoea began to fall in the first month of implementation of the programme and was consistently lower than in the controls for the next two months. During the following 35 weeks of investigation, the incidence of diarrhoea in the hand washing group was half of that in the control group.

The survival of rotavirus in the immediate environment of young animals has been implied, although no attempts have been made to detect the virus. Piglets weaned at one day of age into a previously fumigated "nursery" were free of rotaviral infection (Lecce et al. 1978). However when the nursery was continuously used without further fumigation, rotaviral infection steadily built up causing a progressive increase in the incidence of vomiting, diarrhoea and death among the piglets. In a closed dairy herd, thorough cleansing and disinfection of the calf house were reported to have broken a cycle of rotaviral infection (McNulty and Logan 1983). Bovine rotavirus NCDV was found to survive for seven months in the laboratory at an average ambient temperature of 18 to 20°C (Woode and Bridger 1975).

Investigations have also focused on factors such as temperature and relative humidity which may influence the survival of rotavirus in the environment. Raphael et al. (1985) tested the survival time of human rotavirus in tap and raw water at 20°C and 4°C and found that rotavirus survived much longer at 4°C than at 20°C. Moe and Harper (1983) also observed that the infectivity of rotavirus in aerosol was lost more rapidly at high temperatures than at low temperatures. Furthermore, Hejkal et al. (1984) found lower levels of rotavirus in sewage in the summer months than during winter and spring. This coincides with the fact that rotaviral infection occurs more in winter than in the summer in temperate climates. In the winter months, rotavirus was detected in 50-80% patients with diarrhoea and in the summer, rotavirus was not detected at all or only sporadically (Bryden et al. 1975, Holdaway et al. 1982, Kapikian et al. 1976, Konno et al. 1978). A climatological analysis of the occurrence of acute diarrhoea associated with rotaviral infection was carried out in hospitals in a northern Japanese city over a period of seven years. The frequency of infection was related to the environmental temperature. Rotaviral infection appeared abruptly when the mean temperature fell below 5°C (November or December), reached a peak when it was less than 0°C (January and February) and waned when the temperature rose above 20°C (June and July) (Konno et al. 1983). In contrast, rotaviral infection occurs all year around in tropical countries (Hieber et al. 1978, Soenarto et al. 1981), with 30-40% of diarrhoeic patients shedding rotavirus.

Humidity has also been reported to have a profound effect on the survival and spread of rotavirus. Rotaviral infection was more common after a month of cold or dry weather than after a month of warm and wet weather (Black et al. 1980, Brandt et al. 1982). A similar trend was

observed by Paul and Erinle (1982), Georges et al. (1984) and Echeverria et al. (1983) where the rate of rotavirus infection was higher in the dry months than in the wet months. Dry weather aids in the formation of aerosols while cold weather may result in overcrowding indoors. Both factors could aid the spread of infection.

1.3.4 Transmission of rotaviral infections

The mode of rotaviral transmission is thought to be by the faecal-oral route (Mata et al. 1983, Rodriguez et al. 1979, Woode and Bohl 1981). However, transmission of rotaviral infection by the respiratory route is possible. A high prevalence of respiratory symptoms has been reported in patients with rotavirus associated diarrhoea (Lewis et al. 1979, Holdaway et al. 1982); the spreading nature of rotaviral infection is similar to that of influenza (Foster et al. 1980); and rotavirus has been detected in respiratory aspirates and nasopharyngeal secretions (Santosham et al. 1983).

Group A rotaviruses mainly infect children and young animals and these infected young individuals become important disseminators of infection (Flewett 1983). Infected children have been reported to be the most important reservoir for nosocomial and intrafamilial rotaviral infections. Thus, Grillner et al. (1985) described an outbreak in 3 to 49 day old babies in a neonatal ward after the introduction of an infected baby from an obstetrical ward. None of the medical personnel, nor the mothers, were shedding the virus at that time. Dearlove et al. (1983) reported that 76 out of 196 children admitted to a baby care unit during a one year period developed rotaviral infection after one baby was infected with rotavirus and developed diarrhoea. In a study of rotaviral infection in the family environment, Grimwood et al. (1983) also observed that 46% of members from 28 families with infected children became infected with rotavirus while only one did so in 18 uninfected families.

As described in section 1.3.1, rotaviral infections have been occasionally detected in adults and in older animals, especially dams with young animals. The importance of rotaviral infection in adults in maintaining and transmitting the virus to others, especially to the young, is not known. Ryder et al. (1986) observed that the rates of infection in children were the same regardless of whether their parents had had rotaviral infection. Rates of infection were higher in parents of children with rotaviral infection than in those of children without

such infection. Therefore, children, not adults, are the more important reservoir for rotaviral infection. Nevertheless, Bolivar et al. (1978) and Kapikian et al. (1976) suggested that adults may serve as carriers of rotavirus and act as reservoirs under certain circumstances.

The detection of rotavirus in drinking water, in domestic sewage and on surfaces in day care centres (see section 1.3.3) indicates that the environment is another potential source for rotaviral infections. Diarrhoea associated with group A rotavirus due to drinking contaminated water has been reported in many communities, affecting both tourists and residents of the area (Baker et al. 1979, Lycke et al. 1978, Murphy et al. 1983, Steinmann 1981).

In cattle, outbreaks of rotaviral infection and associated diarrhoea in dairy and beef herds occur each year in the calving season (Murakami et al. 1987, White et al. 1970, Woode 1978). During the calving season, rotavirus is transmitted between calves by direct or indirect contact (Woode 1978). The way in which rotavirus is maintained during the intervals between calving seasons is not known. Rotavirus has been detected in adult cattle, especially in heifers and cows (McNulty and Logan 1983, Bridger and Woode 1975, Murakami et al. 1987). However, most of these infections occurred after contact with rotavirus infected calves. Therefore it is unlikely that adult cattle are carriers. On the other hand, the large amount of virus shed by infected calves (Woode 1978) and the survival of rotavirus in faeces for as long as seven to nine months at room temperature (Woode and Bridger 1975, Woode 1978) indicates that the virus could survive in the environment for at least the interval between calving seasons, and so be the source of infection for the calves born in the following season.

1.4 IMMUNITY TO, AND VACCINATION AGAINST, INFECTION WITH GROUP A ROTAVIRUS

1.4.1 Transfer of maternal antibody

Transfer of maternal antibody from the human mother to baby occurs both in utero and via colostrum and milk. McLean and Holmes (1980) found that antibody titres to rotavirus in babies' cord sera reflected those in their mothers' sera. In colostrum, most antirotaviral antibody is present in the IgA fraction with lower levels in the IgG and IgM fractions. The level of IgA in milk declines and reaches a steady low

level by three or four days post partum (pp). By this time IgM almost disappears. IgA is detectable in infants' faeces from two days after birth and is observed only in breast-fed babies. Blacklow et al. (1976) reported that maternally-derived antibody to rotavirus in the serum of children declined rapidly after birth, and reached its lowest level by three to six months of age.

In most other animal species, transfer of maternal antibody does not occur in utero (Payne and Marsch 1962), but only via colostrum and milk. Hess and Bachmann (1981) investigated the transfer of maternal antirotaviral antibody in pigs during the first 14 days pp. It was found that antibody against rotavirus in sow's colostrum was four to 16 times higher than that in serum. Antibody levels in milk decreased 2 to 32 fold in some sows, while the level was maintained in others, throughout a 14 day period. IgA was detected in all colostrum and milk samples. IgG was present in colostrum and milk in the first week and then declined rapidly. IgM was not always detectable, certainly not in milk at about 14 days pp. Piglets were born free of specific antibody, but soon after consumption of colostrum, their antibody levels were comparable with those in colostrum, but were higher than those in sow's sera. The maternally-derived antibody in piglets' sera declined rapidly in the first 14 days after birth, while levels of antibody in sow's sera remained high during the same period. Askaa et al. (1983) also reported that antirotaviral antibodies were present in both colostrum and milk but declined rapidly within three to four days after farrowing.

1.4.2 Serological response after natural infection

Humoral immune responses have been detected in patients after rotaviral infection. Paired serum samples (acute and convalescent) from people with non-bacterial gastroenteritis usually showed a four fold or greater increase in antibody titres to rotavirus (Bishop et al. 1984, Gust et al. 1977, Hieber et al. 1978, Holdaway et al. 1982, Puerto et al. 1987). Seroconversion has also been detected in patients over 80 years old following rotaviral infection (Heimer and Cubitt 1983). However, infants or very young children, less than four months old, responded poorly. Thus, Gust et al. (1977) failed to detect rises in antibody in two infants (10 days and 4 months of age) although rotavirus was detected in their diarrhoeic faeces. Whether or not this is due to interference by maternal antibody is not known. The 10 day old baby did have a CF antibody titre of 1:32 in the initial sample.

IgM is first detected as early as three days after natural infection (Heimer and Cubitt 1983). This is followed by the production of IgG and IgA antibodies (Riepenhoff-Talty et al. 1981). Levels of serum antibody following natural infection persisted for as long as two years without significantly altering (Gust et al. 1977). Antibodies to rotavirus have also been detected in the faeces after natural rotaviral infection (Inouye et al. 1984, Riepenhoff-Talty et al. 1981, Stals et al. 1984), but were transient (Kapikian et al. 1983, Sonza and Holmes 1980). The latter authors (Sonza and Holmes 1980) reported that IgA, IgM and IgG levels in faeces reached peak titres between two and four weeks after infection and then dropped back to undetectable levels after two months.

Antibody responses in pigs have also been studied by experimental infection with the OSU strain (Corthier and Vannier 1981). Antibody in the faeces was first detected as immune complexes, then in free form. Production of coproantibody in pigs was also transient and was detected only from days 3 to 16 post infection (pi). In pigs, both IgA and IgM were found in comparable levels in faeces while IgG was not detected. In contrast, IgG and IgA antibodies were detected in serum at 24 days pi while IgM was not found at that time. This might have been due to the decline of IgM to undetectable levels by 24 days pi.

Serological responses to both homologous and heterologous strains of rotavirus have been observed in both naturally and experimentally infected subjects. In an outbreak associated with human serotype 1 infection, Linhares et al. (1981) found that antibodies to homologous serotype 1 and heterologous serotype 3 strains were significantly increased, especially in persons with preexisting serotype 3 antibodies. Chiba et al. (1986) also found that 77%, 62% and 23% of children infected with human serotype 3 developed antibodies to human serotypes 1, 4 and 2, respectively. When piglets were infected in utero with human serotype 1 (Wa strain) only an homologous antibody response was detected at birth; however 21 days after birth, the homologous antibody level increased four fold or greater and significant rises in heterologous neutralizing antibodies to serotypes 3 and 4 were also detected while antibody titres to serotype 2 were very low (Hoshino et al. 1987c). Piglets infected postnatally with human rotavirus also developed both homotypic and heterotypic antibody responses. Heterotypic neutralizing antibody responses were also found in calves infected with NCDV both in utero and postnatally (Wyatt et al. 1983b). All the heterologous antibody responses were found to be lower than the

homologous responses. The heterotypic responses are most likely due to sharing of VP3, the minor viral component for neutralization.

1.4.3 Protective effects of colostrum and milk

A protective effect of colostrum or milk against rotaviral infection in human babies has not been demonstrated in all studies. More bottle-fed babies were found to be infected with rotavirus than breast-fed babies (Chrystie et al. 1975, McLean and Holmes 1981, Totterdell et al. 1976). However, Weinberg et al. (1984) found no significant differences between breast- and bottle-fed babies in the numbers of those infected with rotavirus or the average age at infection. This difference may have been due to the fact that different age groups of children were involved in the studies. Chrystie et al. (1975), McLean and Holmes (1981) and Totterdell et al. (1976) studied newborn babies and found that babies became infected with rotavirus during the first four weeks of age. While Weinberg et al. (1984) studied children from birth to one year of age, and found that a similar percentage of children in breast- and bottle-fed groups became infected with rotavirus at six to eight months of age. Equivocal results have also been reported in terms of the correlation of antibody level and protection. McLean and Holmes (1981) found that the protection afforded by colostrum and milk against rotaviral infection in breast-fed babies was due to the high levels of antirotaviral antibodies (secretory IgA). Significantly less infants with high levels of antirotaviral secretory IgA became infected with rotavirus than those with low antibody levels during the first five days of age (McLean and Holmes 1981). Totterdell et al. (1982, 1983) also detected antirotaviral antibodies (IgA and IgG) in expressed milk and these antibodies were capable of aggregating rotavirus in vitro, but no difference was found between the antibody level in milk from mothers whose babies shed rotavirus and from mothers whose babies were rotavirus free. The discrepancy observed may have been due to infants in different groups being exposed to different doses of rotavirus (Totterdell et al. 1982).

Askaa et al. (1983) reported that colostrum from gilts had lower levels of antibody to rotavirus than that from sows, and piglets born of gilts became infected with rotavirus earlier (5 days of age) than those born of sows (14 days of age). Bovine colostrum was also found to protect piglets against rotaviral infection (Bridger and Brown 1981). Lambs fed with a large amount of ewe's colostrum did not develop clinical disease and delayed viral shedding for several days after

challenge. Lambs fed with colostrum for four days as part of their diet were protected from both infection and diarrhoea (Snodgrass and Wells 1978).

1.4.4 Protective roles of circulating and local antibodies

The protective roles of both circulating and local antibodies against group A rotaviral infections are not fully understood. Some investigators have correlated serum antibody levels, while others correlated faecal antibody levels, with protection against infection or related illness. Thus Ryder et al. (1985, 1986) reported that people with CF antibody titres equal to or greater than 1/4 had less chance of rotaviral infection or related diarrhoea than those with titres less than 1/4. Chiba et al. (1986) found that children with neutralizing antibody titres equal to or greater than 1/128 were resistant to reinfection although this protective effect was of short duration, lasting only for half a year. In studies with animals, Sheridan et al. (1983) observed that sero-positive mice did not develop diarrhoea after experimental infection with EDIM although viral shedding and serological responses were detected while sero-negative mice developed severe diarrhoea. However, Ward et al. (1986) could not obtain this positive correlation. Human volunteers, with antibody titres ranging from less than 1/2 to greater than 1/1600, did not always resist infection after challenge with an infectious dose of virus.

Correlation of the levels of faecal antibody with resistance to infection has also produced conflicting results. Lambs fed with ewe's colostrum (100 ml) within a few hours of birth did not resist challenge although these lambs had serum antibody titres between 1:40 and 1:80. By contrast, lambs fed with ewe's colostrum for four days after birth were protected against challenge (Snodgrass and Wells 1978). The authors claimed that the important protective factor was the continuing presence of antibody in the gut lumen. Furthermore, Sheridan et al. (1983) found that IgG antibody titres equal to or greater than 1/512 in the gut before inoculation protected one-day-old mice from developing diarrhoea after challenge with EDIM. The production of coproantibodies (specific IgA) after infection coincides with the cessation of viral shedding and clinical recovery in both porcine and human species (Corthier and Vannier 1983, Stals et al. 1984), which indicates that the presence of specific antibodies (IgG or IgA) in the gut plays a major role in the intestinal resistance to rotaviral infection. However, other workers (Eydeloth et al. 1984, Riepenhoff-Talty et al.

1981) claimed that the presence of specific antibody in the gut lumen alone is not sufficient to limit the duration of viral shedding or of diarrhoea. Moreover, Riepenhoff-Talty et al. (1981) found that the highest concentration of secretory IgA was detected in faeces of patients with prolonged diarrhoea and viral shedding.

The wide discrepancies reported for the correlation of antibody levels in both the serum and the gut lumen with protection may have been due to the differences in study design, tests used for detection of antibody, and serotypes of virus. It is, however, believed that gut immunity is of primary importance against infection caused by enteric pathogens such as rotavirus. Antibody in the serum is also involved in protection but predominantly by transfer of serum antibody into the gut lumen (Besser et al. 1988).

Many serotypes of rotaviruses have been identified in human and animal species (see section 1.3.2) so it is likely that immunity which develops after infection with one serotype may not neutralize in vitro or protect in vivo against infection with another serotype. Most of the protective studies in both man and animals have shown that only homotypic antibody protected against rotavirus infection or related diseases, even a minor serotype difference was sufficient for the lack of cross-protection (Bohl et al. 1984, Chiba et al. 1986, Gaul et al. 1982, Losonsky et al. 1986b, Woode et al. 1983, Yolken et al. 1978c). However, protection against heterologous serotype has also been reported. In a study of human rotavirus in gnotobiotic piglets, Hoshino et al. (1987c) found that piglets inoculated with Wa strain in utero were protected against clinical disease when challenged at birth with either homologous and heterologous serotypes although no neutralizing antibody to heterologous rotaviruses was detected. The heterotypic immunity observed in these studies may be due to the sharing of VP3, which is also involved in neutralization. This was supported by the report of Offit et al. (1986c) that a MAb (2G4) developed to VP3 of the OSU strain not only neutralized the homotypic strain OSU and heterotypic strains SA11, RRV and UK in vitro but also protected mice in vivo against diarrhoea after challenge with any of these viruses.

1.4.5 Control of rotaviral infection and related diseases

Control of rotaviral infection and related illness is important because of its great impact on the health and welfare of man and animals (see section 1.1). Basic measures for controlling diarrhoeal diseases

are also applicable to the control of rotaviral infection. These include general management practices such as environmental sanitation and adequate water supplies free of contamination, and specific measures such as vaccination of susceptible populations (Ryder et al. 1985). Practical measures such as fumigation, thorough cleaning of wards, handwashing of staff and patients, restricting visitors and isolating infected patients have been applied in hospitals and day care centres (Black et al. 1981, Dearlove et al. 1983, Flewett 1983, Grillner et al. 1985, Madeley et al. 1978). These measures have been reported to dramatically reduce the incidence of rotaviral infection and related diarrhoea and to stop the spread of infection. Similar measures such as fumigating and thoroughly cleaning animal sheds have also been reported to break the cycle of rotaviral infections in pigs and cattle (Lecce et al. 1978, McNulty and Logan 1983).

So far, no satisfactory vaccine has been developed to immunize man or animals against rotaviral infection. This has been largely due to the complexity of rotaviral antigens, the lack of cross protection for different serotypes of rotaviruses, the poor understanding of protection offered by serum and faecal antibodies and the unsuitability of animal models for evaluating vaccine against human rotaviral infection. Nevertheless, some heterologous protection has been observed and this has prompted some investigators to use animal rotaviruses as vaccines for human immunization. Selected viruses which are avirulent or attenuated for man could be useful if they afford protection against human rotaviral infections.

Passive immunization of young animals can be achieved either by feeding animals with colostrum or milk containing antibody, or vaccinating dams with a suitable antigen. Feeding young animals with colostrum and milk containing antibodies to rotavirus has been reported to protect against experimental infection (Bridger and Brown 1981, Bridger and Woode 1975, Lecce et al. 1976, Snodgrass and Wells 1978) especially when a large quantity of colostrum was fed at one time, or a small quantity was fed for a few days. An interesting observation from these studies is that, although feeding colostrum prevented viral shedding and related diarrhoea, it did not prevent the generation of active immunity after challenge. This finding indicates that feeding colostrum to young animals, especially those which can not get a normal supply of colostrum or are born of sero-negative dams, would give protection against rotaviral infection and associated diarrhoea during the neonatal period. This would not interfere with the production of

active immunity. By immunizing cows with each of the four human rotavirus serotypes, Brussow et al. (1987) were able to produce and concentrate antirotaviral antibodies to human rotaviruses from pooled colostrum and milk of vaccinated cows. These antibody preparations have been shown to neutralize human rotavirus serotypes 1 to 4 in vitro. If the neutralizing effect can also be demonstrated in vivo, the concentrated antibodies would be an ideal supplement for babies' diets. The same technique can also be applied to young animals to combat rotaviral infection and related losses.

To ensure that neonates get sufficient antirotaviral antibody from colostrum and milk, it would be useful to boost the mothers' immunity to rotavirus before parturition. By cross foster nursing, Offit and Clark (1985) found that newborn mice, which sucked dams immunized orally or parenterally with SA11, were protected against diarrhoea when challenged with SA11. Those which sucked on sero-negative dams were not protected. However, this approach would not be possible in man since rotaviral infection can cause clinical illness in adults and it could also be transmitted from mothers to their children.

The approach reported by Sheridan et al. (1984) of vaccinating mice before parturition with a non-infectious empty capsid of SA11 would be feasible for vaccinating human mothers. In that study, Sheridan et al. (1984) found that the empty capsids elicited high titres of rotaviral specific IgG, and to a lesser degree IgA, in colostrum and milk. Mice born of immunized dams acquired high levels of antibodies and resisted challenge with the murine rotavirus EDIM while those born of control mice shed the virus and developed diarrhoea within two days of challenge.

A modified live rotavirus-coronavirus vaccine ("Calf Guard") developed by Norden Laboratories was approved in 1979 in the USA to vaccinate cows before parturition. The initial vaccination was carried out four to 11 weeks before calving with the second vaccination 22 days later. Field trials have led to equivocal results. Hudson (1981) claimed that this vaccine significantly reduced both calf mortality and the incidence of diarrhoea in vaccinated herds when compared with herds without vaccination, and calves of vaccinated cows required less treatment when they developed diarrhoea than affected calves from non-immunized cows. In contrast, Waltner-Toews et al. (1985) found that the vaccine was not effective in preventing calf diarrhoea or calf mortality. Differences in either the incidence or the course of

diarrhoea were not found between calves from vaccinated or non-vaccinated cows when vaccination was either carried out in cows randomly within a herd or in all cows from randomly selected herds. Furthermore, no significant antibody rises were detected in colostrum between vaccinated and control cows (Myers and Snodgrass 1982, Waltner-Toews et al. 1985). It thus appears that this vaccine is not effective in preventing rotavirus associated diarrhoea in calves.

Active immunity against rotavirus can be achieved by immunizing children with attenuated or avirulent rotavirus vaccines which are capable of eliciting an antibody response before the children reach 6 to 24 months of age. Several attempts have been made to develop vaccines, using classical methods such as the "Jennerian" approach for man, with bovine or simian rotaviruses (Kapikian et al. 1986a).

The bovine rotavirus vaccine (RIT 4237 strain) is derived from the bovine rotavirus NCDV (Mebus et al. 1971). The RIT 4237 strain is a cold-adapted mutant that was attenuated during 147 passages in bovine embryonic kidney cells (Kapikian et al. 1986a). When the vaccine was tested in human adult volunteers and children, it was found safe for use in man (Vesikari et al. 1983a, b). No gastrointestinal or constitutional symptoms or changes were found in vaccinated subjects. However seroconversion was rarely detected possibly due to the presence of preinoculation antibodies. When the vaccine was given to children between six and 12 months of age, by one or two oral doses before the occurrence of rotavirus epidemics (Vesikari et al. 1984, 1985), about 50% of vaccinees seroconverted and 82-88% were protected against clinical diarrhoea lasting for more than 24 hours, during subsequent epidemics of rotaviral infection. However, the vaccine did not protect against mild diarrhoea lasting less than 24 hours in these children. Finally the vaccine was given to newborn babies at five days of age, aimed to stimulate an immunity before they reach the age when infection is most common (Vesikari et al. 1987). At one month after vaccination, seroconversion was detected in only 33% of vaccinees. During a period of 16 months, no difference was found in the incidence of rotaviral gastroenteritis between the vaccine and control groups although the severity of gastroenteritis was modified in vaccinated children. Thus the protective effects of this vaccine is doubtful.

The simian rotavirus vaccine has been prepared from the rhesus rotavirus (RRV) strain MMU18006 (Stucker et al. 1979), which has been passaged nine times in primary or secondary monkey kidney cells and

seven times in DBS-FR_hL-2 cells (Kapikian et al. 1985). The vaccine was also found to be safe in adults (Kapikian et al. 1985, 1986b). Ninety-four percent of 31 adults also developed a serological response to the vaccine. However, when the vaccine was tested in children aged from 4 months to 12 years, more children from the vaccinated group had fever (over 38°C), that clustered on days 3 or 4 post vaccination, rhinorrhoea and developed watery stools than those receiving the placebo (Anderson et al. 1986, Kapikian et al. 1986b, Losonsky et al. 1986a, Vesikari et al. 1986). Shedding of the virus was detected in vaccinated children for up to eight days and 86 to 100% of vaccinees developed a four fold or greater antibody response to the vaccine. This side effects could not be eliminated, by reducing the dose of vaccine from 10⁵ and 10⁷ to 10³ and 10⁴ plaque forming units (PFU) (Perez-Schael et al. 1987, Rennels et al. 1987). Furthermore, only half of those receiving the dose of 10³ developed antibody response. Therefore, further attenuation of this vaccine is required.

A vaccine aimed to reduce rotaviral infection and related losses in calves was developed from NCDV (Mebus et al. 1971) which was attenuated by passaging it approximately 200 times in foetal bovine kidney cells with the last 60 passages at 29 to 30°C (Mebus et al. 1973). Administration of this attenuated vaccine to hysterectomy- derived colostrum-deprived calves at 6 to 7 hours after birth did not cause diarrhoea but stimulated antibody production against the virus. Prelicensing and postlicensing field trials demonstrated that oral vaccination of calves with this vaccine at birth or 5 days after birth significantly reduced morbidity (from 50% to 17%) and mortality (from 9% to 1%) in vaccinated herds compared with that prevailing before vaccination (Mebus et al. 1972, Twiehaus et al. 1975). The beneficial effects from this vaccine could not, however, be demonstrated when double blind field trials were carried out (Acres and Radostitis 1976, Blackmer 1976, Thurber et al. 1977), since morbidity and mortality due to neonatal diarrhoea were not significantly different between vaccinated and placebo-receiving groups. However, the overall morbidity and mortality in both groups were significantly less than that recorded in previous years. Furthermore, protective effects of the vaccine could be demonstrated in sequential trials (Blackmer 1976, Thurber et al. 1977). Morbidity and mortality were significantly reduced in vaccinated calves compared with that recorded before vaccination was commenced or in unvaccinated controls in the time between each trial. The controversial results from these studies led these authors to conclude that the double blind trials were not appropriate for evaluation of this

modified live-virus vaccine. This was because vaccinated animals could spread the vaccine virus to placebo-recipients or unvaccinated controls in the same herds, so that there were no real controls. On the other hand, unvaccinated controls, if naturally infected, may shed large amounts of virulent virus which could overwhelm the resistance induced by vaccine in vaccinated animals. These two effects could equalize the morbidity and mortality rates in the vaccinated and control groups.

A modified live-virus porcine rotavirus vaccine has been shown in prelicensing trials to reduce the incidence of diarrhoeal disease and improve weight gain if administered before weaning (Westercamp 1986). In the first trial, piglets vaccinated with two oral doses or two intramuscular doses at 7 or 21 days of age gained an average of 4.7 lb (2.1 kg) and 5.9 lb (2.7 kg) more weight than control pigs, respectively, by 7 weeks postweaning. Control pigs generally developed severe scours, subsequently became dehydrated and required treatment for an average of 4 days. Vaccinated piglets developed only mild scours that did not require therapy. In the second trial on 1750 piglets, those which were vaccinated once via drinking water at weaning, had less severe diarrhoea and gained a mean of 4 lb (1.8 kg) more weight than the controls.

Modern approaches to the development of rotavirus vaccines, such as subunit and recombinant virus vaccines, have also been attempted with encouraging results. Empty-capsid vaccine prepared from SA11 by gradient centrifugation has been described earlier in this section for immunizing pregnant mice (Sheridan et al. 1984). Since both the proteins (VP3 and VP7) responsible for neutralization are located on the outer capsid (Mason et al. 1983), such vaccine may be able to stimulate immunity against several serotypes of rotaviruses.

The gene encoding VP7, the major neutralization protein, has been fully sequenced and compared between different serotypes (Charpilienne et al. 1986, Green et al. 1987, Richardson et al. 1984). The gene encoding for VP7 of SA11 rotavirus (gene 9) has been cloned and inserted into the plasmid PUMA91 (Arias et al. 1986). The fused gene vector PUMA93Z was then used to transform the MX614E strain of E.coli. Two hybrid polypeptides were produced in this system and were found to induce neutralizing antibodies to SA11 when injected into mice. If these hybrid polypeptides can be produced in large quantities, they could be used for immunoprophylaxis.

The bacterial strains containing the cloned rotaviral gene responsible for neutralization could also be used as a recombinant vaccine. If the vaccine is administered orally, the bacteria could transiently colonize the small intestine, and express the rotaviral antigen in the local environment. This may stimulate local immunity and provide protection (Flores et al. 1984). Another approach to the production of recombinant vaccines is to use a virus vector such as vaccinia virus. This approach has been used to produce recombinant vaccines for other viruses (Mackett et al. 1984, Panicali and Paoletti 1982) and has also been used recently to express the VP7 gene of SA11 (Andrew et al. 1987). A cDNA copy of the VP7 gene was incorporated into the vaccinia virus genome under the control of the vaccinia promoter. The recombinant vaccinia virus directed the synthesis of rotaviral protein in infected cells and the product was secreted. When recombinant vaccinia virus was injected into rabbits, infected animals generated antibodies which were able to recognize SA11 in an ELISA test and to neutralize the virus in a PRN assay. The neutralizing antibodies produced are serotype-specific since they reacted only with serotype 3 viruses when tested against representative strains of all four human serotypes. Now that two viral proteins (VP3 and VP7) have been shown to be involved in virus neutralization, it is necessary to take this, as well as serotype variations, into consideration when attempts are made to develop rotavirus vaccines, for use in human or animal species. The vaccinia virus vector may offer substantial advantages since a large non-essential region of the virus genome could accommodate multiple gene insertions (Andrew et al. 1987, Perkus et al. 1985), and thus allow the production of a single vaccine which could induce immunity against all serotypes of rotaviruses in man and animals.

1.5 NON-GROUP A ROTAVIRUSES

1.5.1 Introduction to non-group A rotaviruses

Before 1980, all rotaviruses detected from different animal species were thought to share a common group antigen (Flewett and Woode 1978, Woode et al. 1976b). This view has changed since Bridger (1980) and Saif et al. (1980) discovered viruses in pig faeces which were morphologically similar to, yet antigenically distinct from, the conventional rotaviruses. These non-conventional rotaviruses have since been detected in man (Rodger et al. 1982), cattle (Chasey and Davies 1984), lambs (Chasey and Banks 1984), chickens (McNulty et al. 1981),

turkeys (Saif et al. 1985) and ferrets (Torres-Medina 1987). They are commonly referred to as rotavirus-like viruses (Bridger 1980), pararotaviruses (Bohl et al. 1982), or simply atypical rotaviruses (Bridger and Brown 1985, Chasey and Davies 1984). Pedley et al. (1983, 1986) made the initial effort to distinguish these atypical from typical rotaviruses. On the basis of cross-reactivity of group antigen, genome profiles by PAGE and one-dimensional terminal fingerprinting, most rotaviruses isolated so far were classified into one of the following groups, A, B, C, D, E, F and possibly G, with the typical rotavirus as group A rotavirus. In pigs, groups A, B, C and E have so far been recovered and their schematic genomic patterns are shown in Fig. 1-2.

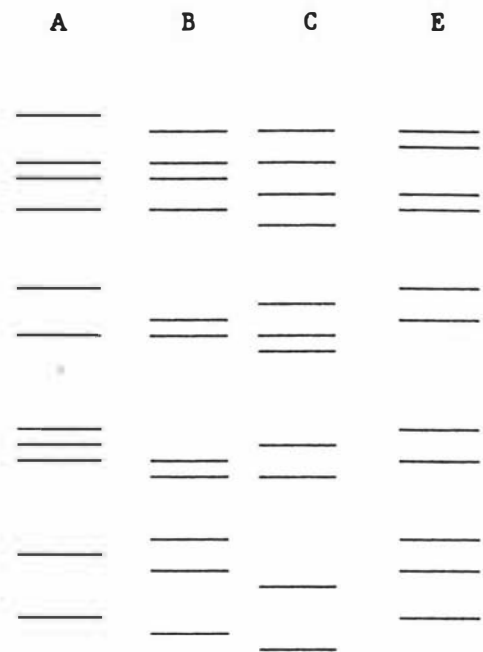


Fig. 1-2 Schematic RNA patterns of rotavirus groups A, B, C and E.

1.5.2 Group B rotaviruses

Group B rotavirus was first reported by Bridger (1980) in faeces of three week old pigs affected by PWD. It was found that the virus resembled typical rotavirus morphologically but the convalescent sera from the infected pigs failed to react with the then known rotaviruses. Further analysis by PAGE revealed that the virus contained 11 segments of double-stranded RNA with an overall size range similar to that of the conventional rotavirus but with an unusual electrophoretic pattern (Bridger et al. 1982). In comparison with the conventional rotavirus, segments 5 and 6 of the newly recognized virus ran much closer together

and the triplet of segments 7, 8 and 9 was replaced by a doublet. At the position of segment 10, there was a pair of bands in the new virus. Pedley et al. (1983) suggested the designation of group B rotavirus.

Group B rotavirus has been detected to date in man (Hung et al. 1984), pigs (Bridger 1980, Bridger et al. 1982), cattle (Chasey and Davies 1984), lambs (Chasey and Banks 1984), and rats (Vonderfecht et al. 1984, 1988). This virus has been reported to be responsible for outbreaks of diarrhoea in adults in China (Hung et al. 1984). Chasey and Banks (1984) also reported that group B rotavirus was responsible for 17 individual outbreaks of diarrhoea in lambs in England and Wales during the first three months of 1984. Other workers have observed only a mild diarrhoeal syndrome in young animals associated with infection by group B rotavirus (Bridger 1980, Chasey and Banks 1984, Theil et al. 1985). Experimental infections of gnotobiotic pigs (Theil et al. 1985), a colostrum-deprived lamb (Chasey and Banks 1984), and gnotobiotic calves and lambs (Snodgrass et al. 1984) have produced diarrhoea and anorexia commencing 15 to 24 hours post infection.

Serological evidence indicated that 86% of pigs surveyed had antibodies to group B rotavirus (Bridger and Brown 1985). However the virus has so far only been detected in the UK (Bridger 1980, Chasey and Banks, 1984), Brazil (De San Juan et al. 1987), China (Hung et al. 1984) and the USA (Theil et al. 1985). Following experimental infection of lambs with calf group B rotavirus and piglets with lamb rotavirus, all infected animals shed the virus and developed diarrhoea within 24 hours of inoculation (Snodgrass et al. 1984).

1.5.3 Group C rotavirus

In 1980, Saif et al. (1980) detected rotavirus-like virus together with calicivirus-like and 32 nm virus-like particles in diarrhoeal faeces of young piglets. Later the rotavirus-like virus was purified in gnotobiotic piglets by eliminating the other two viruses, and was tentatively referred to as pararotavirus (Bohl et al. 1982). The virus did not cross-react with, and had a different electrophoretic pattern from, the group A and B rotaviruses and was classified as group C rotavirus (Pedley et al. 1983). The characteristic of group C rotaviral patterns by PAGE is that segment 7 runs close to segment 6 rather than to segment 8 as in group A rotavirus.

Group C rotavirus has so far only been detected in pigs (Saif et

al. 1980, Chasey and Davies 1984), humans (Dimitrov et al. 1983, Rodger et al. 1982) and ferrets (Torres-Medina 1987). This virus is usually associated with mild diarrhoea of young individuals. Experimental infections of gnotobiotic piglets with group C rotavirus have resulted in diarrhoea at 17 to 30 hours after infection (Bohl et al. 1982, Snodgrass et al. 1984). Piglets less than 5 days of age were more severely affected than older pigs and some deaths occurred in the young but not in the older pigs (Bohl et al. 1982). However the human group C rotavirus failed to infect 5 day old gnotobiotic pigs although these pigs were later infected with porcine group C rotavirus and developed diarrhoea (Bridger et al. 1986).

Preliminary investigations by serology suggested that group C rotaviral infection in human populations was sporadic (Bridger et al. 1986, Espejo et al. 1984), yet it has been detected in Australia (Rodger et al. 1982), Brazil (Pereira et al. 1986), Bulgaria (Dimitrov et al. 1983), France (Nicolas et al. 1983), Hungary (Szucs et al. 1987), Mexico (Espejo et al. 1984), and the UK (Bridger et al. 1986). In contrast, Bridger and Brown (1985) reported that 77% of pigs in the UK had antibodies against group C rotavirus, but infection of pigs with this virus has only been recorded in Belgium (Pedley et al. 1983), the UK (Chasey and Davies 1984) and the USA (Bohl et al. 1982).

1.5.4 Other groups of rotaviruses

The designation of group D rotavirus was given by Pedley et al. (1986) to a chicken rotavirus (132) detected in 1981 (McNulty et al. 1981). Serological evidence indicated that 70% of chickens had been infected with the virus. This was similar to the prevalence of group A rotaviral infection in chickens (McNulty et al. 1984a).

A rotavirus detected in pigs by Chasey et al. (1986) was classified as group E rotavirus (Pedley et al. 1986). The virus caused only mild diarrhoea even in gnotobiotic piglets. Serological evidence indicated that only pigs over 10 weeks of age were infected and none of the animals surveyed under 10 weeks of age had antibodies to the virus (Chasey et al. 1986).

The virus strains A4 and 555 which were identified in chickens (McNulty et al. 1984b) have been designated as group F and possibly group G rotaviruses by Bridger (1987). Other unclassified rotaviruses include those strains detected in chickens (Bellinzoni et al. 1987b),

turkeys (Theil et al. 1986, Theil and Saif 1987) and pheasants (Gough et al. 1985).

1.6 AIM AND SCOPE OF THE PRESENT STUDY

From the preceeding review, it is clear that there are gaps in our knowledge concerning the epidemiology of rotaviral infections and the immunity they induce. A better understanding of these aspects would greatly assist the construction and development of efficient control measures to combat rotaviral infections and related diseases in the future. The aim of the present study was to investigate the epidemiology of rotaviral infections and the immune responses in pigs following natural infection. It is hoped that the information obtained from this investigation will improve the understanding of the epidemiology of rotaviral infections not only in pigs but also in other species of animals including man.

Before the actual investigations were carried out, tests for the detection of rotaviruses in pig faeces and antirotaviral antibodies in serum and faecal samples of pigs were either standardized or developed in the laboratory. This is described in chapter two.

The investigations on the epidemiology of group A rotaviral infection including the patterns of shedding and transmission, and the antibody profiles in serum and in faeces from birth to two months of age in relation to natural infection from pigs at Massey University Piggery are described in chapters three and four.

The study of the pattern of shedding of group A rotavirus was extended to another five New Zealand piggeries and isolates of group A rotaviruses from these five piggeries and from Massey Piggery were compared by PAGE. In addition, non-group A rotaviruses were also detected in these piggeries. All these results are summarized in chapter five.

The implications of all these findings to the control of rotaviral infection in pigs and other animals is discussed in chapter six.

Chapter Two

GENERAL MATERIALS AND METHODS AND

STANDARDIZATION OF VIROLOGICAL AND SEROLOGICAL TECHNIQUES

2.1 GENERAL MATERIALS AND METHODS

General materials and methods applied in this study are described in this section. The adaptation and standardization of ELISA tests used for the detection of group A rotaviral antigen and antibody are described in sections 2.2 and 2.3, respectively. Other specific procedures will be given in relevant chapters.

2.1.1 The piggery

Most of the study described in this thesis was carried out in a 60-sow piggery run commercially by Massey University. This piggery will be referred to as Massey Piggery. There were approximately 600 pigs of all ages on the premises at any one time. Management is conventional with three distinct rearing stages, i.e. sucking, weaning and fattening. In the farrowing unit, up to four sows are accommodated in each of four farrowing rooms. Piglets in this piggery are usually weaned at about five weeks of age, then transferred to a separate weaner house. At eight to ten weeks of age, weaner pigs are moved to fatterer houses where they are kept until slaughter at pork or bacon weight, usually at 20 to 22 weeks of age. General cleaning by hosing with pressurized water is carried out daily throughout the piggery. Cleaning and disinfection with a commercial potassium hydroxide disinfectant (Multikleen, KW Products Ltd., Auckland, New Zealand) is undertaken as a routine procedure in the farrowing rooms after each batch of piglets has been weaned.

2.1.2 Collection and processing of faecal samples

Faeces, faecal swabs (cotton wool) and intestinal contents collected from pigs were taken directly to the laboratory for processing. In the laboratory, these samples were extracted with phosphate buffered saline (PBS) at a ratio of one to four. After homogenization on a Whirlimixer (Fisons Scientific Apparatus, Loughborough, England), the samples were centrifuged at 1,000 g for 10

min. The supernatant was harvested and stored at -70°C .

2.1.3 Collection and processing of blood samples

Blood was collected from piglets by venipuncture of the anterior vena cava and from sows by venipuncture of the ear vein using a sterile needle and sterile evacuated glass tube ("Neo-Tube", Nipro). The tubes were kept at 4°C overnight and then centrifuged at 1000 g for 10 min before the serum was poured off and stored at -20°C .

2.1.4 Cell cultures

Throughout the project, MA-104, a monkey kidney cell line, was passaged serially by conventional methods and used for virus titration, serum neutralization and production of viral antigen for ELISA. Growth medium consisted of 5 to 10% (v/v) foetal calf serum (FCS) in Eagle's Minimum Essential Medium (MEM) with the addition of penicillin (100 units/ml), streptomycin (10 mg/ml), kanamycin (10 mg/ml) mixture (PSK) to 1% (v/v). The maintenance medium was the same as the growth medium except that FCS was omitted. All the materials used for cell culture including media, culture flasks, microtitre plates and glassware were sterilized by ultrafiltration, γ -irradiation or by autoclaving as appropriate.

MA-104 cells were usually passaged in 20 ml plastic tissue culture flasks (Nunc, InterMed, Denmark) with approximately 1×10^5 cells/ml. Confluent monolayers were formed after incubation at 37°C in a CO_2 incubator for 3-5 days. For further passage, cell monolayers were trypsinized with antibiotic-trypsin-versene (ATV) mixture and viable cells were counted by the trypan blue exclusion method. The suspended cells were then distributed into culture flasks, culture tubes at approximately 1×10^5 cells/ml or into microtitre plates at 1.75×10^5 cells/ml according to requirement.

2.1.5 Production of virus stocks

Porcine rotavirus isolate SW20/21 (Woode et al. 1976a) was kindly supplied by Dr. J.C. Bridger, Institute of Animal Health, Compton Laboratory, Compton, Newbury, Berks, RG16 ONN, England.

The virus was cultured in MA-104 cells. Confluent monolayers in culture flasks (20 ml) were washed twice with PBS, the medium was then

changed to MEM without serum but with 0.5 µg porcine trypsin (type IX, Sigma, St Louis, Mo) per ml and the flasks were inoculated with 2 ml of virus stock (approximately 0.5 tissue culture infective dose (TCID) per cell). Viruses were harvested when most cells detached from the culture flask wall, usually within five days after infection and were stored at -70°C.

The same procedure was used for the production of viral antigen for the ELISA test, except that an equal number of control cell cultures were included and treated identically but were not infected with virus.

2.1.6 Titration of rotaviruses

For titration of rotaviruses, confluent monolayers of MA-104 cells were prepared in 96-well microtitre plates (Nunc, InterMed, Denmark) by dispensing 100 µl of cell suspension containing 1.75×10^5 cells per ml into each of the 96 wells. After the monolayers were fully developed, each well of the microtitre plate was inoculated with 100 µl of a serial 10-fold dilution of virus preparation in maintenance medium containing 0.5 µg trypsin per ml. Four wells were inoculated with each dilution and an appropriate number of control wells were included. Inoculated plates were incubated at 37°C for six days and then examined for the development of cytopathic effect (CPE). Virus titres were calculated by the method of Reed and Muench (1938) and expressed as a TCID₅₀/ml.

2.1.7 Virus neutralization

The procedure used was similar to that described for virus titration. Pig sera were inactivated at 56°C for 30 min prior to use and then doubling dilutions made in MEM starting from 1/2. One hundred µl of each serum dilution was mixed with an equal volume (100-1000 TCID₅₀) of virus suspension diluted in MEM, containing 0.5 µg trypsin per ml, and incubated at 37°C for one hour. The mixture was then transferred onto preformed monolayers of MA-104 cells which were incubated at 37°C for six days for the development of CPE. The titre was recorded as the highest dilution of test serum which totally neutralized the virus. All sera were titrated in quadruplicate, and on each plate a virus back titration was performed.

2.2 DETECTION OF PORCINE GROUP A ROTAVIRUS IN FAECES BY USING

A COMMERCIAL ELISA TEST, AND COMPARISON WITH EM AND PAGE

2.2.1 Introduction

Since rotaviruses are not easily adapted to cell culture, other methods such as ELISA, EM and PAGE have been developed to detect the virus in clinical specimens (see 1.2.5). Among these, the ELISA test is the most commonly used for the detection of rotavirus in faeces. Many ELISA tests, complete with all reagents, are available commercially. At the time when this project was initiated, there were at least two such kits on the market. One is the ELISA kit manufactured by Dakopatts (Copenhagen, Denmark) and the other the Rotazyme by Abbott Diagnostics (North Chicago, Il). Both of the tests were originally developed for the detection of human group A rotavirus and were reported to be highly sensitive and specific compared with EM (Grauballe et al. 1981, Hammond et al. 1982). The ELISA kit from Dakopatts was selected for the investigation into the epidemiology of porcine rotaviral infection in this study because it had been evaluated for the detection of group A rotaviruses in cattle (Grauballe et al. 1981) and pigs (Askaa and Bloch 1981). This kit has also been widely used by others (Askaa et al. 1983, Utrera et al. 1984) for the detection of porcine group A rotavirus.

Before the ELISA kit was used for the present investigation, it was evaluated for its sensitivity and specificity in this laboratory and the results were compared with those obtained by EM and PAGE.

2.2.2 Materials and methods

Faecal samples: Eighty nine faecal samples were collected from pigs of various ages from Massey Piggery, and from another piggery nearby. Faeces were taken to the laboratory and processed as described in section 2.1.2.

Detection of group A rotavirus by the ELISA kit: The ELISA kit was purchased from Dakopatts (Denmark) and consisted of polystyrene plates (Nunc-Immuno Plate I, InterMed, Denmark), catching antibody produced in rabbits against human group A rotavirus, control antibody (preimmune serum preparation), conjugate which is rabbit antirotaviral antibody

conjugated with horse radish peroxidase, and blocking agent (normal rabbit serum). The test was performed according to the manufacturer's instructions with slight modifications. Briefly, alternate wells in the plates were coated with 100 μ l of catching antibody or control antibody diluted 1/50 in 0.05M carbonate-bicarbonate buffer (pH 9.6). After incubation for an hour at room temperature, the plates were washed ten times with PBS containing 0.5% tween 20 (PBS-Tween) (pH 7.2) using a semi-automatic MINIWASH Washer Aspirator (Dynatech, Virginia, USA) and shaken dry. Faecal extracts (100 μ l) from each sample, as prepared in section 2.1.2 were directly added to two wells, one precoated with antirotaviral antibody and another with control antibody. The plates were again incubated and washed as above and 100 μ l of blocking agent diluted 1/25 in PBS-Tween plus 1% bovine serum albumin (BSA) was added to each of the wells. Half an hour later 100 μ l of conjugate diluted 1/250 was added to the plates which were incubated for another 30 min and washed as above. The plates were rinsed with 0.1M citric acid-phosphate buffer (pH 5) before substrate was added. The substrate was freshly prepared in citric acid-phosphate buffer consisting of 0.04% (w/v) ortho-phenylenediamine (OPD) (E. Merck Darmstadt, Germany) and 0.012% H_2O_2 . The reaction was stopped after 15 min by the addition of 1M H_2SO_4 . Finally the colour reaction was read using a SLT-210 automatic multi-plate photometer (SLT-Labinstruments, Austria). Samples with an ELISA specific absorbance (ESA) value (test value - control value) of 0.100 or over were considered to be positive for group A rotaviral antigen.

Detection of rotavirus by EM: Negative staining was used to detect rotavirus in faeces. Faecal extracts as described in 2.1.2 were further processed for EM. Five ml of each sample was centrifuged at 4000 g for 30 min. The supernatant was laid onto a 45% (w/v) sucrose cushion and centrifuged at 150,000 g for 2 h. The pellet was then suspended in 0.1 ml of distilled water.

One drop (0.1 ml) of 1% BSA was added to a carbon-formar coated grid, followed by a drop of virus. After washing with distilled water, 2% phosphotungstic acid (pH 7.2) was then added. Excess fluid was absorbed with filter paper at each step and the grid was finally examined under a Phillips EM 201 (Phillips, Eindhoven, the Netherlands). Ten minutes was allowed to examine each grid and samples with at least three clear rotaviral particles were considered to be positive.

Detection of rotavirus by PAGE: For electrophoresis, the procedure

described by Rodger and Holmes (1979) and Dyall-Smith and Holmes (1984) was used. Half ml of faecal extract as prepared in 2.1.2 was mixed with 0.5 ml of 0.1M sodium acetate buffer (pH 5) containing 1% sodium dodecyl sulfate (SDS), and 0.5 ml phenol-chloroform (3:2) mixture. After mixing for one minute, the samples were centrifuged for two minutes at maximum revolutions (10,000 g) on an Eppendorf micro-centrifuge 5415. The supernatant was removed and used for electrophoresis. Ten percent polyacrylamide slab gels with a 3% stacking gel were prepared. After loading the samples, electrophoresis was conducted at a constant current of 10 mA for each gel for 16 h at 4°C, using the discontinuous buffer system described by Laemmli (1970), but without SDS. The gel was then stained with silver nitrate as described by Herring *et al.* (1982) to visualize the RNA bands. The gel was fixed in 10% ethanol containing 0.5% acetic acid for 30 min, then stained with 0.011M silver nitrate for 1 h in the dark. After rinsing twice with distilled water, the gel was developed using a 1% (v/v) formaldehyde solution containing 0.75M potassium hydroxide. The reaction was stopped by transferring the gel to a 5% acetic acid solution. RNA bands shown on the gel were photographed.

Comparison of the three tests for detection of group A rotavirus: The results from the three tests were compared for sensitivity and specificity using the results from the EM examination as a bench mark. Furthermore the sensitivity of these three tests were compared for detection of rotavirus with serially diluted samples. For this purpose, three faecal extracts and one cell adapted rotavirus SW20/21 (Woode *et al.* 1976a) were serially diluted ten-fold up to 10^{-4} with PBS and each dilution was then processed for ELISA, EM and PAGE as described above.

2.2.3 Results

Of the 89 samples examined, 28 yielded an ESA value of 0.1 or over by the ELISA test, 26 showed rotaviral particles under the EM and 20 gave typical rotavirus RNA bands on PAGE. A total of 29 samples were found to be positive for rotavirus by one or more of the three tests. Table 2-1 shows the results obtained with the combinations of these tests.

Table 2-1. Detection of rotavirus in faeces by ELISA, EM and PAGE

Tests	Number of specimens
ELISA ⁺ , EM ⁺ , PAGE ⁺	20
ELISA ⁺ , EM ⁺ , PAGE ⁻	5
ELISA ⁺ , EM ⁻ , PAGE ⁺	0
ELISA ⁺ , EM ⁻ , PAGE ⁻	3
ELISA ⁻ , EM ⁺ , PAGE ⁺	1
ELISA ⁻ , EM ⁻ , PAGE ⁻	60
TOTAL	89

Of the 29 samples with rotavirus detected, only one was negative by ELISA, but positive by EM. However, this sample showed a RNA pattern by PAGE similar to that reported for group C rotavirus (Pedley *et al.* 1983). Therefore, the ELISA test had a sensitivity of 100% and a specificity of 96.8%, and the PAGE had a sensitivity of 80.8% and a specificity of 100% in detecting group A rotavirus when compared with EM.

Those EM positive samples (apart from the one with group C rotaviral pattern by PAGE) gave a mean ESA value of 1.3360 (ranging from 0.102 to 2.000) in the ELISA test. The three samples giving ESA values over 0.1 but being negative by EM had ESA values of 0.114, 0.129 and 0.790, respectively. The remaining samples had an average ESA value of 0.0127 ± 0.01773 (mean $\pm$ standard deviation (SD)).

Table 2-2 shows the results of these three tests in detecting group A rotavirus in four serially diluted samples. It can be seen that the ELISA test is approximately ten-fold more sensitive than EM which in turn is ten-fold more sensitive than PAGE.

Table 2-2. Detection of group A rotavirus by ELISA, EM and PAGE on four serially diluted samples

Dilution of samples	Numbers of samples positive by		
	ELISA	EM	PAGE
10^0	4	4	4
10^{-1}	4	4	3
10^{-2}	4	2	0
10^{-3}	2	0	0
10^{-4}	0	0	0

2.2.4 Discussion

This study shows that the ELISA kit developed originally for the detection of human group A rotavirus is also highly sensitive and specific for the detection of porcine group A rotavirus under the experimental conditions described. Grauballe *et al.* (1981) evaluated this test and found that its sensitivity for the detection of group A rotavirus in faeces from man and cattle was 96% and 98%, respectively, when compared with EM. Askaa and Bloch (1981) reported that the ELISA kit was also very sensitive (about 90%) for the detection of group A rotavirus in pig faeces. In the present study, the test was found to be 100% sensitive and 97% specific compared with EM for detecting group A rotavirus in faeces of pigs.

Both Grauballe *et al.* (1981) and Askaa and Bloch (1981) encountered some samples which were positive by EM but negative by ELISA. In this study, one such sample was also found. However, this sample possessed a RNA pattern similar to group C rotavirus when analysed by PAGE. In those tests carried out by Askaa and Bloch (1981) and Grauballe *et al.* (1981), however, samples with positive results in EM but negative in ELISA were not verified by PAGE and these might have been due to the presence of non-group A rotaviruses.

The setting of a positive-negative cut-off point is a critical

factor in the determination of sensitivity and specificity of a particular test. The manufacturer of the Dakopatts ELISA kit recommends that an ESA value of 0.100 should be used as the cut-off point. The results from this study support this recommendation since all the samples positive by EM produced ESA values over 0.100. Furthermore those samples which did not yield ESA values equal to or greater than 0.100 and which were also negative in EM had a mean ESA value of 0.0127. If the cut-off point were set at the mean of the negatives plus three times the SD of the mean, then 0.070 should have been used as the cut-off point in this study. The failure to detect rotavirus by EM in three samples which had ESA values over 0.100 may have been due to the greater sensitivity of the ELISA test rather than to it being less specific in detecting group A rotavirus than the EM. These samples produced ESA values within the range of ESA values obtained with those samples positive in EM. This claim is further supported by the fact that the ELISA was approximately ten times more sensitive than EM for detecting group A rotavirus in the serially diluted samples.

In summary, the three tests used for the detection of group A rotavirus have both advantages and disadvantages. The ELISA kit is highly sensitive and specific but is limited to detection of group A rotavirus only. The EM has the advantage over ELISA of detecting the presence of both group A and non-group A rotaviruses, but it can not distinguish one from another. PAGE is the only one of the three tests which can distinguish group A from non-group A rotaviruses, but its sensitivity is relatively low. In this study the sensitivity of PAGE was found to be 80.8% for detecting group A rotavirus. It was therefore decided to use the ELISA kit throughout the project for the investigation of the epidemiology of group A rotaviral infection in pigs; EM was used to verify the presence of rotavirus in some equivocal samples and PAGE to detect variations between group A rotaviruses, and to distinguish group A from non-group A rotaviruses.

2.2.5 Conclusions

1. The ELISA kit from Dakopatts developed originally for the detection of human group A rotavirus was found to be very sensitive and specific in detecting porcine group A rotavirus. When compared with EM, it has a sensitivity of 100% and a specificity of 96.7% in detecting group A rotavirus.

2. The PAGE technique has a sensitivity of 80.8% and a specificity of 100% in detection of group A rotavirus. This technique has the advantages over EM and the ELISA test of detecting variations in RNA patterns of rotavirus and distinguishing group A from non-group A rotaviruses.

3. When serially diluted samples were examined by these three tests, it was found that the ELISA test was approximately ten times more sensitive than EM, which is in turn approximately ten times more sensitive than PAGE.

4. The ELISA test from the Dakopatts is a valuable tool in investigation of the epidemiology of group A rotaviral infection in both man and animals.

2.3 THE DEVELOPMENT OF AN ELISA TEST FOR DETECTING ANTIBODIES

AGAINST GROUP A ROTAVIRUS IN PIG SERA

2.3.1 Introduction

A variety of serological tests have been developed for measurement of antibodies against group A rotavirus in human and other animal species. These include complement fixation (CF) (Blacklow et al. 1976, Kapikian et al. 1975), indirect immunofluorescence (IF) (Blacklow et al. 1976, Bryden et al. 1977), counter-immunoelectrophoresis (CIEOP) (Bryden et al. 1977, Linhares et al. 1981), immunoadhesion haemagglutination (IAHA) (Kapikian et al. 1983), serum neutralization using either plaque reduction (PRN) (Matsuno et al. 1977) or fluorescent focus reduction (FFN) assays (Chiba et al. 1986), immune electron microscopy (IEM), radio-immunoassay (RIA) (Watanabe and Holmes 1977) and ELISA (Corthier and Franz 1981, Ghose et al. 1978, Hess and Bachmann 1981, Yolken et al. 1978b). Following the successful cultivation of human and some animal rotaviruses (Sato et al. 1981, Urasawa et al. 1981), ELISA has become the most widely used test among these antibody assay systems. Compared with other antibody assays, the ELISA test is easier to perform, more sensitive and more economical (Bishop et al. 1984, Ghose et al. 1978). In this section the development and standardization of an ELISA test for the measurement of antibodies to group A rotavirus in pig sera is described.

2.3.2 Materials and methods

Preparation of antigen: The virus used as the ELISA antigen in this study is strain SW20/21 of porcine rotavirus (Woode et al. 1976a) which was propagated in MA-104 cells as described in 2.1.5. The antigen was purified as follows: The cultures were frozen and thawed three times to release all cell associated virus. Equal volumes of infected and uninfected cells were then treated by the polyethylene glycol (PEG) precipitation method. Basically, 500 ml of each material from infected and uninfected cell cultures were adjusted to 1.0 M NaCl and 8% (w/v) PEG (of average molecular weight 6000-7500). The preparations were stirred for 2 hours at 4°C, then left to stand overnight. The precipitate that formed was recovered by centrifugation in a Sorvall GSA rotor at 8000 rpm (10,000 g) for 30 min and resuspended in 30 ml of 0.1M Tris buffer. The resuspended virus was mixed with an equal volume of

chloroform and then centrifuged for 30 min at 1000 g. The aqueous phase was removed and the chloroform phase was further extracted with 0.1 M Tris buffer and centrifuged as above. The two aqueous phases were combined and pelleted through a 45% sucrose cushion at 100,000 g for 90 min. The pellet was suspended in 10 ml of 0.001 M Tris buffer and used as the ELISA antigen.

ELISA procedures: The procedure for this indirect ELISA was based on that of Engvall and Perlmann (1971) as modified by Voller et al. (1979). Tests were performed in 96 well Nunc polystyrene microplates.

The detailed procedures were as follows: 100 μ l of antigen or control antigen diluted in 0.05M carbonate-bicarbonate buffer (pH 9.6) were added to appropriate wells in Nunc plates. These plates were incubated at 37°C for 3 h and washed six times with PBS-Tween using the Miniwash Aspirator. Each well was then filled with 100 μ l PBS-Tween-1%BSA and incubated at 37°C for 30 min. After emptying the wells, 100 μ l of each serum sample, appropriately diluted in PBS-Tween-1%BSA, was added and the plate was incubated overnight at 4°C. The plates were washed ten times as above. The enzyme labelled antiglobulin conjugate, diluted in PBS-Tween-1%BSA, was added to all wells and the plates incubated at 37°C for 2.5 h before being emptied and washed as above. Substrate solution (100 μ l per well) was added and the plate was incubated in the dark for 30 min at room temperature. The substrate used was 0.04% w/v OPD and 0.012% H_2O_2 in phosphate-citrate buffer (pH 5.0) prepared immediately before use. After the reaction was stopped with 1 M H_2SO_4 , the absorbance value of each well was measured at a wavelength of 490 nm using the SLT-210 automatic multi-plate photometer. Two wells that had been incubated without antigen, serum or conjugate but containing substrate were included in each plate as blanks.

Standardization of ELISA: Chequer-board titrations were performed to determine the optimum concentrations of antigen, serum and conjugate for routine use.

For the determination of optimum antigen concentration, both viral and control antigens (at dilutions from 1/50 to 1/800) were titrated against a positive and a negative reference serum (both at dilutions from 1/100 to 1/6400). Both sera had been titrated in a serum neutralization (SN) test and the positive serum had a SN antibody titre of 1:64 against SW20/21. The conjugate (see below) was diluted to

1/1000. "No antigen" and "no serum" controls were included on each plate.

The optimal dilution of antigen was then used for the determination of optimal dilutions of serum and conjugate. The conjugate used in this study was sheep anti-pig IgG, IgM, IgA (whole molecules) conjugated with horse-radish peroxidase (Eivai Bios Laboratories). Four dilutions of conjugate (1/500, 1/1000, 1/2000 and 1/4000) and eight doubling dilutions of serum from 1/50 to 1/6400 were titrated against viral and control antigens. Both serum and conjugate were diluted in PBS-Tween-1%BSA. Control wells of "no serum" incubated with PBS-Tween-1%BSA only during the serum incubation step were included for each conjugate dilution against viral and control antigens.

Titration of test sera: Twenty four selected sera, used in these titrations, were collected from piglets and sows from the piggery described in section 2.1.1. These sera were assayed for rotaviral neutralizing antibodies in MA-104 cells as described in section 2.1.7. Each of these sera, in eight doubling dilutions from 1/50 to 1/6400, was titrated against the optimum dilution of a viral antigen and against a control in the ELISA test. In each assay, a positive serum was included as a control.

2.3.3 Results

Standardization of the ELISA test: After titration of viral and control antigens at different dilutions against serum and conjugate, it was found that the negative serum from the SN test had high optical density (OD) values at lower dilutions of viral antigen and serum. OD values from the control antigen were very low at all dilutions of serum. It was therefore decided that a dilution of viral antigen which produced an OD value over 1.000 with the positive serum diluted 1/100 would be used routinely. In this study a 1/200 dilution of viral antigen satisfied this requirement, and the same dilution of the control antigen was also used.

Eight two-fold dilutions of the positive serum, starting at 1/50, were titrated with the optimum dilution of viral and control antigens (1/200), and the conjugate at a dilution of 1/1000. The lowest dilution of serum gave an OD value within the range of the ELISA reader (ie 0-2.000) and the highest dilution produced an ESA value less than 0.1. The titration of the test sera (see below) demonstrated that most sera

tested were titratable within this dilution system. Those sera which still yielded ESA values above 0.100 at a dilution of 1/6400 were further diluted and retested. Non-specific reactions between viral antigens and the conjugate and between control antigens and serum were minimal (OD values < 0.050).

Therefore, viral and control antigens at 1/200, conjugate at 1/1000 and doubling dilutions of serum from 1/50 to 1/6400 were chosen for the titration of test sera.

Titration of test sera: Each of the 24 test sera, at doubling dilutions from 1/50 to 1/6400, was titrated against the viral and control antigens.

The endpoint titres were used to express the results of the ELISA test in this study. For the determination of antirotaviral antibody titres in individual sera, an ELISA specific absorbance (ESA) value was calculated by subtracting the absorbance of the control antigen well from that of the test antigen well. The ESA values at different dilutions of sera were then plotted against the reciprocal of the dilutions. Titration curves of eight out of the 24 sera are shown in Fig. 2-1. These curves flattened towards the highest dilutions of serum, especially when the ESA value reached 0.100 or less. Thus an ESA value of 0.100 was selected as the positive-negative cut-off point. The

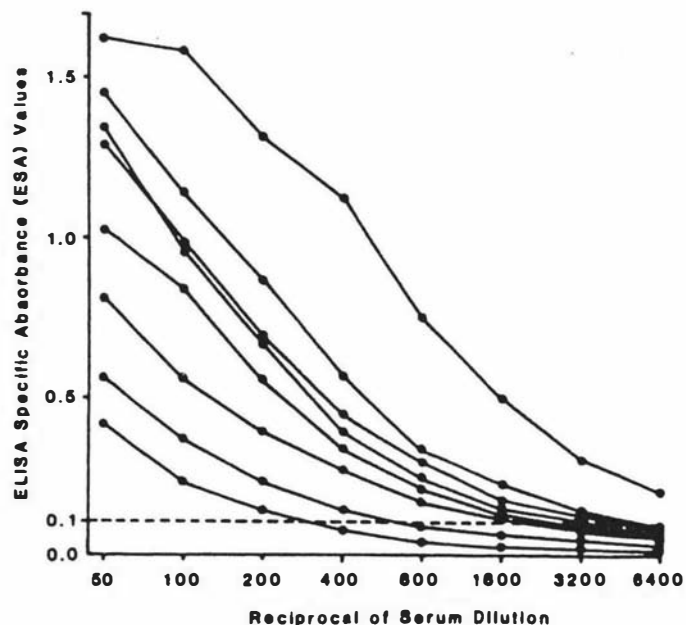


Fig. 2-1. Titration curves of eight test serum samples.

highest dilution of a serum sample giving an ESA value of 0.100 or greater was recorded as the ELISA antibody titre for that sample. A serum sample with an ESA value less than 0.100 at a dilution of 1/50 was considered negative, and the lowest ELISA titre possible became 1/50. Control antigen absorbences were always low and never exceeded 0.100.

All these sera were also assayed for SN antibodies to rotavirus in MA-104 cells. The SN titres and the ELISA titres of these 24 sera are summarized in table 2-3.

Table 2-3. Reciprocal antibody titres against group A rotavirus

Serum No.	SN	ELISA
1	128	12800
2	32	6400
3	8	3200
4	4	1600
5	4	800
6	-*	400
7	-	400
8	-	200
9	32	3200
10	8	1600
11	4	800
12	-	800
13	32	12800
14	16	6400
15	8	3200
16	8	1600
17	32	3200
18	32	3200
19	32	3200
20	32	3200
21	32	6400
22	32	6400
23	16	3200
24	16	3200

* Antibody was not detected.

Among the 24 sera, 20 were positive in both the SN and ELISA tests with ELISA titres between 1/800 and 1/12800. Four samples which were negative in the SN test gave positive results in the ELISA test with titres between 1/200 and 1/800. The antibody titres obtained in the ELISA test were 100 to 400 (average 220) times greater than those obtained in the SN test. The correlation coefficient of the titres obtained from the two tests was 0.82.

The positive serum sample which was included as a control gave very consistent results with titres of 1/6400 on nine occasions and 1/12800 only once.

2.3.4 Discussion

ELISA tests have been developed for detecting antibodies to group A rotavirus in sera of many species including man and pigs (Bishop et al. 1984, Corthier and Franz 1981, Ghose et al. 1978, Yolken et al. 1978b). These tests are usually more sensitive than other assay systems such as complement fixation and fluorescent-antibody assays. The ELISA test described in this section for the measurement of antibody against group A rotavirus in pig sera was also more sensitive than the SN test.

Rotaviruses have a double-shelled structure, composed of an outer capsid and an inner capsid (Bridger and Woode 1976, Flewett et al. 1974a, b). Two proteins (VP7 and VP3) on the outer capsid are responsible for neutralization specificity of rotavirus (Greenberg et al. 1983a, c, Kalica et al. 1981b, Offit and Blavat 1986). The SN test, therefore, detects only antibodies specific to these viral proteins. The ELISA test may detect not only the neutralizing antibody, but also antibodies induced by other viral components such as proteins located on the inner capsid. The increased sensitivity of ELISA over SN in this study may, therefore, be due to the wider range of antibodies detected. Samples from four animals with undetectable SN antibodies yet possessing ELISA antibodies may indicate that small amount of antibodies, insufficient to neutralize virus, may still be detected by ELISA.

The setting of the "cut-off point" between positive and negative is a crucial element in every assay for specific antibody activity. In the ELISA system, it is usual to set the minimum positive response value at two or three times the mean of the negative group, or at the mean negative value plus two (or three) SD of the mean if more reference sera

are tested. However, a more statistically valid method is to determine the upper limit of normal by setting this at the 97.5th or 100th percentile (Savigny and Voller, 1980). All these methods require a number of known negative sera. Unfortunately, most pigs become infected with rotavirus early in their life and possess some levels of antibody. Even those without SN antibody may have ELISA antibody at a detectable level. Four such sera, without detectable SN antibody, gave positive results in the ELISA test. Thus in this study, the setting of ESA at 0.1 as the positive-negative cut-off point was based on the titration curves of those 24 test sera since the titration curves from most sera became flatter when their ESA values reached 0.1 or below. Furthermore, non-specific reactions between serum and control antigen never exceeded an OD value of 0.100. This cut-off point has nevertheless been used by many authors for detection of both rotaviral antigen and antibody (Bishop et al. 1984, Grauballe et al. 1981).

It was decided to use the ELISA test described in this section for the detection of rotaviral antibodies in sera and faeces of pigs for the studies described in chapter four.

2.3.5 Conclusions

1. The ELISA test developed in this study is more sensitive than SN in detecting antirotaviral antibodies in pig serum. Those samples with detectable SN antibodies all had ELISA antibodies. Samples with undetectable SN antibody also had some levels of antirotaviral antibodies detected by the ELISA. The increased sensitivity of the ELISA test over SN is due at least in part to the fact that ELISA detects a wider range of antibodies than SN.
2. Comparing the titres of 24 pig sera as detected by the SN test and the ELISA test revealed a correlation coefficient of 0.82 between the two tests.
3. The results of the ELISA test are reproducible.
4. The procedures used in this study, including PEG precipitation, chloroform treatment and ultracentrifugation, appeared to be efficient for the purification and concentration of rotavirus from cell cultures, and yielded a suitable antigen preparation for the ELISA test.

5. The ELISA test has potential use for sero-epidemiological investigation of rotaviral infection in pigs.

Chapter Three

EPIDEMIOLOGY OF PORCINE GROUP A ROTAVIRAL INFECTION

3.1 INTRODUCTION

Since the detection of group A rotavirus in pig faeces in 1975 (Rodger et al. 1975a), infection by this virus has been found to be widespread in pig breeding herds, including New Zealand (Askaa et al. 1983, Debouck and Pensaert 1983, Durham et al. 1979, Svensmark 1983, Utrera et al. 1984). Two common syndromes in pigs have been associated with infection with group A rotavirus. First, there is the syndrome referred to as milk scours, white scours or 3-week-old scours in young sucking piglets (Bohl 1979, Bohl et al. 1978, Mouwen 1971). Secondly, there is the syndrome characterized by postweaning diarrhoea (PWD) in newly weaned pigs (Barrow et al. 1979, Lecce and King 1978, Tzipori et al. 1980a).

Shedding of group A rotavirus is common in sucking and weaner piglets from two to six weeks of age, which coincides in time with the two diarrhoeal syndromes observed in pigs (Askaa et al. 1983, Debouck and Pensaert 1983, Utrera et al. 1984). Shedding of rotavirus is a rare occurrence in piglets less than 10 days of age. Shedding of rotavirus by fatter pigs (over two months of age) has been demonstrated after both experimental infection (Bernard et al. 1984) and under natural conditions (Grom et al. 1984). Reports on the shedding of rotavirus by sows have been inconsistent; Debouck and Pensaert (1983) found that faeces from sows were always free of rotaviral antigen, while Benfield et al. (1982) detected rotavirus in sows both before and after farrowing.

The detection of rotavirus in sows before and after farrowing (Benfield et al. 1982) indicates that adult pigs, with high levels of antirotaviral antibody, can shed the virus in their faeces at a time when piglets are particularly susceptible to infection. This led the authors to conclude that adult pigs are of primary importance in initiating infection in piglets. Rotavirus has also been detected in gilts and sows with diarrhoea (Corthier et al. 1980, McNulty et al. 1976c). After observing a steady build up of rotaviral infection in piglets weaned in a fumigated nursery, Lecce et al. (1978) hypothesized that there was a postpartum transmission of a small amount of virus from

the dam to the young, which caused an asymptomatic infection due to the passive immunity conferred by colostrum. If these piglets were weaned at one day of age to a nursery, their immunity would wane rapidly and they would become capable of amplifying the infection. This, in turn, would lead to infections of increasing severity which would spread from young to young when newly weaned piglets were continually added to the nursery if it was not subjected to further fumigation.

Group A rotavirus has been detected in drinking water (Gerba et al. 1984, Smith and Gerba 1982) and domestic sewage (Hejkal et al. 1984, Steinmann 1981). Waterborne outbreaks of gastroenteritis in man associated with group A rotavirus have also been reported (Freij et al. 1978, Morens et al. 1979, Lycke et al. 1978). Furthermore human rotavirus has been recovered from environmental surfaces of day care centres (Keswick et al. 1983). All these observations indicate that rotavirus in the environment may also be an important source of infection for susceptible individuals. Woode and Bridger (1975) reported that bovine rotavirus could survive in the laboratory at an average temperature of 20°C for seven months. This also suggests that rotavirus shed by animals could survive for a long time in the environment and then be transmitted to other animals.

The studies described in this chapter were carried out to investigate the shedding pattern of group A rotavirus in pigs under natural conditions from birth to the age of slaughter. The pattern of transmission of the virus from sows to piglets, from piglets to piglets, and from the environment to piglets was also studied.

3.2 PATTERNS OF SHEDDING OF GROUP A ROTAVIRUS IN NATURALLY INFECTED PIGS

3.2.1 Introduction

Several studies on the shedding pattern of group A rotavirus in piglets have been conducted (see above). However, the usefulness of the information obtained is limited because only pooled faeces from litters, rather than faeces from individual pigs, were examined. Furthermore, sampling was only carried out on a weekly basis (Askaa et al. 1983, Debouck and Pensaert 1983).

The purpose of the present study was to obtain information on the prevalence of group A rotaviral infection in pigs at Massey Piggery by means of a cross-sectional survey, and then to observe the daily pattern of shedding in individual piglets by sampling a cohort of piglets from birth to the time of slaughter.

3.2.2 Materials and methods

Animals and sampling procedures: For the cross-sectional survey, faecal samples were collected from all pigs in the piggery on one day and taken directly to the laboratory for processing.

In the cohort study, four pregnant sows (two gilts and two multiparous sows) were introduced to the farrowing unit at one week prior to the projected date of farrowing. These four sows were kept in one single farrowing room as shown in Fig. 3-1. Sows No. 1 and 3 were multiparous sows and sows No. 2 and 4 were gilts. Sow No. 1 farrowed first and was followed by sows No. 2 and 3 the next day. Sow No. 4 farrowed three days later. After farrowing, four piglets from each litter were selected at random to form a cohort. Eight of these 16 piglets comprising two from each litter were weaned at 24 days of age, and the rest were left on two sows. Four piglets from each of the weaned and unweaned groups were then killed at 32 days of age and the remaining unweaned piglets were weaned at five weeks of age. At weaning, piglets were transferred to a controlled temperature house and fed a commercial weaner diet ad libitum. The piglets were weighed at two, three, four and six weeks of age.

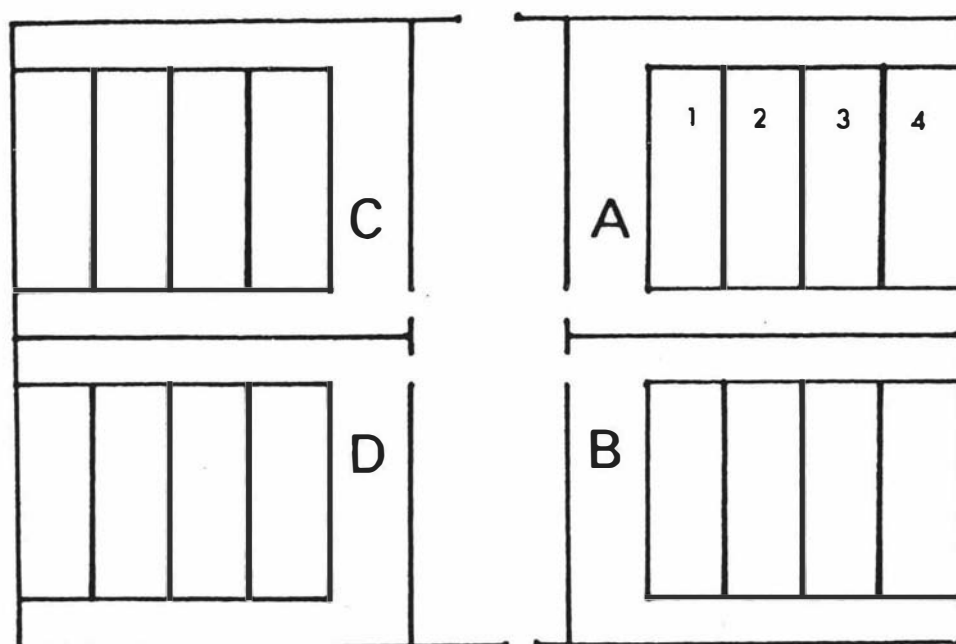


Fig. 3-1. The schematic plan of the farrowing unit and the position of the four sows (1, 2, 3, and 4). A, B, C, and D are the four farrowing rooms in the unit. Piglets from other rooms (B, C and D) were not included in this study.

Faeces from sows were collected daily from one week before farrowing until after the piglets were weaned. After birth, faeces or faecal swabs were taken from the selected piglets every day up to two months of age. Thereafter, faeces were collected once a week until these animals were slaughtered at 20 to 22 weeks of age. Faeces as well as ileal contents were taken from piglets killed at 32 days of age and from the other piglets which were slaughtered later. All samples were stored at -70°C until processed.

Detection of group A rotavirus: Faecal samples and intestinal contents collected were processed and examined for group A rotavirus using the Dakopatts ELISA kit (section 2.2.2).

Detection of haemolytic *Escherichia coli* (*E.coli*): Diarrhoeic faeces from sucking pigs and all faecal samples from pigs from the day of weaning to 15 days after weaning were examined for the shedding of haemolytic *E.coli*. Fresh faeces were plated out on to split nutrient agar plates containing 5% sheep red blood cells in the upper layer, and

were incubated aerobically overnight at 37°C. The approximate proportion of β -haemolytic coliform colonies among the aerobic flora was estimated. The results were recorded as + (<25%), ++ (26-50%), +++ (51-75%), ++++ (76-95%) and V (pure growth).

3.2.3 Results

Cross-sectional survey: Results from the cross-sectional survey are summarized in table 3-1. Forty seven (9%) of 521 animals sampled were shedding rotavirus at the time of sampling. Rotaviral infection was detected only in sucking and weaner piglets with prevalence rates of 19.3% and 14.4%, respectively. No rotavirus was detected in pigs over two months old, including fatteners pigs, sows and boars. Of the 62 sows, 15 were with piglets, 28 were pregnant, 10 were non-pregnant gilts and 8 were "dry" sows.

Table 3-1. Prevalence of rotaviral shedding at Massey Piggery as detected by ELISA

	Numbers tested	Numbers positive	Prevalence (%)
Suckers	161	31	19.3
Weaners	111	16	14.4
Fatteners	183	0	0
Sows	62	0	0
Boars	4	0	0
Total	521	47	9

Table 3-2 shows the age distribution of infection in the group of sucking piglets. The earliest detection of infection with the virus was at two weeks of age, and the prevalence rate then increased with age to reach a maximum at five weeks of age, just before weaning.

Diarrhoea was observed in eight (17%) out of the 47 pigs shedding rotavirus. Six of these were sucking and two were weaner pigs. Two of

the 16 weaner pigs had diarrhoea when shedding rotavirus, and diarrhoea without rotaviral shedding was also observed in eight other weaner pigs.

Table 3-2. Age distribution of rotaviral shedding in the sucking piglets

Age	Numbers tested	Numbers positive	Prevalence(%)
1st week	30	0	0
2nd week	39	1	2.5
3rd week	36	3	8.3
4th week	26	10	38.5
5th week	30	17	56.7

Cohort study: All of the 16 piglets which were examined on a daily basis shed rotavirus before 40 days of age (table 3-3). Piglets No. 1-4, 5-8, 9-12, 13-16 were with sows No. 1, 2, 3 and 4, respectively. Apart from piglets in litter 3 (piglets No. 8-12), all piglets in each of the other three litters shed rotavirus within three to five days of one another. The average age at which rotavirus was first detected was 19 days, ranging from 13 to 39 days. The duration of shedding in individual animals varied between one and 12 days with a mean duration of 7.4 days. In five of the piglets, rotavirus could be detected intermittently at intervals of between one and 11 days. The total duration of shedding in this population was 30 days.

In the sucking period (0 to 23 days of age), 13 of the 16 piglets became infected with rotavirus at an average of 16.5 days of age. Yellowish watery diarrhoea was observed in 11 of the 13 piglets either on the same day as, or one to three days after, rotaviral shedding commenced. Diarrhoea persisted in these animals for one to nine days with an average of four days. Vomiting was observed in two piglets but no signs of dehydration were observed. All the piglets recovered without treatment. Haemolytic E.coli was not detected in any of the diarrhoeic faeces collected from sucking piglets.

Table 3-3. Age at first detection of rotavirus and duration of shedding in 16 piglets

Pig No.	First Detection (age in days)	Duration (days)
1	19	8
2	18	7
3	22	4
4	19	3
5	15	11
6	15	11
7	16	7
8	18	5
9	39	4
10	16	1
11	24	11
12	27	4
13	13	12
14	16	11
15	13	10
16	15	9
Mean (SD)	19 (6.6)	7.4 (3.5)

Among the eight piglets weaned at 24 days of age, three continued to shed, two re-shed and two started to shed rotavirus for the first time after weaning. All these piglets started to shed haemolytic E.coli from five to eight days after weaning. The non-haemolytic flora were soon replaced by a pure growth of haemolytic E.coli. The duration of shedding of haemolytic E.coli in surviving animals was three to eight days. Five piglets developed watery diarrhoea six to eight days after weaning, coinciding with the shedding of haemolytic E.coli, but at this stage only one piglet was concurrently shedding rotavirus. This diarrhoea was much more severe than that seen before weaning. Depression and dehydration was observed in diarrhoeic piglets and three piglets were given antibiotic therapy. These animals responded to the treatment, and diarrhoea ceased one to three days afterwards. The relationships between the shedding of rotavirus and haemolytic E.coli and the occurrence of diarrhoea in the weaned pigs are summarized in table 3-4. In the unweaned group, four animals were still shedding rotavirus at this time. Neither haemolytic E.coli nor diarrhoea was detected in these animals.

When the eight piglets were killed at 32 days of age, rotavirus was detected in the faeces and gut content of one piglet, and haemolytic E.coli was detected in the faeces as well as in the intestinal contents of three piglets. Of the remaining four piglets weaned at five weeks of age, only two shed rotavirus for one and four days respectively, and three shed haemolytic E.coli at three to seven days after weaning with a duration of four to seven days. Two piglets developed diarrhoea three days after haemolytic E.coli was detected, but neither shed rotavirus.

During the period of investigation, weekly weight gain of diarrhoeic piglets was half of that of their non-diarrhoeic counterparts. In the third week of the sucking period, the average weight gain in pigs without diarrhoea was 1.6 kg (1.2-2.2 kg) compared with 0.9 kg (0.5-1.6 kg) in piglets with diarrhoea ($P < 0.01$).

During the second week after weaning, non-diarrhoeic piglets gained a mean of 1.4 kg (0.8-2.9 kg) while those with diarrhoea gained only 0.7 kg (0.2-1.4 kg) ($P < 0.05$). Of the four piglets weaned at five weeks of age, two with diarrhoea gained only 1.0 kg in the first week after weaning in contrast to 1.7 kg gained by two without diarrhoea.

Table 3-4. Relationships between shedding of rotavirus and haemolytic E.coli and the occurrence of diarrhoea in weaned pigs

Pig No.	Detection of		
	Rotavirus	Haemolytic E. coli	Diarrhoea
1	1-3*	7-9	8-9
2	2-3	8-10	-**
3	6-7	7-11	8-9
4	-	7-9	8-9
5	2-13	5-12	8-9
6	6-9	6-9	-
7	1-5	6-11	-
8	1-3	6-14	6-9

* Days after weaning.

** No rotavirus or diarrhoea was detected.

The differences in rotaviral shedding and development of diarrhoea between piglets born of gilts and those of multiparous sows are summarized in table 3-5. In the sucking period, more piglets born of gilts shed rotavirus and had diarrhoea than did those born of multiparous sows. The former also became infected with rotavirus earlier and shed the virus longer than the latter. In addition, the two gilts were seen to be dirtier, due to contamination of faecal materials, than the multiparous sows especially on their abdomens and udders.

Rotaviral antigen was not detected in the remaining eight piglets from two months of age to the time of slaughter (at 20 to 22 weeks). No rotavirus was detected in the ileal contents of these pigs collected at slaughter.

Table 3-5. Differences between piglets born of multiparous sows and gilts in relation to rotaviral shedding and diarrhoea before weaning

	Piglets of	
	multiparous sows	gilts
Rate of rotaviral shedding	5/8	8/8
Mean age at first detection (days)	18.8	15
Mean duration of shedding (days)	3.5	7
Rate of diarrhoea	2/8	8/8
Mean age of appearance of diarrhoea (days)	19	17.6
Mean duration of diarrhoea (days)	1.5	4.6

Based on the results of the ELISA test, none of the sows shed group A rotavirus during the whole period of investigation.

3.2.4 Discussion

The duration of shedding of a pathogen in individual animals is an important factor in both the speed and pattern of transmission of an infection within a population. In other studies, gnotobiotic or colostrum-deprived piglets, experimentally infected with group A rotavirus, were found to shed the virus for five to seven days (Crouch and Woode 1978, McNulty *et al.* 1976c). In the present study, it was found that individual piglets shed rotavirus for 1 to 12 days after natural infection, with a mean duration of 7.4 days. This appears to be the only recorded data on the duration of shedding of rotavirus under natural conditions.

In both the cross-sectional and cohort studies, rotavirus was detected in sucking piglets with the prevalence rates increasing with age during this period. No rotavirus was detected in piglets less than

one week old. These observations on the general pattern of rotaviral shedding in piglets are in agreement with most other workers (Askaa et al. 1983, Debouck and Pensaert 1983, Utrera et al. 1984). In herds of Danish pigs, Svensmark (1983) also observed this trend, but the prevalence in each of the age groups was much higher than that observed in this present study. The author (Svensmark 1983) also reported a high prevalence rate (23%) in pigs under one week of age. The differences between his study and this present study may have been either because the farming system in Denmark is more intensive than in New Zealand, or because pooled samples from a litter rather than faeces from individual piglets were used for examination of group A rotaviruses in Svensmark's study, or both.

Weaner pigs have also often been found to shed rotavirus, particularly where early weaning is practised (Barrow et al. 1979, Lecce et al. 1982). In the cross-sectional study, rotaviral antigen was detected in 14.4% of weaner pigs (5-8 weeks old) in the piggery. Seven of the eight pigs in the cohort study which were weaned at 24 days of age shed rotavirus after weaning.

Shedding of rotavirus by older pigs, especially by sows, is potentially important in the epidemiology of the viral infection because they could be the primary source of infection for young susceptible piglets. In the present study, all piglets ceased shedding rotavirus before two months of age. Rotaviral antigen was not detected in these animals from then up to the time of slaughter. Furthermore, none of the sows shed the virus during the whole period of investigation. In an epidemiological study conducted by Debouck and Pensaert (1983), faeces from sows were also always free of rotaviral antigen. However, rotaviral infections have been detected in fatteners under natural conditions (Grom et al. 1984) and have been established in such animals (Bernard et al. 1984) by experimental inoculation. Rotavirus has also been recovered from gilts and sows with or without diarrhoea (Benfield et al. 1982, Corthier et al. 1980, McNulty et al. 1976c).

Recurrent shedding of rotavirus by litters of piglets three to four weeks after its first detection has been reported (Debouck and Pensaert, 1983). Five piglets in the study reported here also shed rotavirus intermittently at intervals of one to 11 days during the investigation period. It was not possible to determine whether this pattern represented reinfection or persistent infection at an undetectable level.

Rotaviral infection in pigs has been associated mostly with a diarrhoeic syndrome commonly known as white scours, milk scours or three-week-old scours (Bohl 1979, Bohl et al. 1978, Debouck and Pensaert 1983). This association was also observed in the present study. Among the 13 piglets shedding rotavirus in the sucking period, 10 developed diarrhoea soon after rotavirus was first detected.

Rotavirus has also been associated with PWD in newly weaned piglets, especially in those weaned at an early age (Barrow et al. 1979, Lecce and King 1978, Tzipori et al. 1980a). However, other factors such as dietary change and infection with pathogenic E.coli are also involved in this syndrome (Lecce et al. 1983, Tzipori 1985). In this present study, PWD was also observed in piglets six to eight days after weaning and this syndrome occurred soon after the aerobic faecal flora shifted to nearly a pure growth of haemolytic E.coli. At this stage only one piglet was shedding rotavirus. This observation is in agreement with Lecce et al. (1982) and Tzipori et al. (1980b).

It is, therefore, concluded from these studies that diarrhoea in the sucking piglets was closely associated with rotaviral infection while rotavirus did not appear to play an important role in PWD. In the latter condition, haemolytic E.coli was probably responsible. The haemolytic E.coli recovered from the weaner pigs in this study has been identified as O-serotype 138 (Hampson et al. 1988). This serotype has often been associated with PWD (Tzipori 1985).

It has been well documented that piglets born of gilts are more susceptible to rotaviral infection than are those born of multiparous sows (Askaa et al. 1983, Svensmark 1983). In this study, piglets born of gilts were also found to become infected with rotavirus earlier, to shed the virus for a longer period and to suffer more severe diarrhoea than those born of multiparous sows. This is possibly due to a lower level of antibody to rotavirus in colostrum from gilts than from multiparous sows as has been reported by Askaa et al. (1983). The present observation that the gilts were dirtier than multiparous sows is of interest because rotaviral infection of susceptible animals occurs through contact either with individuals shedding rotaviruses in their faeces or with a contaminated environment (Woode and Bohl 1981). The dirtiness of the gilts was mainly due to faecal material. As this contamination was especially around the mammary glands, it would have provided a potentially rich source of rotaviral infection, and possibly other enteric pathogens, for the piglets.

3.2.5 Conclusions

1. The prevalence of group A rotaviral infection at Massey Piggery was 9% with 19.3% in sucking piglets and 14.4% in weaner pigs. Rotaviral antigen was not detected in fattener pigs, sows and boars.
2. In the sucking period, piglets less than one week of age did not shed group A rotavirus possibly due to the presence of maternal antirotaviral antibody. Piglets began to shed group A rotavirus at two weeks of age, the prevalence then increased with age and reached a peak at five weeks of age.
3. The cohort study revealed that all piglets shed group A rotavirus before 40 days of age. The average age at which rotavirus was first detected was 19 days, and ranged from 13 to 39 days.
4. The average duration of shedding was 7.4 days in individual piglets, and ranged from one to 12 days. The total duration of shedding in the cohort was 30 days.
5. Recurrent shedding of group A rotavirus occurred in five of the 12 piglets at one to 11 days after the first shedding ceased.
6. All piglets ceased shedding group A rotavirus by two months of age. Pigs did not shed rotavirus from two months of age to the time of slaughter. Rotavirus was not detected from any of the four sows from one week before farrowing until their piglets were weaned.
7. Diarrhoea observed in sucking piglets was associated with shedding of group A rotavirus since this diarrhoea occurred soon after rotavirus was first detected in their faeces.
8. Diarrhoea observed in weaner pigs six to eight days after weaning (PWD) was not associated with the shedding of group A rotavirus since few piglets shed the virus at that time. However, all piglets shed haemolytic E.coli five to seven days after weaning, just before PWD occurred. Thus, haemolytic E.coli may have been responsible.
9. Diarrhoea observed in sucking piglets was milder than that in weaner piglets. Depression and dehydration was observed in the latter but not

in the former group.

10. Weekly gain in weight in diarrhoeic piglets was only half of that in non-diarrhoeic counterparts.

11. In the sucking period, more piglets born of gilts shed group A rotavirus and developed diarrhoea than those born of multiparous sows. Piglets of the former also shed the virus earlier and for a longer period than those of the latter group. These differences may be due to lower levels of antibody to rotavirus in colostrum and milk from gilts than from multiparous sows as suggested by other workers. Gilts were observed to be dirtier than multiparous sows due to contamination with faecal material, especially around the mammary glands, thus they may have provided a richer source of rotavirus for their piglets than did multiparous sows.

3.3 NATURAL TRANSMISSION OF GROUP A ROTAVIRUS WITHIN A PIG POPULATION

3.3.1 Introduction

The previous cohort study involving 16 piglets showed that all of these animals became infected with group A rotavirus before 40 days of age. The duration of shedding was approximately eight days in individual piglets and 30 days in the group as a whole. Rotaviral antigen was never detected in faeces from pigs over two months of age. Faeces from sows were always free of group A rotaviral antigen although they were also sampled daily from one week before farrowing until weaning. However, in this study, only four piglets from each of the four litters were monitored, so it was not possible to observe how the virus was transmitted from piglet to piglet and from litter to litter.

A further investigation was therefore carried out in the same piggery using all the pigs from five litters (a total of 50 piglets) for two main reasons: firstly, to determine whether or not the pattern of shedding of group A rotavirus in pigs was as previously observed; secondly, to study the transmission of rotavirus from piglet to piglet and from litter to litter under natural circumstances.

3.3.2 Materials and methods

Management of animals: Approximately one week prior to their expected date of parturition, five sows were introduced to the farrowing unit and were located as shown in Fig. 3-2. One sow (No.1) was housed in room C together with three other sows with piglets which were not included in this study. Piglets from these three sows were two weeks old when sow No. 1 was moved into the room. The other four sows (Nos. 2, 3, 4, 5) were housed together in a separate room A. All the five sows farrowed within two days. Fifty piglets survived the post-neonatal period, and these piglets formed the study cohort.

Twenty four piglets (four to five randomly selected from each of the five litters) were weaned at 26 days of age and were kept in individual cages close to one another in a controlled temperature house where they could be monitored individually. The rest were mixed, left on two sows and weaned ten days later. Four piglets from the unweaned, and eight from the weaned group, were killed at 33 days of age to compare the detection of rotavirus in the faeces and in the intestines.

The surviving piglets from the early and later weaned groups were redistributed into four pens within the same weaner room according to their sizes at 40 days of age.

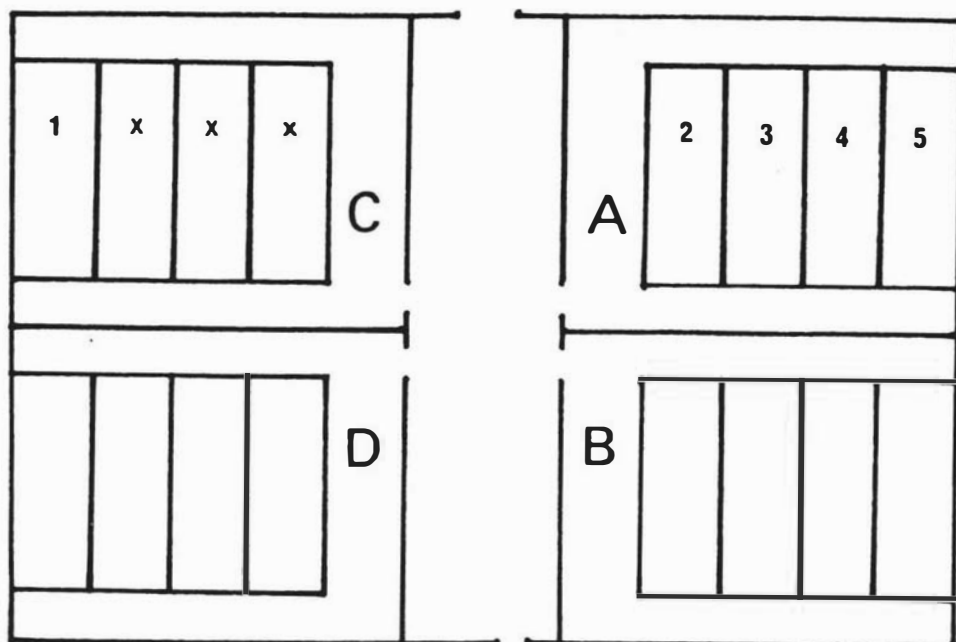


Fig. 3-2. The relative location of the five sows in the farrowing unit.
X are litters not included in this study. Piglets in rooms B and D were not studied.

Sampling procedures: Faeces from sows were collected daily from the time when they were transferred into the farrowing unit to the time of weaning.

Faeces or faecal swabs were taken from all the piglets every day from birth to 60 days of age. Faeces as well as ileal contents were taken from piglets killed at 33 days of age. All these samples were stored at -70°C until examined.

Detection of group A rotavirus: All the faecal samples were examined for group A rotaviral antigen by the Dakopatts ELISA kit (see section 2.2). Selected samples were also compared for their genomic patterns by PAGE as described also in section 2.2.

Detection of haemolytic E.coli: Faeces from all pigs from the day

of weaning to 15 days after weaning were examined for the shedding of haemolytic E.coli as described in section 3.2.2.

3.3.3 Results

All the piglets became infected with group A rotavirus before 35 days of age (table 3-6). The age at which piglets first shed rotavirus was 19 to 34 days with a mean of 27 days. The average duration of shedding in individual piglets was eight days ranging from two to 13 days.

Table 3-6: Pattern of shedding of rotavirus in five litters of piglets

Litter No.	No.of pigs	Average age at first detection		Average Duration of shedding*	
		(days)	(Range)	(days)	(Range)
1	11	22	(19-24)	8.5	(5-13)
2	9	26	(22-28)	9.0	(6-13)
3	9	27	(26-29)	7.1	(5-11)
4	11	30	(25-34)	6.8	(2-10)
5	10	31	(27-34)	8.3	(7-11)
Total	50	27	(19-34)	8.0	(2-13)

* During the first episode of shedding.

Piglets born of sow No. 1, which was housed in farrowing room C, were the first among the five litters to shed group A rotavirus (at 19-24 days of age). Two days later, piglets from sow No. 2, housed closest to the door in farrowing room A started to shed the virus. The remaining three litters (sows No. 3,4,5) started to shed rotavirus at about the same time, three to four days after the second litter. At this stage, the piglets were at the point of being weaned (early weaned group) or of being mixed with other litters of piglets (unweaned group).

One piglet from litter 4 shed rotavirus on the day of being weaned. Two piglets from litter 3 and one from litter 4 began to shed the virus one day after weaning. All piglets in litter 3 shed rotavirus within three days of weaning. In the other two litters (4 and 5), piglets from the weaned group shed group A rotavirus earlier (28 ± 1.4 days of age) than those left unweaned (32.6 ± 2.88 days of age) ($P < 0.005$). The number of new cases occurring each day is shown in Fig. 3-3 for the five litters both individually and as a group. In each of the litters, one or two piglets were always infected earlier than the rest, and then infection spread to other piglets. It took four to ten days for rotaviral infection to spread to all piglets in each litter and 16 days to infect the whole pig population investigated.

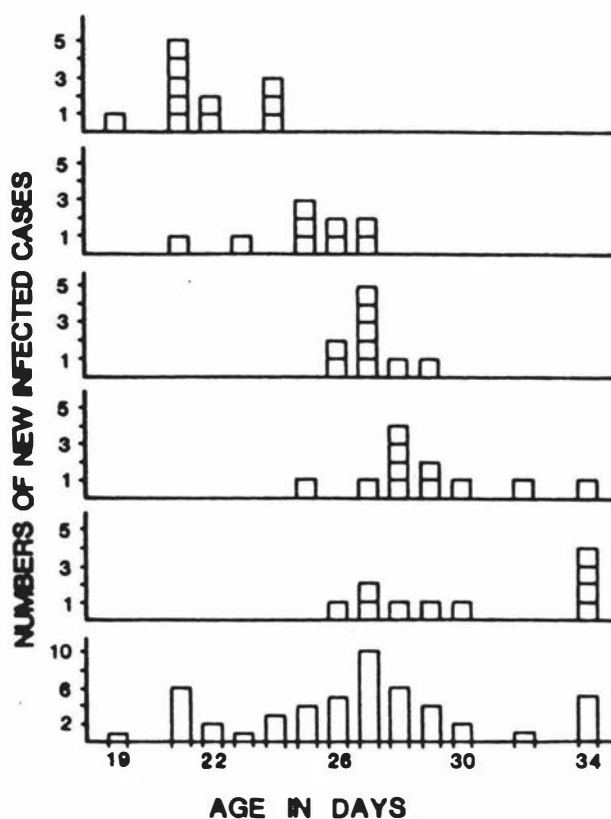


Fig. 3-3. New cases of rotaviral infection in each of the five litters and in the cohort. From top to bottom are litters 1,2,3,4,5 and the cohort.

The prevalence of rotaviral infection is depicted in Fig. 3-4 for the five individual litters and in Fig. 3-5 for the total pig population studied. A second peak of rotaviral shedding was observed in 18 of the 38 surviving piglets (47%) at around 47 days of age, ranging from 43 to 52 days. The second period of shedding was first recorded in

one piglet three days after all piglets were mixed and redistributed in four pens of a weaner house at 40 days of age. At this time, two piglets, one each from litters 4 and 5 were still in their first episode of infection. Among the 18 animals which shed rotavirus for a second time, five, three, one, two and seven were from litters 1, 2, 3, 4 and 5, respectively. This second shedding occurred in individual piglets 6 to 20 days (mean 12.6 days) after the first shedding had ceased. The duration of shedding of group A rotavirus was 34 days in total for these five litters of piglets. By 53 days of age, rotaviral antigen was no longer detected in any of these animals.

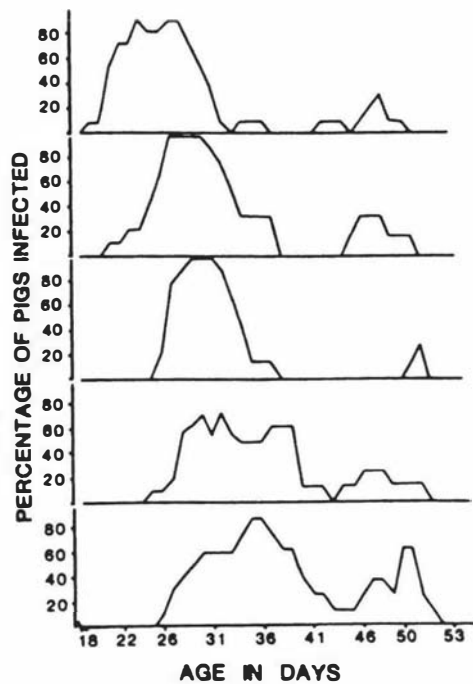


Fig. 3-4. Pattern of rotaviral shedding in the five litters.

From top to bottom are litters 1,2,3,4 and 5.

Among the 12 piglets killed at 33 days of age, five were still shedding rotavirus in their faeces, and rotaviral antigen was also detected in their intestinal contents. Rotaviral antigen was not detected in the intestinal contents of the remaining seven piglets which were not shedding rotavirus in their faeces at this time.

Ten ELISA positive samples from the five litters during the two period of shedding at 19-35 and 43-48 days of age were selected and examined by PAGE. All of the samples showed identical electrophoretic patterns (Fig. 3-6).

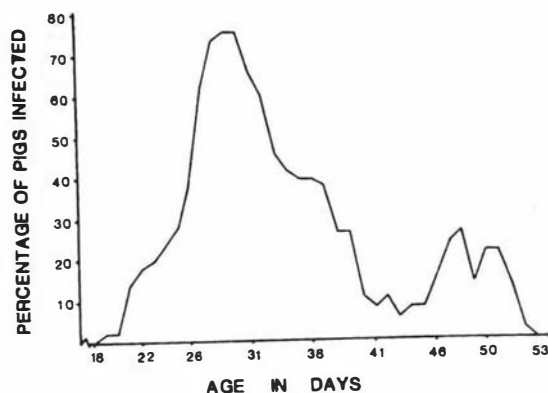


Fig. 3-5. Pattern of rotaviral shedding in the cohort.

Haemolytic E.coli (near pure growth) were detected in all (24) of the early weaned piglets three to eight days (mean 5.3 days) after weaning. Haemolytic E.coli were also detected in eight of the 26 unweaned piglets at 26 to 34 days of age. These unweaned piglets shed haemolytic E.coli at a lower level (25-75% of the aerobic faecal flora). When the surviving pigs (22) from the unweaned group were weaned at 37 days of age, all shed haemolytic E.coli (near pure growth). Those which shed haemolytic E.coli before weaning, either continued to shed or re-shed at about ten days after weaning. For those which did not shed haemolytic E.coli before weaning, haemolytic E.coli were first detected at an average of 6 days after weaning.

During the study period, 44 episodes of diarrhoea were observed in 39 (78%) of the 50 piglets. Seventeen piglets developed diarrhoea in the sucking period soon after rotavirus was first detected. Two piglets concurrently shed haemolytic E.coli. Twenty one out of the 24 piglets weaned at 26 days of age developed diarrhoea after weaning. Among these, seven piglets were shedding rotavirus, 11 were shedding haemolytic E.coli, two were concurrently shedding rotavirus and haemolytic E.coli, and one did not shed either. When those piglets were weaned at 37 days of age, six of the 22 developed diarrhoea at three days after weaning. At this time, these pigs were shedding haemolytic E.coli in their faeces. The duration of diarrhoea in individual piglets was from one to eight days with an average of three days. Diarrhoea

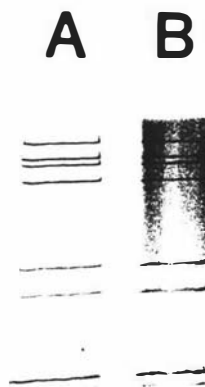


Fig. 3-6. Electrophoretic patterns of two representative group A rotaviruses detected during the two peaks of shedding at 19-35 (A) and 43-48 (B) days of age.

observed in piglets before weaning was much milder than that seen in weaned piglets. Depression and dehydration was generally associated with diarrhoea in weaner, but not in sucking piglets.

Neither rotavirus nor diarrhoea was observed in any of the sows that were sampled daily throughout the period of investigation.

3.3.4 Discussion

Results from this study confirm the pattern of rotaviral shedding in pigs observed in the previous study (section 3.2). All piglets became infected with group A rotavirus before 40 days of age and ceased shedding by two months of age. The duration of shedding in individual piglets was about eight days.

The age distribution of rotaviral infection in pigs observed in both this study and the previous study described in section 3.2 is in agreement with most other workers (Askaa et al. 1983, Bohl et al. 1978, Liprandi et al. 1987, Utrera et al. 1984). Rotaviral shedding occurred mainly in piglets between three and eight weeks of age. Younger piglets are occasionally reported to be infected (Debouck and Pensaert, 1983, McNulty et al. 1976c). Rotaviral antigen was not detected in piglets under ten days of age in the piggery under study, possibly as a result of protection by antibody present in colostrum and milk.

At Massey Piggery, group A rotavirus was not detected in pigs over two months of age. None of the sows investigated shed rotavirus as detected by ELISA or developed diarrhoea. This was despite the fact that faeces from these sows were collected and examined daily from a week before farrowing until weaning. These results are in contrast to those reported by other workers. Grom et al. (1984) found that 15% of fattener pigs and 10-33% of sows were shedding group A rotavirus. Corthier et al. (1980) and McNulty et al. (1976c) detected rotaviral antigen from diarrhoeic gilts and sows, and Benfield et al. (1982) demonstrated rotavirus in faeces from sows both before and after parturition. The discrepancy between the results of the present study and those of others could be due to the following reasons. Firstly, the ELISA test used in our studies may be less sensitive than tests used by others and therefore failed to detect low amounts of rotavirus shed by older animals. This, however, is unlikely since Corthier et al. (1980) also employed an ELISA test for detecting rotaviral antigen. Both

Benfield et al. (1982) and McNulty et al. (1976c) identified rotavirus by electron microscopy (EM). The sensitivity of the ELISA test (Dakopatts, Copenhagen) has been reported to be more sensitive than EM (Grauballe et al. 1981). The earlier work in this study also revealed that the ELISA test was approximately ten times more sensitive than EM (section 2.2). Secondly, rotavirus detected in this piggery under investigation may be less virulent than that reported by others. Variation in virulence has been reported among bovine group A rotaviruses by Bridger and Pocock (1986) and Caprio et al. (1981). This variation could also exist among porcine rotaviruses. Thirdly, the sows and gilts that were studied in the present study might have had sufficient immunity to protect them from further infection. This could be partially supported by the findings of the work described in section 3.2 and this section in this thesis, in which it was shown that piglets under ten days of age were protected from rotaviral infection, presumably by antirotaviral antibody in colostrum and milk. Thus, their dams would have had high levels of antibody. This has been further investigated and the results will be described in chapter four. The fourth possibility could be that in other studies, different serotypes or strains were introduced, infecting sows and gilts which lacked immunity to the new serotypes or strains. It has been reported in man that infection with one serotype may not necessarily protect against infection with another serotype (Yolken et al. 1978c). To date more than three serotypes have been reported to infect pigs (Bohl et al. 1984, Nagesha and Holmes 1988, Paul et al. 1988).

Biphasic shedding of rotavirus among litters of piglets at intervals of three to four weeks was reported by Debouck and Pensaert (1983). In the previous cohort (section 3.2), five out of 12 piglets developed a second period of shedding of rotavirus at intervals of one to 11 days. In the present study, about half the piglets (18 out of 38) shed group A rotavirus for a second time six to 20 days after the first shedding had ceased. This is possibly a feature of rotaviral infection in pigs, which may arise due to a weak immune response to the first infection. Bishop et al. (1983) have also reported that neonatal rotaviral infection did not confer immunity against reinfection although it did protect against the development of severe disease during subsequent infection. The second period of shedding observed in this study probably represents a reinfection rather than a persistent infection, even though the viruses detected during the two phases showed identical electrophoretotypes. This seems likely because (1) rotavirus could not be detected in the intestinal contents of seven piglets which

had previously shed rotavirus, but which were no longer shedding in their faeces when they were slaughtered at 33 days of age, (2) the identical electrophoretotypes may not necessarily have identical nucleotide sequences (Clarke and McCrae 1982); furthermore, antigenic drift has been detected by monoclonal antibody studies of rotavirus strains isolated three months apart even though the strains showed identical electrophoretic pattern by PAGE (Coulson *et al.* 1985), (3) the second shedding began three days after redistribution of all the piglets and at this stage, two piglets were still shedding during their first episode of infection. These two piglets might have served as the source of rotavirus for the reinfection of the other pigs in the mixed group.

Again in this study the association between the occurrence of diarrhoea and the shedding of rotavirus was observed in piglets before weaning. Diarrhoea after weaning (PWD) was more related to the shedding of haemolytic E.coli than to rotavirus.

Group A rotaviral infection is ubiquitous and enzootic in pig breeding herds (Bohl, 1979). Virtually all of the piglets that were followed in this study became infected early in their life. However, the duration of shedding of group A rotavirus in infected animals was only about eight days and rotaviruses were not demonstrated in pigs over two months old. None of the sows investigated shed rotavirus during the period of farrowing. This raises the question of how rotavirus is maintained in the piggery. The natural pattern of transmission observed in this study throws light on the problem. The litter of piglets which first became infected was housed in farrowing room C with three older litters which were not included in this study. Four days before the first litter became infected, diarrhoea was observed in the older piglets and rotaviral antigen was detected in diarrhoeic faeces from them (data not shown). Two days after the first litter was infected, the second litter, which was the closest to the door in farrowing room A, began shedding rotavirus and then infection occurred in other litters in the same room. The mechanism of rotaviral transmission from one room to another may have been by aerosol, by movement of personnel, or both. The farrowing house in the piggery under investigation is cleaned every day by hosing with high pressure water and this could create aerosols of dust and faeces. Aerosols could contain infectious particles at the time when pigs are shedding rotavirus in their faeces. The airborne infection could also explain the simultaneous shedding of rotavirus in the remaining three litters four days after rotavirus was first detected

in litter 2. The simultaneous shedding of rotavirus in these three litters of piglets may also have been due to the mixing of piglets at the point of weaning. Since the four farrowing rooms are adjacent to one another, the continuous farrowing in the unit provides a continuous supply of susceptible young piglets and hence maintains the cycle of rotaviral infection. Usually, in each of the litters, one or two piglets began to shed rotavirus earlier than the rest and then infection spread among others in the same litter, and finally spread to piglets of other litters. From these observations, it is concluded that continuous transmission of rotavirus from piglet to piglet, from litter to litter and from room to room is a major factor in maintaining the cycle of rotaviral infection in this piggery.

3.3.5 Conclusions

1. The results from this study confirm the pattern of shedding of group A rotavirus observed in the previous study (section 3.2). Piglets less than ten days of age did not shed group A rotavirus. All piglets shed the virus before 40 days of age, with the age at first detection ranging from 19 to 37 days. Piglets ceased shedding before two months of age. Rotaviral antigen was not detected in faeces of any of the five sows.

2. The duration of shedding in individual piglets was two to 13 days with a mean duration of eight days. The duration of shedding in the cohort was 34 days. These results are also similar to those observed in the previous study (section 3.2).

3. Nearly half of the piglets investigated shed group A rotavirus for a second time starting at six to 20 days after the first shedding had ceased. The duration was about three days in individual piglets during the second episode. This was possibly due to a weak immune response to the first episode of infection.

4. The second period of shedding represented reinfection rather than persistent infection.

5. The source of rotavirus for the second infection was from piglets which were still shedding rotavirus during their first episode of infection.

6. Transmission of rotavirus occurred from room to room by airborne

infection or by movement of personnel. Rotavirus was first detected in litter No. 1 housed in room C, and then in litter No. 2 housed closest to the door in room A.

7. Transmission of rotavirus occurred from litter to litter. Piglets in litter No. 1 shed rotavirus four days after rotavirus was detected from other pigs in the same room. Piglets in litters No. 3, 4 and 5 began to shed rotavirus four to five days after rotavirus was detected in piglets in litter No. 2.

8. Transmission of rotavirus occurred from piglet to piglet. In each litter, usually one or two piglets became infected with group A rotavirus earlier than the rest, infection then spread to other piglets within the same litter.

9. The simultaneous shedding of rotavirus in litters No. 3, 4 and 5 were due to either airborne infection or due to the effects of mixing at the point of weaning.

10. All piglets weaned at 26 and 37 days of age shed haemolytic E.coli three to eight days after weaning.

11. Forty-four episodes of diarrhoea was observed in 39 (78%) of the 50 piglets during the study. Diarrhoea which occurred in sucking piglets was associated with the shedding of rotavirus while diarrhoea which occurred in weaner piglets was associated with shedding of haemolytic E.coli.

12. The continuous transmission of rotavirus from piglet to piglet, from litter to litter and from room to room is believed to be a major factor in maintaining the cycle of rotaviral infection in this piggery.

3.4 DETECTION AND SURVIVAL OF GROUP A ROTAVIRUS IN THE PIGGERY

ENVIRONMENT

3.4.1 Introduction

Waterborne outbreaks of gastroenteritis associated with group A rotavirus have been reported in man (Freij et al. 1978, Lycke et al. 1978, Morens et al. 1979) and the virus has been detected in drinking water (Gerba et al. 1984, Smith and Gerba 1982) and in domestic sewage (Hejkal et al. 1984, Smith and Gerba 1982, Steinmann, 1981). Human rotavirus has also been recovered from environmental surfaces of day-care centres (Keswick et al. 1983). These studies indicate that rotavirus in the environment may be an important source of infection for susceptible populations.

In the pig population, rotaviral infection is widespread, but disease is limited to piglets up to and immediately after weaning. In studies of the epidemiology of group A rotaviral infection in pigs (see section 3.2 and 3.3), it was consistently found that all pigs became infected before 40 days of age and that the average duration of shedding in individual piglets was approximately eight days. Virus was never detected in faeces of pigs over two months old. It is unlikely that, direct transmission via faeces between infected and non-infected piglets is the only way by which rotaviral infection is maintained in a herd. The present study was carried out to determine whether or not rotavirus was present in the environment and thereby show if fomites could serve as a possible source of infection for new litters of piglets.

3.4.2 Materials and methods

Environmental sampling: Samples were collected from the piggery described in section 2.1.1. Dust and dry faeces were collected from a farrowing house, a weaner house, a disused weaner house, a fatterer house and a sow house. After the farrowing house had been routinely cleaned and disinfected, dry faeces were collected from the floor space between the farrowing pen and the wall. Dry faeces were also collected from the floor of a disused weaner house which had been free of piglets for three months at the time of sampling. Faecal material was collected from the floor of other houses. Dust from all the houses was collected by scraping the surfaces of the wall, and metal and wooden fittings.

Samples of sewage (faecal sludge) were collected from the drain in the farrowing house. Sewage from other houses was not available for collection. Faeces were also taken from nine sows at around the time of farrowing. All samples were stored at -70°C until examined.

Processing of samples: Forty grams each of dry faeces or dust were diluted in PBS (pH 7.2) to a final volume of 100 ml. Each of these diluted samples and 100 ml of sewage were processed by the Freon extraction method described by Brashear and Ward (1981). Samples were mixed with an equal volume of chilled Freon (F22) by continuous stirring and centrifuged at 1000 g for 30 min. After the supernatant had been removed, the samples were re-extracted with PBS. The combined aqueous phases were then centrifuged at 60,000 g for 2 h and the final pellet was resuspended in 5 ml of Earle's balanced salt solution. The samples were treated with 5 ml of ether for 30 min, centrifuged at 1000 g for 15 min, and finally stored again at -70°C until assayed.

Stock virus: Two stock group A rotaviruses were used as positive controls: a cytopathic porcine rotavirus SW20/21 (Woode *et al.* 1976a) and a non-cytopathic porcine rotavirus (PRV) purified from faeces of pigs infected with group A rotavirus from Massey Piggery. For PRV purification, faeces were collected from piglets which were shedding rotavirus as detected by the ELISA test and were extracted with PBS at a ratio of 1/4. After centrifugation at 5000 g for 30 min, the supernatant was harvested and passed through a $0.45\ \mu\text{m}$ millipore filter (MFS, Dublin, CA, USA).

Culture and neutralization of rotavirus in samples: Processed samples (0.2 ml) were inoculated onto MA-104 cell monolayers in quadruplicate. Two inocula from each sample were mixed with serum from a sow which had previously been shown to have a neutralizing antibody titre of 1:64 against SW20/21 strain of rotavirus. The mixture of samples and antiserum was incubated at 37°C for 1 h before inoculation onto cells. Samples were allowed to adsorb to the cells for one and a half hours before being decanted and discarded. Maintenance medium without serum, but with $0.5\ \mu\text{g}$ trypsin per ml, was added to the monolayers. The cultures in roller tubes were maintained at 37°C for 3 days and then harvested. After being frozen and thawed three times, the culture fluids were examined by the ELISA test as described below. Monolayer cultures of PRV and SW20/21 grown in the same manner were included as controls.

Detection of rotavirus: All the samples, before and after culture, were examined by the Dakopatts ELISA kit (see section 2.2) and original samples were examined by EM.

Survival of porcine rotavirus (SW20/21) in culture medium and in pig faeces: One ml of rotavirus SW20/21 cultured in MEM was dispensed into each of ten sterile plastic containers which were then left on a shelf at room temperature (20-25°C). Two containers were taken from the shelf to a -70°C freezer at day one and at every month thereafter up to four months. All the samples were later titrated on MA-104 cells in microtitre plates as described in 2.1.6.

For the determination of survival of rotavirus in pig faeces, 100 g of sow faeces which were free from rotavirus as detected by the ELISA test were collected and seeded with 100 ml SW20/21 in MEM. After mixing, the faecal preparation was dispensed equally into ten glass bijous bottles and kept on a shelf as for the suspension of the virus in MEM. Two samples were taken at day one and each month thereafter up to four months and stored at -70°C. For assay of the viral infectivity in these samples, a 25% (w/v) suspension was made in MEM. After mixing, the samples were centrifuged at 3000 g for 30 min. The supernatant was passed through a 0.45 µm millipore filter and then titrated as described in section 2.1.6.

3.4.3 Results

Detection of rotavirus in the environment: Table 3-7 summarizes the results of the detection of rotavirus from the samples by the ELISA test. Rotaviral antigen was detected only from rooms where young piglets were kept (i.e. farrowing and weaner rooms) but not from fattener and sow houses. Fresh faeces from sows were also negative in the ELISA test.

Under EM, rotaviral particles were observed from two of the original samples which were positive in the ELISA test (sewage from the farrowing house and dust from the disused weaner house).

Table 3-7. Detection (+) of rotavirus by ELISA in samples collected from various sites at Massey Piggery

House	Dust	Dry faeces
Farrowing*	+	+
Weaner	+	-
Disused weaner	+	+
Fattener	-	-
Sow	-	-

* Rotaviral antigen was also detected in the sewage sample collected from the farrowing house.

Replication and neutralization of rotavirus from the samples: After incubation for 3 days, CPE was observed in cells inoculated with SW20/21 but not in any of the monolayers inoculated with the samples or with PRV. When the culture fluids were examined by the ELISA test, four out of the six samples classified as positive before culture were also shown to be positive after culture, with the culture fluids giving ESA values of the same order as those of the original samples (table 3-8). The ESA values of the other two samples which were positive before culture fell below the criterion for positives after culture. The two positive controls (PRV and SW20/21) also showed positive reactions both before and after culture. All the positive reactions on cells were neutralized by antirotaviral antibody. None of the samples with negative reactions before culture showed positive reactions in the ELISA test after culture.

Table 3-8. ESA values given by environmental samples before and after culture on MA-104 cells with or without antirotaviral serum

Sample	Before culture	Cultured with serum	Cultured without serum
Dry faeces/Farrowing	<u>0.242</u>	0.001	0.039
Dust/Farrowing	<u>0.160</u>	0.000	<u>0.208</u>
Sewage/Farrowing	<u>0.735</u>	0.002	<u>0.619</u>
Dry faeces/Weaner	0.023	0.005	0.015
Dust/Weaner	<u>0.213</u>	0.003	<u>0.201</u>
Dry faeces/Disused weaner	<u>0.112</u>	0.003	0.036
Dust/Disused weaner	<u>0.508</u>	0.002	<u>0.211</u>
Dry faeces/Fattener	0.005	0.000	0.001
Dust/Fattener	0.007	0.001	0.002
Dry faeces/Sow	0.008	0.002	0.004
Dust/Sow	0.001	0.004	0.000
PRV	<u>1.680</u>	0.004	<u>1.061</u>
SW20/21	1.229	0.000	1.200

Note: ESA values equal to or greater than 0.100 were regarded as positive (underlined).

Viral survival in medium and in faeces: Fig. 3-7 shows the results of the survival of SW20/21 in medium and in faeces. Rotavirus in both preparations survived for the four month period with a reduction in titre of about $2 \log_{10}$ TCID₅₀. Virus in these two preparations showed a similar rate of decline in titre with $0.5 \log_{10}$ each month.

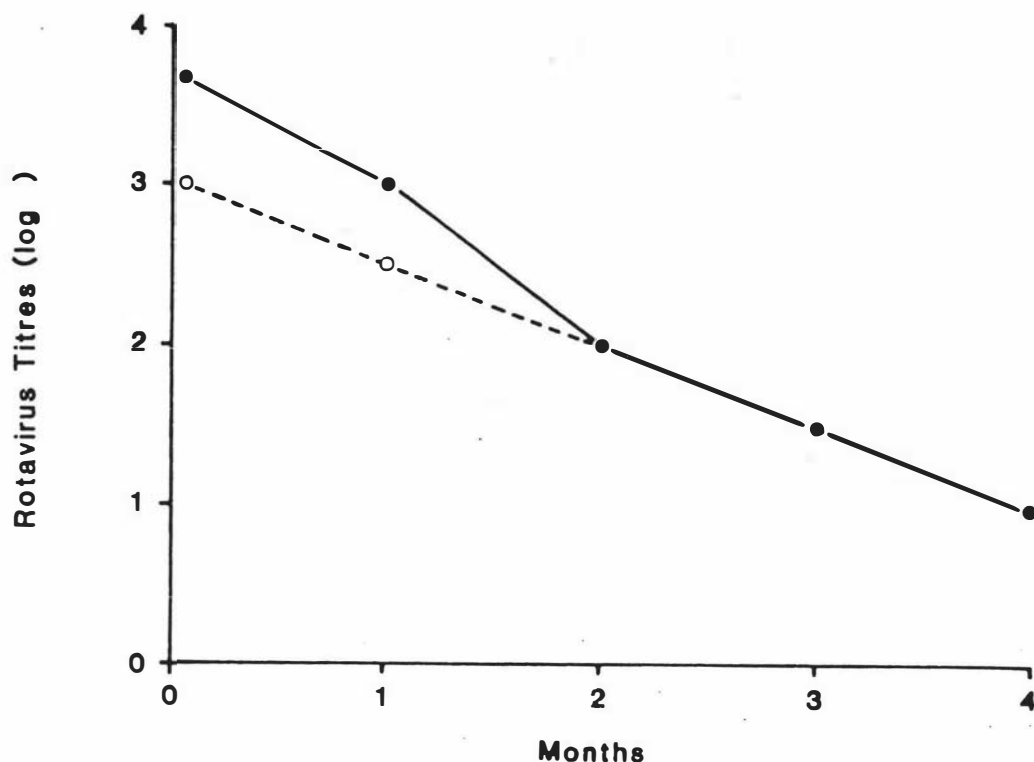


Fig. 3-7. Porcine rotavirus (SW20/21) survival in medium (---) and in faeces (—) held at room temperature (20–25°C).

3.4.4 Discussion

Since rotaviruses are not readily adapted to growth in cells with CPE, many techniques such as EM and ELISA have been developed to detect rotaviral antigen from field samples. However, neither of these tests can distinguish virus which is infectious from that which is non-infectious. Furthermore, Agbalika *et al.* (1985) claimed that the ELISA test alone could lead to false-positive results, especially in detecting rotavirus from sludge. To overcome these difficulties, the combinations of cell culture, neutralization and ELISA tests were used in this study. The positive reaction found in samples both before and after culture, and the inactivation of the infectivity on cell monolayers when inocula were preincubated with antirotaviral antibody, demonstrated that the ELISA reactivity detected in the samples from the environment of the piggery was, in most cases (4/6), due to the presence

of infectious rotavirus.

The survival of human rotavirus has been investigated in different types of waters (Raphael et al. 1985). It was found that the titre of rotavirus was reduced by $2 \log_{10}$ in raw water after 10 days, and in tap water after 64 days, when held at 20°C . No significant drop in titre was found during a 64 day period in filtered water. In the present study, strain SW20/21 of porcine rotavirus was found to survive at about 20°C for a period of at least four months with a reduction in titre of $2 \log_{10}$. The longer survival time of the porcine rotavirus in medium and faeces in this study, compared with that of human rotavirus in tap and raw water (Raphael et al. 1985) may be due to the protective effects of proteins present in the medium and faeces. Bovine rotavirus (NCDV) has also been found to survive for a long time (seven months) in the laboratory at an average temperature of 18 to 20°C (Woode and Bridger 1975).

Group A rotaviral infection is enzootic in pig breeding herds. In studies described in section 3.2 and 3.3, it was shown that all of the piglets became naturally infected with the virus by the age of 40 days, with most becoming infected before weaning. The way in which rotaviral infection is maintained in the pig herd has not been fully elucidated. There are a few reports in the literature of rotavirus being recovered from adult pigs (sows) (Benfield et al. 1982, Corthier et al. 1980). If this was a common occurrence, such infected sows could act as a source of infection for young pigs. However, in light of the present work, this now appears unlikely at least in the New Zealand situation. Previous studies (sections 3.2 and 3.3) showed that piglets were all naturally infected with rotavirus before six weeks of age and had eliminated infection before they were two months old. No infected adult pigs (including sows) were detected in the herd examined. Debouck and Penseart (1983) also failed to detect rotaviral antigen in faeces from sows. Transmission of rotavirus from piglet to piglet and from litter to litter has been observed. This suggests that continuous transmission of virus from one group of young piglets to another is important in maintaining the cycle of rotaviral infection in a piggery. In the present study, rotaviral antigen was readily demonstrated in dust and dry faeces from the farrowing house which had been cleaned and disinfected, and from a disused weaner house which had been free of piglets for three months. Rotavirus was only recovered from those environments (sucking and weaner houses) in which rotaviral infection was also detected in pigs. Furthermore, porcine rotavirus can survive

in faeces for a long period (more than four months). The environmental persistence of rotavirus appears to be another important factor in maintaining the cycle of rotaviral infection in a piggery. Young, susceptible piglets could become infected with rotavirus from their environment when their maternal antibody wanes to an unprotective level. They may then transmit the virus to other piglets.

Thus any future control programs for group A rotaviral infection must take much greater cognizance of the potential importance of environmental sources of virus.

3.4.5 Conclusions

1. Group A rotavirus is present in the environment (farrowing and weaner houses) where pigs are found to shed rotavirus. Rotaviral antigen was not detected in fatterner and sow houses, which coincides with the fact that rotavirus was not demonstrated in fatterner pigs or sows.
2. The virus detected in the samples collected from the environment of Massey Piggery was infectious as replication occurred in MA-104 cells.
3. The method developed for recovering indigenous viruses from raw wastewater sludge (Brashear and Ward 1981) appeared to be efficient for recovering rotavirus from environmental samples.
4. Group A rotavirus can survive in the environment for a long time. The SW20/21 strain of porcine rotavirus survived for at least four months with a reduction in titre of $2 \log_{10}$.
5. The detection of rotavirus in the environment of Massey Piggery and the survival of porcine rotavirus (SW20/21) in MEM and faeces for at least four months suggests that environmental persistence of rotavirus is another important factor in maintaining the cycle of rotaviral infection in piggeries.

Chapter Four

TRANSFER OF MATERNAL ANTIBODY AGAINST ROTAVIRUS FROM SOWS

TO PIGLETS AND SEROLOGICAL RESPONSES FOLLOWING NATURAL INFECTION

4.1 INTRODUCTION

Since rotavirus is a major cause of diarrhoea in the young of many species of animals including man, efforts have been made to investigate both passive and active immunity against rotaviral infection. Human infants acquire maternal antibodies in utero and via colostrum and milk from their mothers (McLean and Holmes 1980). Antirotaviral antibody levels in babies' sera reflect those of colostrum and their mothers' sera. Specific antirotaviral antibodies are also present in the faeces of breast-fed babies two days after birth. After natural infection with rotavirus, antibody responses, with four-fold or greater increases in antibody titres, are usually detected in sera and similar responses are also observed in faeces of infected subjects (Bishop et al. 1984, Grimwood et al. 1988, Sonza and Holmes 1980).

Transfer of maternal antirotaviral antibody from sow to piglet has also been investigated during the first 14 days after farrowing (Hess and Bachmann 1981). Antibody responses in sera and in faeces have also been studied in experimentally infected piglets (Corthier and Vannier 1983). In chapter three, the patterns of shedding and transmission of rotaviral infection were studied by following piglets from birth through the period of greatest susceptibility. In this chapter, the dynamics of antirotaviral antibody responses of these animals, including the transfer and persistence of maternal antibody and the development of active immunity following natural infection, was investigated using the standardized ELISA test (section 2.3). Attempts were also made to correlate the levels of antibody in the serum and/or in the faeces with the occurrence of natural and experimental infections with rotavirus.

4.2 MATERIALS AND METHODS

Animals: Three groups of animals were used for this study and all pigs were from the Massey Piggery described in 2.1.1. Group 1 comprised four sows (two gilts and two multiparous sows) and 16 piglets (four from each sow) described in section 3.2. Group 2 comprised five sows (one

gilt and four multiparous sows) and 20 piglets (four from each sow) described in section 3.3. The management of these animals is described in detail in sections 3.2 and 3.3, respectively.

Group 3 comprised two sows (multiparous) and their piglets (total 18). Sow No. 1 had 10 and sow No. 2 had 8 piglets. These piglets were weaned at 32 days of age. Faecal samples were collected from sows in this group from 3 days before farrowing until weaning and from piglets from 4 to 80 days of age. All these samples were extracted with PBS as described in section 2.1.2.

Natural infection with rotavirus: Shedding of rotavirus in piglets of groups 1 and 2 was summarized in section 3.2 and 3.3, respectively. Shedding of rotavirus in piglets of group 3 was also examined by the Dakopatts ELISA kit (section 2.2). Shedding of haemolytic E.coli was also detected as described in 3.2.2.

Experimental infection with rotavirus: Four piglets from group 3 were experimentally inoculated at 66 days of age with rotavirus purified from faeces collected in the piggery. For virus purification, 100 g of faecal material from pigs shedding rotavirus was collected and diluted to 25% in PBS. After homogenization, the sample was centrifuged at 5000 g for 30 min. The supernatant was harvested and passed through a 0.45 µm millipore filter (MFS, Dublin, CA, USA). The final preparation was used as the inoculum. Each piglet was inoculated intranasally with 1 ml of the virus preparation.

To confirm the infectivity of the virus preparation, four piglets at three weeks of age, which were free from rotaviral infection, were also inoculated intranasally with 1 ml of the preparation. Faeces from these four piglets and their littermates (total nine piglets) were collected daily for eight days after inoculation. The faeces were examined for group A rotavirus by the Dakopatts ELISA kit. The genomic pattern of the virus preparation was compared with that of the virus shed by these younger piglets, after infection, using PAGE as described in section 2.2.

Samples: Colostrum was collected from the nine sows within 24 hours post partum (pp). Samples were adjusted to pH 4.6 with 0.1 M HCl and then centrifuged at 3000 g for 30 min. The whey was collected and stored at -20°C until use. Milk samples were collected from one sow in group 1 at 10 and 18 days pp and from four of five sows in group 2 at 18

days pp. These milk samples were processed in the same way as for colostrum.

Blood samples were collected from piglets in group 1 at 5, 14, 21 and 30 days of age, from piglets in group 2 at 9, 17, 25, 33 and 55 days of age, and from piglets in group 3 at 8, 30, 52, 66 and 80 days of age.

Blood samples were collected from sows in group 1 at 2, 8, 14 and 24 days pp, from sows in group 2 at 1, 16 and 30 days pp, and from sows in group 3 at 8 and 21 days pp.

Detection of antirotaviral antibodies: The levels of antirotaviral antibody were measured in colostrum, milk, serum and faeces from piglets of the above three groups by the ELISA test described in section 2.3, using the sheep anti-pig IgG, IgM and IgA (whole molecule) conjugate. Colostrum, milk and serum was doubly diluted from 1/50 to 1/6400, and faecal extracts from 1/10 to 1/1280. If a sample at the highest dilution still yielded an ESA value over 0.100, this sample was further diluted and retested. Class specific antibodies (ie, IgA, IgG and IgM) were also detected by the ELISA tests using conjugates of sheep anti-pig IgA (α -chain only), IgG (γ -chain only) and IgM (μ -chain only), respectively conjugated with horse-radish peroxidase (Eivai Bios Laboratories). These conjugates were used at dilutions of 1/1000. All sera from animals in group 1 and group 3 were also titrated for neutralizing antibodies by the SN test as described in 2.1.7.

4.3 RESULTS

Transfer of maternal antibody from sow to piglet: Fig. 4-1 shows the mean reciprocal ELISA antibody titres against rotavirus in colostrum, sows' sera and piglets' sera. Eight of the nine sows had antirotaviral antibody titres between 1/1600 and 1/12800 in colostrum, while the other one had only 1/200. All the sows had serum antibody titres of 1/1600 to 1/12800 at 1 to 3 days pp. All the piglets acquired maternal antibody and had serum antibody titres of 1/800 to 1/12800 at the age of 3 to 9 days. Comparison of the antibody titres in colostrum, sows' sera and piglets' sera revealed that the antibody levels in piglets' sera were more closely correlated to those in colostrum (with a correlation coefficient of 0.79) than to those in sows' sera (0.50).

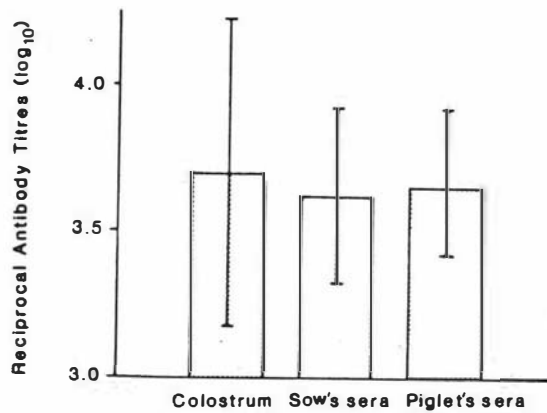


Fig. 4-1. Comparison of antibody titres in colostrum, sows' and piglets' sera. Vertical bars represent SD.

Fig. 4-2 depicts the decline of antirotaviral antibody in mammary secretions with the change from colostrum to milk, and in sow's sera. Antibody titres in milk collected at 18 days pp were 8 to 32 fold lower than those in colostrum. There was little change in serum antibody titres in sows during the period of investigation (one month).

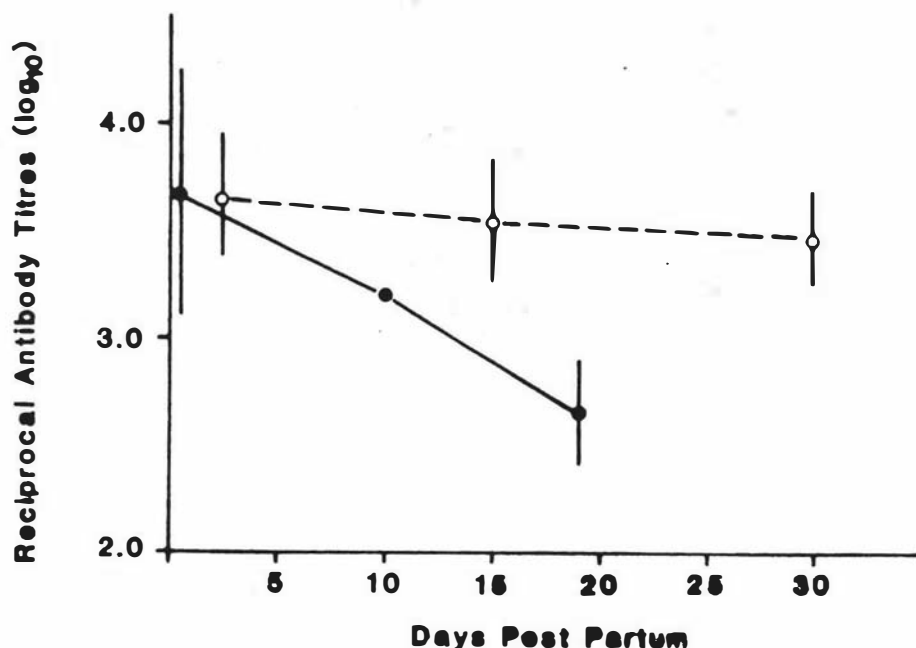


Fig. 4-2. Decline of antibody titres in milk (—) from one to 18 days pp and in sows' sera (---) from parturition to one month pp. Vertical bars represent SD.

After consumption of colostrum, piglets had antibody levels in their sera comparable to those in colostrum. These maternally-derived antibody titres declined rapidly, and in piglets of all groups, had reached the lowest level at about one month of age (Fig. 4-3).

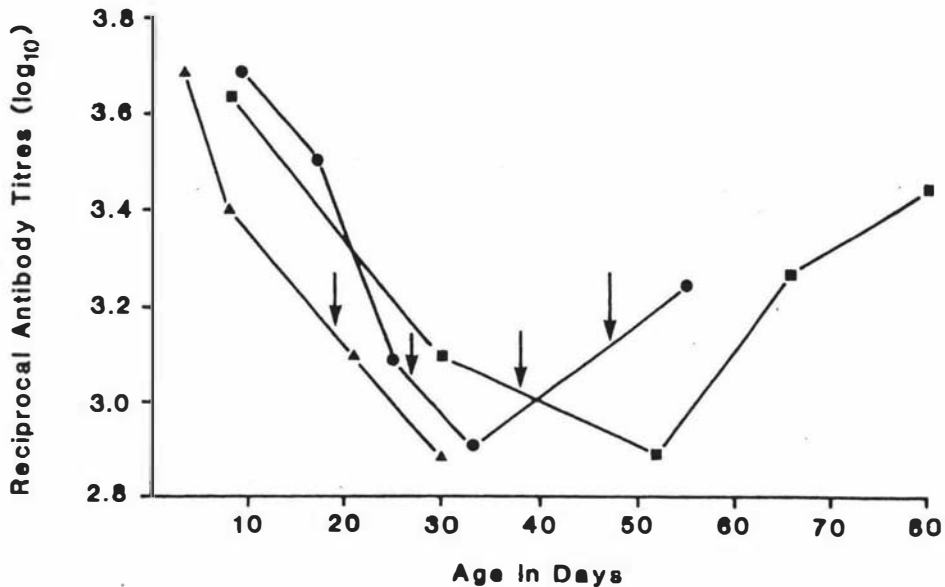


Fig. 4-3. The profile of antirotaviral antibody titres in sera of piglets. (▲—▲) group 1, (●—●) group 2, (■—■) group 3. Arrow indicates average age at which shedding of rotavirus was first detected in the particular group.

Antirotaviral antibodies were also detected in the faeces of piglets soon after birth. The levels and the decline of the copro-antibody in the two litters of piglets in group 3 are shown in Fig. 4-4. High levels of antibody were detected at six and eight days of age but the copro-antibody levels declined rapidly and copro-antibody was not detected at 18 days of age.

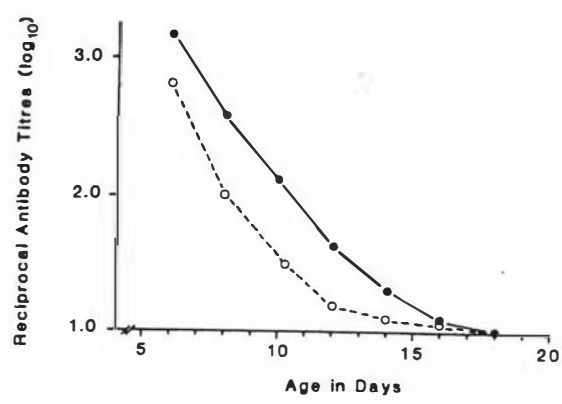


Fig. 4-4. Decline of maternally-derived antibody in faeces of piglets in litter No.1 (---) and those in litter No.2 (—) in group 3.

Class specific antibodies in colostrum and in milk: Table 4-1 summarizes the results for class specific antibodies in colostrum and milk in five sows (one from group 1 and four from group 2). Antirotaviral antibodies of IgA and IgG classes were detected in all of the colostrum samples but IgM was not detected in any of them. Antirotaviral IgA antibody levels were slightly higher than those of the IgG class. In milk samples, both IgA and IgG were detected at a low level. IgM was not detected.

Table 4-1 Reciprocal titres of class specific antirotaviral antibodies in colostrum and milk samples of five sows

Sow No.	Colostrum			Milk (18 days pp)		
	IgA	IgG	IgM	IgA	IgG	IgM
1	25600	12800	<50	200	50	<50
2	1600	1600	<50	100	100	<50
3	25600	25600	<50	200	200	<50
4	3200	800	<50	200	<50	<50
5	6400	3200	<50	800	800	<50

Production of antibody after natural infection:

Group 1: As summarized in table 3-3, 14 of the 16 piglets in group 1 were naturally infected with rotavirus between 13 and 24 days of age (the other two shed rotavirus for the first time at 27 and 39 days of age, respectively). No antibody increase was detected in any of these piglets at 30 days of age by either the ELISA (table 4-2) or the SN tests (table 4-3).

Table 4-2 Reciprocal ELISA antibody titres in sera of 16 piglets in group 1

Piglet No.	Age in days			
	5	14	21	30
1	12800	6400	3200	1600
2	6400	3200	1600	800
3	12800	6400	3200	1600
4	12800	6400	3200	3200
5	1600	800	400	200
6	NT	NT	NT	NT
7	800	400	400	200
8	1600	1600	NT	400
9	3200	1600	800	800
10	3200	1600	800	400
11	3200	1600	800	800
12	3200	1600	800	400
13	12800	6400	3200	1600
14	6400	NT	NT	800
15	12800	NT	3200	1600
16	6400	1600	800	800

NT Not tested.

Table 4-3 Reciprocal SN antibody titres in sera of 16 piglets in group 1

Piglet No.	Age in days			
	5	14	21	30
1	128	32	8	4
2	64	32	16	8
3	128	32	8	4
4	32	16	8	4
5	<2	<2	<2	<2
6	<2	<2	<2	<2
7	4	<2	<2	<2
8	<2	<2	<2	<2
9	16	4	<2	<2
10	64	16	4	4
11	32	8	4	<2
12	32	8	<2	<2
13	32	16	8	8
14	16	8	NT	8
15	32	NT	4	4
16	16	4	2	<2

NT Not tested.

During the period of investigation, no increase in antibody titres was detected by the ELISA test in any of the four sows in this group (table 4-4), but in the two gilts (sows No. 2 and 4), rising titres of antibody against rotavirus were detected by the SN test (table 4-5). Antibody titres did not increase in the two multiparous sows during the same period.

Table 4-4 Reciprocal ELISA antibody titres in sera of four sows in group 1

Sow No.	Days post partum			
	2	8	14	24
1	3200	3200	3200	3200
2	1600	1600	1600	1600
3	6400	6400	3200	3200
4	6400	6400	6400	6400

Table 4-5 Reciprocal SN antibody titres in sera of four sows in group 1

Sow No.	Days post partum			
	2	8	14	24
1	32	32	32	32
2	<2	<2	4	8
3	16	16	16	16
4	4	8	8	16

Group 2: All piglets in group 2 were infected at ages from 19 to 34 days (see table 3-6), but rising antibody titres were not detected in any of these piglets at 33 days of age using ELISA (see Fig. 4-3). At 55 days of age, 70% of these piglets had rising antibody titres with a 2 to 16 fold increase. None of the sows had rising ELISA antibody titres during the period of investigation.

Group 3: None of the piglets in group 3 shed rotavirus before being

weaned at 32 days of age. Immediately after weaning (3-5 days), all of these piglets shed rotavirus (between 35 and 38 days of age). Piglets born of sow No. 2 shed group A rotavirus one to three days earlier than did those born of sow No. 1. The duration of shedding in these piglets was from 4 to 6 days. Nine piglets developed diarrhoea for one to two days soon after rotavirus was detected. Four of the 18 piglets shed haemolytic E.coli two to three days after weaning and another five shed the bacteria 18 to 21 days after weaning. The duration of shedding of haemolytic E.coli was about two days. None of the faecal samples from these piglets showed a pure growth of haemolytic E.coli and none of the piglets developed diarrhoea associated with the infection of haemolytic E.coli.

Fig. 4-5 depicts the profile of antirotaviral antibody in piglets of group 3 as detected by ELISA and SN tests. From the results of the SN test, increases in antibody titres were detected in the sera collected at 52 days of age (14 to 17 days after shedding of rotavirus) from all the piglets of this group. The increase was between four and eight fold in individual piglets. No further increases in antibody titres were detected in these animals at 66 and 80 days of age by the SN test. From the results of the ELISA test, no rising antibody titres were detected in sera collected at 52 days of age from any of these piglets. Thirteen (72%) of the 18 piglets had rising ELISA antibody titres, with a two to eight fold increase in titre in the sera collected at 66 days of age. In eight piglets the antibody titres continued to rise by 80 days of age with a further two to eight fold increase. During the period from 52 to 80 days of age, 14 (78%) of the 18 piglets had rising antirotaviral antibody titres as detected by the ELISA test with a two to 16 fold increase (mean of 7.3 fold). All the piglets in litter No. 2, and four of the 10 piglets in litter No. 1, had rising ELISA antibody titres during the period of investigation.

Following natural infection with rotavirus, none of the piglets from these three groups had rising faecal antibody titres as detected by the ELISA test.

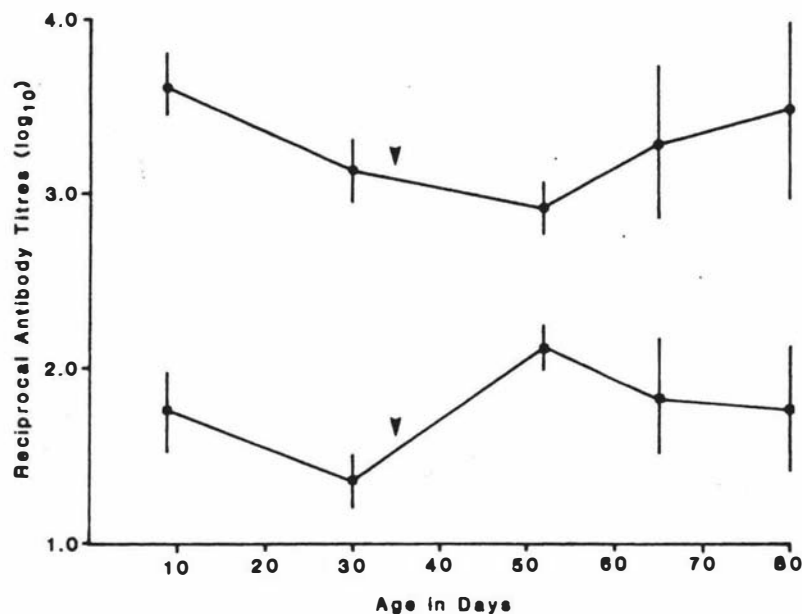


Fig. 4-5. Antirotaviral antibody profile in piglets of group 3 as detected by ELISA (upper line) and SN (lower line). Vertical bars represent SD. Arrows indicate age at which shedding of group A rotavirus was first detected.

Class specific antibodies against rotavirus were also measured in the sera of five piglets in group 3 from birth to 80 days of age (Fig. 4-6). IgA and IgG were detected in all of the serum samples, but IgM was not detected in any of these samples. IgA antibody levels were slightly higher than those of IgG in samples collected 9 days after birth. However, following natural infection, increases in antibody titres occurred more in the IgG fraction than in the IgA fraction.

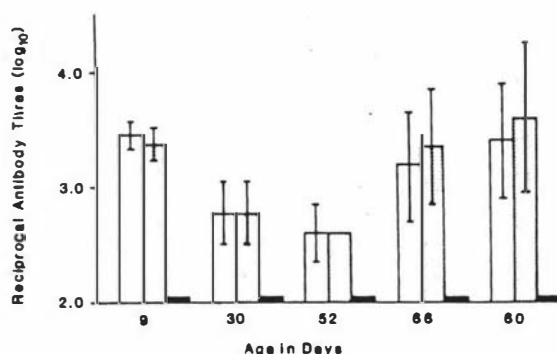


Fig. 4-6. Profile of class specific antibodies against rotavirus in sera of five piglets in group 3 from birth to 80 days of age. (□) IgA, (◐) IgG, (■) IgM. Vertical bars represent SD.

Experimental infection: Four pigs (two from each litter) from group 3 were infected at 66 days of age with one ml of virus preparation as described in the materials and methods. Up to 80 days of age, none of these pigs developed diarrhoea or shed the virus, or had an increase in SN antibody titre (table 4-6). Two piglets had four and eight fold increases in antibody titres against rotavirus as detected by the ELISA test. Neither of the piglets had rising antibody titres at 66 days of age following natural infection at about 38 days of age. None of the other 14 littermates, which were not inoculated, developed diarrhoea or shed the virus. Six of these 14 littermates, however, had rising antibody titres with two to four fold increases by the ELISA test during the 14 day period, but no increases in antibody titre were detected by the SN test.

Table 4-6 Reciprocal of antibody titres in sera of four pigs before and after intranasal inoculation of rotavirus at 66 days of age

Piglet No.	SN		ELISA	
	66*	80	66	80
1	32	32	400	3200
2	64	32	400	400
3	256	256	1600	6400
4	256	256	6400	6400

* Age in days.

When four young piglets (three weeks of age) were inoculated with the same virus preparation, all shed the virus in their faeces three days after inoculation. Other littermates also shed the virus four to six days after the four piglets were inoculated. The virus recovered from these piglets showed the same pattern by PAGE as that of the virus in the inoculum (Fig. 4-7). Sera from these piglets were not tested for antirotaviral antibodies.

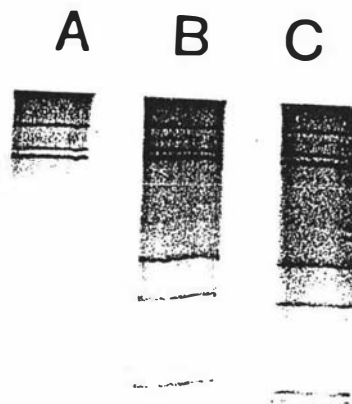


Fig. 4-7. RNA patterns of rotavirus in the inoculum and in the faeces of experimentally infected piglets. Lane A: virus in the inoculum; Lane B: co-electrophoresis of samples in Lanes A and C; Lane C: virus recovered from the faeces of infected animals. Electrophoresis was from top to bottom.

Correlation between levels of antibody and rotaviral infection: The shedding of rotavirus in all piglets, including the second episode of shedding in some piglets in group 2, occurred when the average ELISA antibody titres in the sera were below 1/1600 (reciprocal titre $3.2 \times \log_{10}$) (see Fig. 4-3).

In group 1, rotaviral shedding occurred earlier in piglets from the two gilts than in those from the two multiparous sows (see table 3-5). Piglets from one of the gilts had very low levels of maternal antibody (see table 4-2 and 4-3) associated with the low antibody levels in the colostrum of their dam (1/200), while piglets from the other gilt had ELISA and SN antibody levels comparable to those from multiparous sows. Piglets (No. 9-12) from one multiparous sow (No. 3) shed rotavirus later (see table 3-3), but they did not have higher ELISA or SN antibody titres than those from the other multiparous sow (No. 1) (see tables 4-2 and 4-3).

In group 2, piglets from all the five litters had similar levels of maternal antibody, as measured by the ELISA test, with titres ranging from 1/3200 to 1/12800. Rotaviral shedding occurred first in piglets from sow No.1 which was housed in one farrowing room with other older piglets, then in piglets from sow No.2 which was housed closest to the door of another farrowing room. Finally, piglets from sows No. 3, 4 and 5 started to shed the virus. These piglets were housed with piglets of sow No.2. Therefore, the shedding of rotavirus in piglets of this cohort occurred depending on where they were situated in the farrowing unit (see section 3.3).

In group 3, rotaviral shedding did not occur until piglets were 35 days of age. Serum antibody titres in these piglets at 30 days of age were between 1/800 and 1/1600 as detected by the ELISA test and between 1/8 and 1/32 as detected by the SN test, while faecal antibody had declined to levels undetectable in the ELISA test by 18 days of age. At 66 days of age, four piglets were inoculated with rotavirus purified from faeces from pigs in the same piggery, but none of them became infected as judged by the shedding of the virus, development of diarrhoea or increases in SN antibody titres. However, rises in antibody titres were detected in two piglets by the ELISA test.

4.4 DISCUSSION

Investigations have been conducted to detect antirotaviral antibodies in sow's colostrum and milk (Askaa et al. 1983, Corthier and Franz 1981), and to study the transfer of antibody from sows to piglets (Hess and Bachmann 1981). These investigations are further extended in the present study by measuring the levels of antirotaviral antibody in sow's colostrum, milk and sera, and in piglet's sera and faeces, and by comparing the levels of antibody to episodes of natural rotaviral infection throughout the period of greatest susceptibility.

The results from this study on the levels of antirotaviral antibody in colostrum and milk and on the transfer of antibody from sows to piglets are in agreement with those reported by Hess and Bachmann (1981). The titres of antirotaviral antibody in colostrum and sera from sows, and piglets' sera are comparable, but serum antibody titres in piglets are more closely correlated to those in colostrum (0.79) than to those in sow's sera (0.50). Milk samples contained very low levels of antibody.

Class specific antirotaviral antibodies have also been studied in colostrum. Hess and Bachmann (1981) observed that the levels of IgA and IgG were very high and similar, but IgM was either not detected or detected at a very low level. However, Corthier and Franz (1983) found that the titres of all three classes of antibodies were high in colostrum, but IgG was the predominant fraction. In the present study, both IgA and IgG titres were very high with IgA titres slightly higher than IgG, while IgM was not detected. These results are more similar to those of Hess and Bachmann (1981) than to those of Corthier and Franz (1981).

After consumption of colostrum, antirotaviral antibodies were detected, not only in the serum, but also in the faeces of piglets. The levels of maternal antibody in faeces declined more quickly than those in serum and by 18 days after birth, no faecal antibody was detected. Faecal antibody has also been detected in breast-fed infants two days after birth (McLean and Holmes 1980).

The protective effects of maternally-derived antirotaviral antibody against rotaviral infections have not been fully elucidated. In man, rotaviral infection occurs mostly in young children between six and 24

months of age but very rarely in neonates (less than six months of age) (see section 1.3). This phenomenon has been attributed to maternally-derived antibody either acquired in utero or from colostrum, the presence of enzyme inhibitors in human colostrum and milk (McLean and Holmes 1980, 1981) and the low concentration of proteolytic enzymes in the neonatal gut (Wolf et al. 1981). Breast-fed babies were found to be infected with rotavirus much less frequently than were bottle-fed babies (Chrystie et al. 1975, Totterdell et al. 1976). These authors, however, later found no correlation between the levels of antirotaviral antibody in mothers' colostrum and milk and protection of their babies from infection by rotavirus (Totterdell et al. 1982, 1983). A correlation between protection and the presence of enzyme inhibitors in colostrum and milk was not found in those studies either. Furthermore, no significant differences were observed by other workers (Weinberg et al. 1984) between breast- and bottle-fed babies in either the numbers of babies infected with rotavirus or the average age at infection.

In pigs, rotaviral infection occurs mostly in three to six weeks old piglets, but rarely in piglets under ten days of age. This has also been attributed to the presence of maternally-derived antibodies (Bohl 1979, Debouck and Pensaert 1983, Utrera et al. 1984). It has been reported that colostrum from gilts had lower levels of antirotaviral antibody than that from multiparous sows. Piglets born of gilts contracted rotaviral infection earlier than those born of multiparous sows (Askaa et al. 1983). In the present study, it was found that maternally-derived antibody in serum, as detected by both ELISA and SN tests, declined to a low level at three to five weeks of age and faecal antibody was not detected at 18 days of age. This coincided with the occurrence of infection by rotavirus observed in this study and reported by others (Bohl et al. 1978, Debouck and Pensaert 1983, Utrera et al. 1984). All the piglets in this study shed rotavirus when the mean ELISA antibody titers fell below 1/1600 (equivalent to SN antibody titres of 1/8 to 1/16) in the respective groups. All these results indicate that maternally-derived antibody is protective against natural rotaviral infection during the neonatal period. However, a correlation between the levels of maternal antibody and the occurrence of rotaviral infection was not observed in individual litters of piglets in the present study. Piglets born of the two gilts in group 1 shed rotavirus earlier than those of the multiparous sows. Piglets from one of the gilts had low antibody titres in serum, as detected by both the ELISA and SN tests. Antibody levels of piglets from the other gilt were high and were comparable to those of piglets from the multiparous sows.

Piglets from one multiparous sow had the highest level of ELISA and SN antibodies, but they shed rotavirus only 3-4 days after piglets from the gilts commenced shedding the virus. The piglets from the other multiparous sow did not have such high antibody levels, yet they shed the virus much later than piglets from any other litter. In group 3, piglets from sow No. 1 had lower antibody levels than those from sow No. 2, but piglets from sow No. 2 shed rotavirus earlier than those from sow No. 1. In each of these three groups of piglets, usually one or two piglets from a litter started to shed rotavirus, then the infection spread to other piglets in the same litter and finally to piglets in other litters. The pattern of transmission of rotaviral infection was related to the location of the piglets within the farrowing house but not to the levels of antirotaviral antibody in individual litters. The only exception was with piglets from sow No.3 in group 1. Two of the four piglets investigated shed the virus for the first time at 27 and 39 days of age; this was much later than other piglets in the same group. However, piglets from this litter did not have the highest level of antibody for that groups.

From these results, it is, therefore, postulated that young piglets are usually protected against rotaviral infection during the first one or two weeks of life. As the antibodies from some piglets wane to a low, unprotective level, these piglets could become infected from other infected animals or from a contaminated environment. These initially infected animals are capable of amplifying the infection. The large amount of rotavirus shed by these initially infected animals will eventually overcome the protective effects of even quite high levels of maternally-derived antibody in other piglets of the same group. This postulate is supported by the experiment carried out by Lecce et al. (1978), in which a steady build up of rotaviral infection of increasing clinical severity was observed in piglets when they were weaned at one day of age and introduced continuously to a nursery without it being further fumigated. Substantial evidence to suggest that the protective effect of maternally-derived antibody against rotavirus depends on the level of the challenge dose has been reported in other species of animals. High levels of antibody in colostrum and milk fed to calves did not prevent infection when they were challenged with a high dose of rotavirus (10^{11} particles/g) (Snodgrass et al. 1980). Fahey et al. (1981) found that passive antibody protected lambs from experimental infection with a smaller dose of rotavirus (10^7 particles/ml). Conner and Darlington (1980) also suggested that a buildup of rotavirus to a level exceeding the protective effects of colostral antibody accounted

for the epidemics of rotaviral infection among foals in nurseries in Kentucky.

In the standardization of the ELISA test used in the present study (see section 2.3), a correlation coefficient of 0.82 was achieved between the results obtained by this ELISA test and a SN test. In the present study, it appeared that the ELISA antibody titres correlated with SN titres in samples from animals with past infection and from young animals with passively-acquired antibody. The ELISA test failed to detect an immediate antibody response in two gilts with subclinical rotaviral infection and did not detect rising antibody titres in animals two to three weeks after natural infection as did the SN test. However, the ELISA test detected such a response in 70% of animals, about one month after SN antibody was first detected. As discussed in section 3.3, these differences between the ELISA and the SN tests may be due to the fact that the two tests detect different antibodies. Serum neutralizing antibody has been regarded traditionally as the indicator of exposure to virus and resistance against infection. Serum neutralization, in the case of rotavirus, detects antibodies against the outer capsid proteins (i.e. VP7 and VP3) which are responsible for neutralization specificities (Greenberg et al. 1983a, c, Kalica et al. 1981b, Offit and Blavat 1986). ELISA, on the other hand, detects any antibodies reactive to the antigens coated onto the plates, including the inner capsid as well as the outer capsid proteins. Another explanation could be that animals, after infection, respond to the outer capsid proteins first and then to other viral components such as the inner capsid proteins of the virus. It is also possible that, due to the method of preparation, the ELISA antigen may have consisted predominantly of single shelled particles and thus did not particularly detect antibodies against the outer capsid proteins which were involved in neutralization. Poor affinity of the antibody produced early after infection may also account for the failure of the ELISA to detect an immediate antibody response (Bulter et al. 1978), however this should also apply to the detection of SN antibodies.

Repeated infections with rotavirus have often been reported early in life in children (Bishop et al. 1983, Chiba et al. 1986, Mata et al. 1983) and in other animals (Debouck and Pensaert 1983). In the present study, repeated infection with rotavirus at intervals of about 10 days was observed in five of 16 animals in group 1 and nearly half of the surviving piglets in group 2 during the first two months of their life. This is possibly due to the weak immunity induced by the first

episode of infection since at the second time of shedding, the mean ELISA antibody titres were still under 1/1600. Weak immunity produced by rotavirus has also been described in children. Children with early infection with rotavirus during the first six months of their life had further rotaviral infections during the subsequent three years although the severity of diarrhoea was reduced during subsequent infections when compared with those children who did not experience infection early in their life (Bishop et al. 1983).

In the present study, none of the pigs examined shed rotavirus over two months of age either under natural conditions or after experimental inoculation with purified rotavirus from faeces. The resistance of these older pigs to infection by rotavirus contrasts with reports by other workers. Grom et al. (1984) detected rotavirus in about 15% of fattener pigs (over two months of age) by ELISA. Jestin (1984) successfully infected specific pathogen free (SPF) fattener pigs (over 70 kg) by placing these pigs in contact with rotavirus infected piglets from a non-SPF herd. Bernard et al. (1984) also successfully infected conventionally-reared fattener pigs by experimental inoculation with the OSU strain of rotavirus. These discrepancies may have been due to the immune status of animals studied. It is not surprising that older SPF pigs became infected with rotavirus when they were put in contact with rotavirus infected piglets (Jestin 1984), since these SPF pigs did not have any specific resistance to rotavirus. The susceptibility to infection of conventionally-reared fattener pigs in France with the OSU strain of porcine rotavirus (Bernard et al. 1984) may have been due to the fact that the virus circulating in the pig population in France belonged to a different serotype from that of the OSU strain, which originated in the USA (Bohl et al. 1984). Therefore, any immunity induced by prior exposure to the French strain of rotavirus may not have been sufficient to protect against infection by the OSU strain. In the present study, rotaviruses infecting piglets at Massey Piggery appear relatively stable. A single electrophoretype of rotavirus has been found to persist at Massey Piggery for two years (see chapter five). Immunity resulting from infection with this electrophoretype of rotavirus would therefore be expected to protect against reexposure. In addition, all the piglets had been infected once or twice with rotavirus before two months of age and might therefore have been sufficiently immune to resist rotaviral infection up to the time of slaughter at four or five months of age. Debouck and Pensaert (1983), who studied the shedding pattern of group A rotavirus in pigs from birth to 23 weeks of age, also reported that detection of group A rotavirus in pigs two weeks

after weaning was an exception. Thus, the study by Grom et al. (1984) should be treated cautiously. The more intensive farming system (about 3000 breeding sows in each piggery) reported by Grom et al. (1984) compared with that described in the present study (about 60 breeding sow) may explain the difference in the detection of group A rotavirus in fattener pigs. More intensive systems of farming not only facilitate the transmission of rotavirus infection but also lead potentially to the rapid emergence of new types of rotaviruses due to mutation and/or reassortment.

Recently, rotaviral infection has been demonstrated in five month old pigs in Papua New Guinea (D. Alpers, personal communication 1988). In these places, pigs and man live within the same ecosystem and cross-species transmission may occur. The low standard of hygiene in these communities would facilitate such transmission.

As described in section 3.2 and 3.3, rotaviral shedding in piglets of group 1 and group 2 was closely associated with the mild diarrhoea syndrome (milk scours) in preweaning pigs. Most of those piglets also developed diarrhoea five to seven days after weaning in association with infection by haemolytic E.coli. In piglets of group 3, no rotaviral shedding was detected before weaning. All of these piglets, shed rotavirus three to five days after weaning. Haemolytic E.coli was only sporadically detected in some of these animals. No severe diarrhoea, as observed in piglets of groups 1 and 2 after weaning, was observed in piglets in group 3. This might have been because piglets in groups 1 and 2 were weaned earlier (24-26 days of age) than those in groups 3 (32 days of age) and thus piglets in the former group were more prone to infection with haemolytic E.coli than the latter. Also, piglets in group 3 were not infected with rotavirus before weaning, so it is possible that the gut epithelium had not been damaged and therefore did not favour colonization by haemolytic E.coli, as suggested by Lecce et al. (1982).

4.5 CONCLUSIONS

1. All piglets acquired antirotaviral antibody by sucking colostrum from their dams. The levels of antibody in sera of these animals after sucking were more closely correlated to those in their dams' colostrum than to those in their dams' sera.

2. The levels of antirotaviral antibody in sows' milk declined rapidly post partum (pp) and were eight to 32 fold less in samples collected at 18 days pp than in samples collected within 24 hours pp.
3. The levels of antirotaviral antibody in piglets' sera also declined rapidly and fell to the lowest levels at about one month of age.
4. Antirotaviral antibody was also detected in the faeces of piglets soon after birth. The levels of faecal antibody fell rapidly and were undetectable by 18 days of age.
5. Antirotaviral antibodies of both IgA and IgG classes were detected in colostrum and milk while IgM was not detected in either.
6. Piglets developed antibody response following natural infection by rotavirus. Rising in serum neutralizing antibody was detected at about two weeks after natural infection, but no rising ELISA antibody was detected until one months after natural infection. This difference is presumably related to the two tests detecting antibodies against different viral antigens rather than indicating that the SN test is more sensitive than the ELISA test.
7. Antirotaviral antibodies of both IgA and IgG classes were detected in piglets' sera collected after sucking and following natural infection. Antirotaviral IgM was not detected in any sample. After consumption of colostrum, the levels of antirotaviral antibody of the IgA class were higher than those of the IgG class in piglets' sera. Following natural infection, the levels of IgG was higher than that of IgA.
8. Increasing antibody titres in the sera of the two gilts were detected by the SN test in this study. However, neither rotaviral antigen nor diarrhoea was observed in these two gilts. This suggests that the two gilts had subclinical infection during the farrowing period.
9. No change in antibody titres were detected in sera from any of the other sows investigated.
10. Pigs over two months of age did not become infected with rotavirus after intranasal inoculation as judged by the shedding of virus, the development of diarrhoea or by serological response. This is thought to

be due to the protection conferred by antibodies produced following early natural infection.

11. Correlation between the levels of maternally-derived antirotaviral antibody in piglets and susceptibility to rotavirus was observed in each of the cohorts studied. Natural infection by rotavirus occurred in each of these cohorts when their geometrical mean ELISA antibody titres fell below 1/1600 (equivalent to a titre of 1/8 to 1/16 in the SN test). It is believed that maternal antibody protects against rotaviral infection in piglets at least for the first one week of life.

12. No correlation was observed between the levels of maternal antirotaviral antibody and the occurrence of rotaviral infection in individual litters of piglets. In each of the cohorts, there were some piglets which began to shed rotavirus first and then the infection spread to other piglets in the same cohort regardless of the levels of antibody. It is thus suggested that the build up of the challenge dose of rotavirus by these initially infected piglets overcomes the protective effects of even high levels of antibody in the other piglets.

Chapter Five

EPIDEMIOLOGY OF ROTAVIRAL INFECTIONS

IN OTHER NEW ZEALAND PIGGERIES

5.1 INTRODUCTION

Previous studies on the patterns of shedding and transmission of group A rotavirus in pigs at Massey Piggery demonstrated that pigs became infected with the virus at two to three weeks of age. Infection rates reached a peak at five weeks and shedding of rotavirus ceased before 60 days of age. No group A rotavirus antigen was detected in the faeces of pigs over two months old. Group A rotaviral antigen was never detected in faeces from sows throughout the farrowing period (see chapters three and four).

PAGE has been widely used for diagnosis and epidemiological investigations of infections by rotavirus in various species of animals (Gerna et al. 1987a, Herring et al. 1982, Konno et al. 1984, Liprandi et al. 1987, Lourenco et al. 1981, Todd et al. 1984). By employing this technique, genomic variations among the 11 segments of the RNA of group A rotaviruses have been frequently detected in different individuals during the same outbreak (Lourenco et al. 1981, Konno et al. 1984), and in the same patient during one episode of infection (Spencer et al. 1983b). Variations have also been detected in viruses recovered from both longitudinal (Gerna et al. 1987a, Rodger et al. 1981), and cross-sectional investigations (De San Juan et al. 1986).

In the present investigation, faecal samples were collected from five other New Zealand piggeries to examine the pattern of shedding of group A rotavirus in different age groups of piglets to see whether or not the pattern observed at Massey Piggery is typical of those in other piggeries in New Zealand. These samples were also examined using the PAGE technique to determine the genomic variations of group A rotaviruses in these piggeries. Attention was also paid to the presence of non-group A rotaviruses.

5.2 MATERIALS AND METHODS

Piggeries: Five New Zealand piggeries were selected for this investigation and these were denoted by the numbers 1, 2, 3, 4 and 5, respectively. These piggeries were located in both the North Island and the South Island of New Zealand. Distances between these piggeries were from 30 to 800 Km. Pigs at all these piggeries were reared conventionally with each piggery having 200 to 400 breeding sows. Piglets were weaned at three to four weeks of age.

Samples: Faecal samples from three age groups of pigs (i.e. 15-20, 30-35 and 70-90 days of age) were collected from the five piggeries. In each of the piggeries, faecal samples were taken from ten litters (sucking) or groups (weaned) of pigs for each of the three age groups. Two extra samples were collected from two litters of piglets with diarrhoea at the age of two to four days in piggery No. 4. Collection of faeces from most animals within a litter or group was attempted and samples from each litter or group were pooled. All the faecal specimens were transported to the laboratory within 24 hours of sampling. In the laboratory, faecal samples were processed as described in 2.1.2.

Detection of rotaviruses: All the samples collected were examined for group A rotavirus by the Dakopatts ELISA kits, and by PAGE. Selected samples were also examined by EM. The details of these procedures have been described in section 2.2.

In addition, selected ELISA-positive samples from Massey Piggery obtained in 1985 (section 3.2), 1986 (section 3.3) and 1987 were also subjected to PAGE analysis.

5.3 RESULTS

Pattern of shedding of group A rotavirus as detected by the ELISA test: The sizes of the five piggeries in terms of breeding sows, the age of weaning of piglets, and the prevalence rates of faecal shedding of group A rotavirus among different age groups are summarized in table 5-1. Rotaviral antigen was detected in faeces from all these five piggeries. About 30% of the litters at 15-20 days of age, and 60% of the litters (groups) at 30-35 days of age were shedding rotavirus, but rotaviral antigen was not demonstrated in pigs of 70-90 days of age. The pattern of shedding of group A rotavirus was similar in all five piggeries investigated.

Detection of rotaviruses from samples by PAGE: Among the 152 samples examined, only 28 (18.4%) samples showed an electrophoretic pattern characteristic of group A rotavirus. All of these samples were also positive by the ELISA test.

Table 5-1 Prevalence rates of shedding of group A rotavirus in the five piggeries as determined by the ELISA test

Piggery No.	Number of sows	Age (weeks) of weaning	Prevalence (percentage) of shedding of group A rotavirus		
			15-20*	30-35*	70-90*
1	360	4	60	40	-**
2	280	4	30	70	-
3	200	3.5	20	60	-
4	275	4	30	60	-
5	400	3	10	100	-
Mean			30	66	-

* Age in days.

** No rotavirus was detected.

Genomic variation of rotavirus detected in the five piggeries:

Piggery No. 1: Eleven of the 30 samples collected in this piggery were positive for rotaviral RNA by PAGE. Eight had patterns typical of group A, two of group C and one resembled group B rotavirus. Among the eight group A rotaviruses, two electrophoretotypes were observed and the two group C rotavirus strains also showed differences in mobility. A total of five different electrophoretotypes of rotavirus, belonging to three groups, were detected in this piggery. These patterns are depicted in Fig. 5-1. The two electrophoretotypes of group A rotavirus were found in five and three samples, respectively. These two electrophoretotypes (lanes D and E) differed in segments 2, 4, 5, 8, and

9. The two electrophoretotypes of group C rotavirus (lanes B and C) differed in segments 3, 4 and 7. The RNA pattern shown in lane A is similar to that of group B rotavirus reported in man (Hung et al. 1984), cattle (Chasey and Davies, 1984), lambs (Chasey and Banks, 1984) and pigs (Bridger et al. 1982; De San Juan et al. 1986). Segments 5 and 6 of previously reported group B rotavirus ran together or as a doublet, and segments 7 and 8 ran as a doublet. However, segments 5 and 6 of the virus in lane A ran as a doublet while segments 7 and 8 ran together. Whether this virus belongs to group B rotavirus or to a new group of rotavirus is not known. Identification of this virus by serology is required.

Piggery No. 2: In this piggery, five samples were positive for rotaviral RNA by PAGE, and showed one single electrophoretotype of group A rotavirus. One of these five samples was found to contain both group A and group C rotaviruses.

Piggery No. 3: Five samples from this piggery were also shown to contain rotavirus by PAGE. Four of these were found to contain both group A and C rotaviruses. However, there was insufficient of one of these samples for further analysis. The RNA patterns of rotaviruses in the other three samples, together with that of only group A rotavirus in one sample, are shown in Fig. 5-2. Lane A is a group C rotavirus which was detected in piggery No. 4 (see below). Two of the three samples containing both group A and C rotaviruses (lanes C and D) appeared to have similar RNA patterns. The pattern of group A rotavirus in these two samples is identical to that of the sample containing group A rotavirus only (lane E). The other sample (lane B) appeared to have a group C rotavirus pattern similar to the other two samples, while the pattern of group A rotavirus was different from that of other samples from the same piggery. Segments 5 and 6 in the sample ran more slowly than those in other samples and segment 10 was missing. This sample was positive for group A rotavirus in the ELISA test. Therefore, there were at least two electrophoretotypes of group A rotavirus circulating in this piggery. The pattern of group C rotavirus from this piggery is different from that from piggery No. 4 at least in segments 4, 7, 8 and 9.

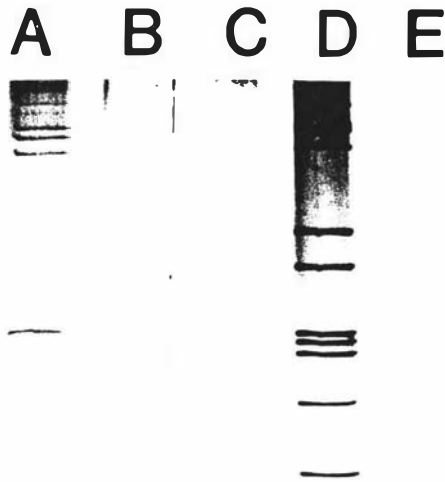


Fig. 5-1. Rotaviruses detected in piggery No. 1. Lane A: Group B-like rotavirus. Lane B and C: Group C rotaviruses. Lanes D and E: Group A rotaviruses. Migration was from top to bottom.

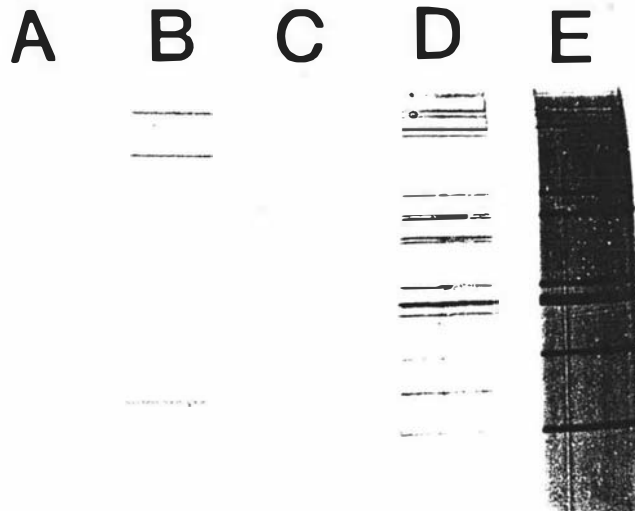


Fig. 5-2. Rotaviruses detected in piggery No. 3. Lane A: Group C rotavirus. Lane B, C and D: Mixed infections between group C and group A rotaviruses. Lane E: Group A rotavirus. Migration was from top to bottom.

Piggery No. 4: In this piggery five out of the 30 samples were found to contain rotavirus RNA as judged by PAGE, with one sample containing both group A and group C rotaviruses. The group A rotaviruses in the five samples had identical electrophoretic patterns. In this piggery, two litters of piglets, at two to four days of age, had diarrhoea at the time when samples were collected from the other pigs. Diarrhoea in these animals was yellowish and watery. Affected piglets were depressed but not dehydrated. Faeces were collected from these two litters and group A rotaviral antigen was not detected by the ELISA test. However, virus particles with characteristic rotaviral morphology were observed in one of the samples under the electron microscope (Fig. 5-3). When analysed by PAGE, the sample showed an electrophoretic pattern typical of group C rotavirus. The pattern of this virus together with those of a group A rotavirus and the mixed infection is shown in Fig. 5-4. The sample which contained both group A and C rotaviruses was detected from pigs at 30-35 days of age. The pattern of group C rotavirus in this sample is identical to that of the group C rotavirus infecting the younger piglets which had diarrhoea.

Piggery No. 5: Only group A rotavirus was detected in this piggery. Two electrophoretotypes of group A rotavirus were possibly circulating, since one of the samples had extra bands between segment 4 and 5 and in the triplet region. This usually indicates a mixed infection of two or more strains of group A rotavirus. The representative pattern of group A rotavirus in this piggery is shown in Fig. 5-5 together with the mixed pattern of possibly two group A rotaviruses.

RNA patterns of group A rotaviruses at Massey Piggery over a period of three years: Samples collected from Massey Piggery over a period of three years were analysed by PAGE, and the results are shown in Fig. 5-6. Rotaviruses detected in this piggery in 1985 and 1986 had identical electrophoretotypes but differed from the pattern of virus recovered in 1987. Co-electrophoresis of these viruses indicated that the differences in mobility were in bands 1, 4, 5, 7 or 8, and 10. The changes in migration rates of the RNA segments were relatively minor.

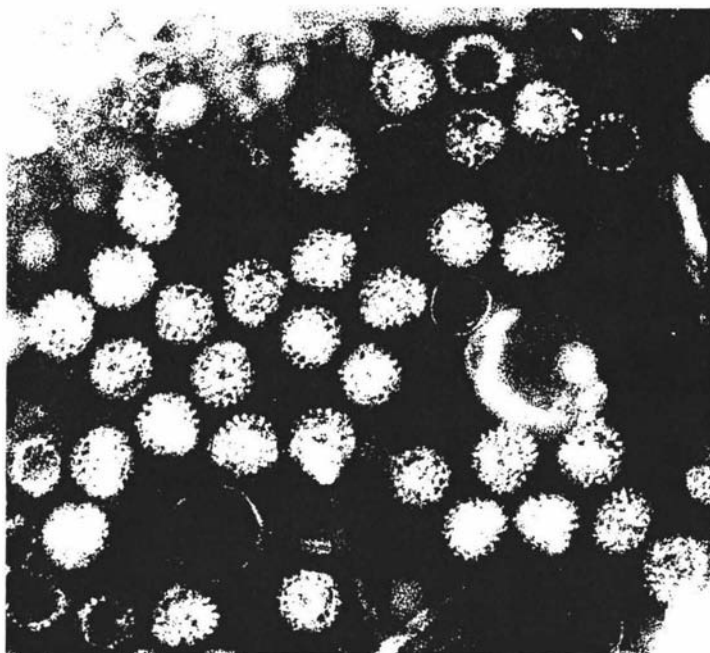


Fig. 5-3. Electron micrograph of rotaviral particles in samples from two to four day old piglets with diarrhoea in piggery No. 4. (x162,400).

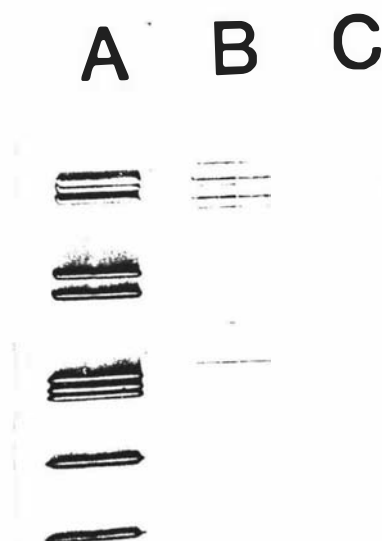


Fig. 5-4. Rotaviruses detected in piggery No. 4. Lane A: Group A rotavirus detected in piggery No. 4. Lane B: Mixed infections between group A and group C rotaviruses. Lane C: Group C rotavirus detected in diarrhoeal pigs at 2 to 4 days of age. Migration was from top to bottom.

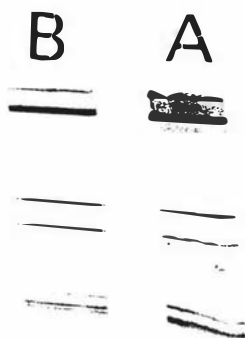


Fig. 5-5. Rotaviruses detected in piggery No. 5. Lane A: Group A rotavirus. Lane B: Mixed infection between two group A rotaviruses. Migration was from top to bottom.

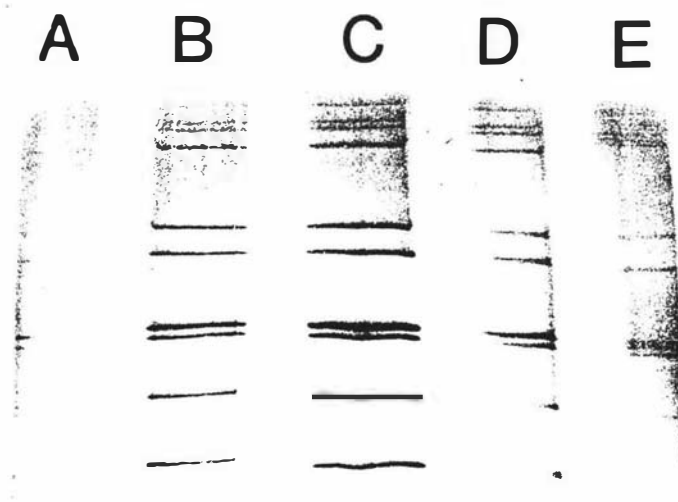


Fig. 5-6. Co-electrophoresis of rotaviruses detected at Massey piggery. A: Virus detected in 1985, B: Co-electrophoresis of viruses in 1985 and 1986, C: Virus detected in 1986, D: Co-electrophoresis of viruses of 1986 and 1987, E: Virus detected in 1987. Migration was from top to bottom.

Electrophoretotypes of Group A rotaviruses detected in pigs in New Zealand: When the rotaviruses from the five piggeries were compared by PAGE with those from Massey Piggery, they all showed different mobilities of their genomic RNA. Therefore, at least eight electrophoretotypes (six from the five piggeries and two from Massey Piggery) were demonstrated in the study. These eight electrophoretotypes are represented diagrammatically in Fig. 5-7 and shown in Fig. 5-8 on a gel. Differences in mobility were detected in most of the segments except in segment 11. Four to seven segment mobility differences were found between each of the eight electrophoretotypes.

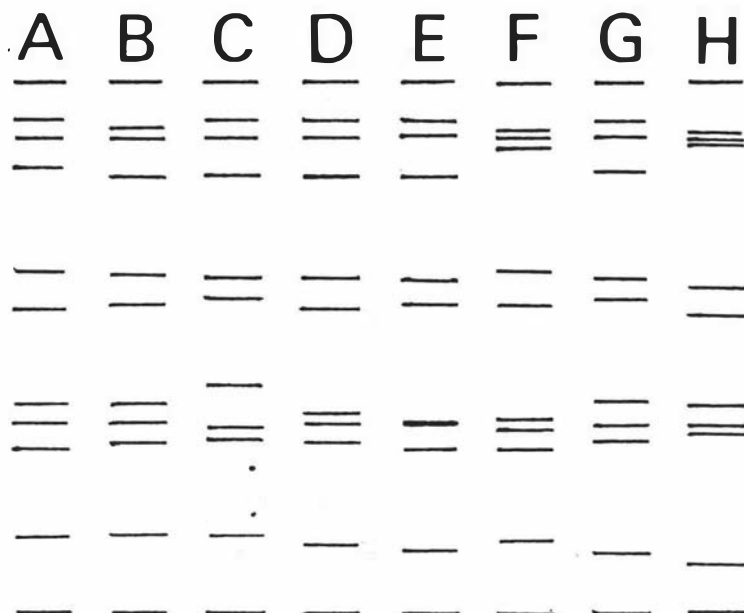


Fig. 5-7. Diagram of the eight electrophoretotypes identified in New Zealand pigs. Lanes A and B: Viruses from piggery no. 1. Lane C: Virus from piggery No. 2. Lane D: Virus from piggery No.3. Lanes E and F: Viruses from Massey Piggery. Lane G: Virus from piggery No. 4. Lane H: Virus from piggery No. 5.

Prevalence of shedding of group C rotavirus in pigs and variation between the group C rotaviruses detected: In the five piggeries studied, group C rotavirus was detected in nine (6%) samples, the second most frequent after infection by group A rotavirus. All the group C rotaviruses, except the one detected in the two to four day old piglets which had diarrhoea, were detected in pigs at 30-35 days of age. By this time, all the pigs had been weaned. No group C rotavirus was detected in pigs at 15-20 and 70-90 days of age. The difference in RNA mobilities between the two group C rotaviruses in piggery No. 1 and between group C rotaviruses detected in piggeries No. 3 and 4 have already been discussed. The group C rotavirus in piggery No. 1 was compared with that detected in piggery No. 4 (Fig. 5-9) and it showed differences in mobilities of segments 3, 5, 6 and 8. Therefore no common electrophoretotypes of group C rotavirus were detected in these piggeries.

5.4 DISCUSSION

The results of the present study indicate that the pattern of shedding of porcine group A rotavirus observed at Massey Piggery (chapters three and four) is typical of that in other piggeries in New Zealand at least in age groups of 15-20, 30-35 and 70-90 days. The prevalence of infection increased with age and was greatest in 28 to 35 day old pigs. In this study, the prevalence rates of shedding of group A rotavirus in the three and five week-old pigs were 30 and 66%, respectively. These rates are comparable to those observed at Massey Piggery in a cross-sectional survey (8.3 and 56.7%) (section 3.2) and in a cohort study (20 and 65%) (see section 3.3). At Massey Piggery, all pigs ceased shedding group A rotavirus by two months of age, and in the other five New Zealand piggeries, rotaviral antigen was not detected in pigs aged from 70 to 90 days either. Similar patterns of shedding of porcine group A rotavirus have also been reported in other countries (Debouck and Pensaert 1983, Liprandi et al. 1987, Svensmark 1983).

Marked variations in genomic patterns have been widely detected in human group A rotavirus by PAGE (Gerna et al. 1987a, Lourenco et al. 1981, Rodger et al. 1981). In a survey carried out in a paediatric hospital in France, Lourenco et al. (1981) noticed that more than one electrophoretype was always found whenever more than four viral samples from one ward were analysed by PAGE. Heterogeneity of RNA segments has also been described in porcine group A rotaviruses by De San Juan et al. (1986) and Liprandi et al. (1987). The latter authors identified



Fig. 5-8. Eight electrophoretotypes detected by PAGE. Lanes A, B, C, D, E, F, G and H: Viruses as in Fig. 5-7. Migration was from top to bottom.

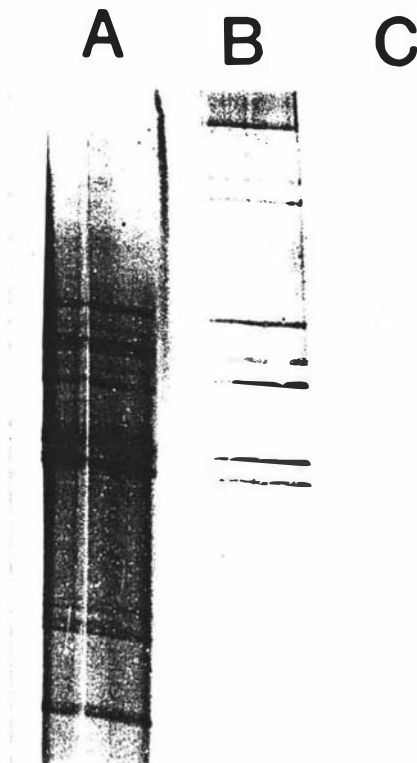


Fig. 5-9. Comparison between group C rotaviruses detected in piggeries No. 1 and No. 4. Lanes A and B: Group C rotaviruses from piggery No. 1. Lane C: Group C rotavirus detected in young piglets in piggery No. 4. Migration was from top to bottom.

eleven electrophoretotypes from samples selected from nine herds. Among those samples analysed, no two electrophoretotypes were found to be identical. In the present study, at least eight electrophoretotypes were demonstrated in six piggeries and more than two electrophoretotypes were found to be simultaneously circulating in at least three of these piggeries.

In longitudinal investigations of rotaviral infections in man over periods of up to four years, one or two electrophoretotypes were always found to be predominant, with others appearing and disappearing during the period of investigation (Chiba et al. 1984, Dimitrov et al. 1984, Rodger et al. 1981). However, in the present investigation, only a single electrophoretotype was identified during 1985 and 1986 at Massey Piggery. This situation resembled that reported by Todd et al. (1984) in a "closed" dairy herd, in which one single electrophoretotype was detected over a period of 16 months. The relative stability of the rotaviral RNA at Massey Piggery during 1985 and 1986 was in contrast to the observation that more than one pattern was identified in three of the other five piggeries. The difference may be attributable to the larger sizes of these piggeries (200 to 400 breeding sows), compared to Massey Piggery (only 60 breeding sows). The opportunity for point mutation and reassortment was presumably much less at Massey Piggery than in the other piggeries. Both mutation and reassortment have been reported to be mechanisms for rapid genetic evolution of rotaviruses (Greenberg et al. 1981, Konno et al. 1984). The consistent pattern at Massey Piggery eventually changed. This was presumably due to point mutations and/or reassortment since five segments were slightly different in mobility between the isolate in 1987 and those detected in 1985 and 1986. Introduction of strains from outside the piggery could not be excluded. However, the new electrophoretotype is so similar to the previous ones at this piggery that it is likely that this was derived from the previous ones. It will be interesting to determine whether, and for how long, the virus with the new pattern will stay unchanged in the piggery.

Common electrophoretotypes of human rotaviruses have been detected in different geographical locations (Dimitrov et al. 1984, Lourenco et al. 1981, Nicolas et al. 1984). Chiba et al. (1984) demonstrated one common electrophoretotype in two districts of Kenya which are 480 Km apart. However, no common electrophoretotypes were found in different pig herds within a six-mile (10 Km) radius in Venezuela (Liprandi et al. 1987). In this present study, no common electrophoretotypes of group A

rotavirus were found in the six New Zealand piggeries between 30 and 800 Km apart. This discrepancy observed between human and porcine rotaviral infections may reflect the extent of the movements of the species concerned.

The epidemiology of infection by rotavirus is complicated by a number of factors. One of these is the fact that the genus consists of a large number of viruses with different antigenic compositions. To date, two subgroups (WHO 1984), seven serotypes (Hoshino et al. 1984) and numerous electrophoretotypes (Estes et al. 1984, Lourenco et al. 1981) have been well established in group A rotavirus alone, with more and more emerging (Clark et al. 1987, Gerna et al. 1987a, Lambert et al. 1984, Matsuno et al. 1985). Further complicating the situation is the discovery of atypical rotaviruses which are morphologically identical to, but antigenically distinct from, group A rotavirus (Bohl et al. 1982, Bridger et al. 1982, Rodger et al. 1982). These atypical rotaviruses have been detected in a wide range of animals including man (Hung et al. 1984, Rodger et al. 1982), calves (Chasey and Davies 1984), pigs (Bohl et al. 1982, Bridger et al. 1982, Chasey et al. 1986), ferrets (Torres-Medina 1987), rats (Vonderfecht et al. 1984), lambs (Chasey and Bank 1984) and birds (McNulty et al. 1981). The atypical rotaviruses have been classified as groups B, C, D, E, F and G on the basis of serological cross-reactivity and genomic profile by PAGE (Bridger 1987, Pedley et al. 1983, 1986). Three groups (B, C and E) of these atypical rotaviruses have been recovered from pigs, with or without diarrhoea (Bridger 1987).

Results from this investigation indicate that the epidemiology of infection by rotavirus is even more complicated. Not only different electrophoretotypes of group A rotavirus, but also different groups (as many as three) of rotaviruses were detected at the same time in one piggery. Mixed infections of group A rotaviruses have been well documented (Estes et al. 1984, Lourenco et al. 1981, Spencer et al. 1983b). This provides the opportunity for genetic reassortment, which is one of the mechanisms responsible for the emergence of new strains (electrophoretotypes) of group A rotavirus (Gombold and Ramig 1986). Mixed infections between a group A and a group B rotavirus was also reported by De San Juan et al. (1986). In the present study, six samples were found to contain both group A and group C rotaviruses. Although these samples were pooled from piglets of a litter or group, it was possible that individual piglets were concurrently infected with rotaviruses of more than one group. Whether or not the mixed infections

between different groups of rotaviruses will lead to emergence of new strains or new groups of rotaviruses is not known and should be investigated in the future.

Infections of pigs with group B and C rotaviruses have been associated with diarrhoea (Bridger 1980, Saif et al. 1980). Experimental infection of gnotobiotic piglets with group C rotavirus has resulted in diarrhoea, and piglets under five days of age were severely affected (Bohl et al. 1982). In the present study, whether or not there was an association between infection with group B and C rotaviruses in the piglets at 30-35 days of age and diarrhoea, is not known. This is because the samples were collected and pooled from litters or groups. However, an association between diarrhoea and infection with group C rotavirus in piglets at two to four days of age is indicated. It is interesting, that in the present study, group A rotavirus was not detected in piglets under ten days of age, while group C rotavirus was associated with diarrhoea in piglets at two to four days of age. The role of group B and C rotavirus in diarrhoea in young piglets needs to be clarified.

Group C rotavirus has so far been reported to only infect man (Rodger et al. 1982), pigs (Bohl et al. 1982) and ferrets (Torres-Medina 1987). The epidemiology of this viral infection has been poorly elucidated in these species. In the human population, preliminary investigations have suggested that group C rotaviral infection is sporadic (Bridger et al. 1986, Espejo et al. 1984), yet it has been detected in Australia (Rodger et al. 1982), Brazil (Pereira et al. 1983), Bulgaria (Dimitrov et al. 1983), France (Nicolas et al. 1983), Hungary (Szucs et al. 1987), Mexico (Espejo et al. 1984) and the UK (Bridger et al. 1986). In pigs, however, 77% of pigs were reported to have antibodies against group C rotavirus in a survey conducted in the UK (Bridger and Brown 1985), but infections with group C rotavirus in pigs have only been recorded in Australia (Bridger personal communication), Belgium (Pedley et al. 1983), the UK (Chasey and Davies 1984), the USA (Bohl et al. 1982), and now in New Zealand. In the present study, group C rotavirus was detected in four of the five piggeries and in nine (6%) of the 152 samples examined. It was the second most frequently detected rotavirus after group A rotavirus. These results, together with the survey of Bridger and Brown (1985) suggest that group C rotaviruses may be as widespread as group A rotavirus in pigs, despite the low rate of detection of group C rotavirus by PAGE.

An even lower frequency of detection of group C rotavirus has been reported by other authors. Nicolas et al. (1983) detected group C rotavirus in only one of 350 isolates of human rotavirus in France. In a study carried out in Mexico City, only one out of 658 samples from infants and children contained group C rotavirus (Espejo et al. 1984). A similar situation was also found in pigs. Chasey and Davies (1984) observed only one sample with a group C pattern after analysing 280 faecal samples from pigs by PAGE. The low rate of detection of group C rotavirus contrasts with the serological evidence of group C rotaviral infection at least in pigs (Bridger and Brown 1985). Compared with that of group A rotavirus, the low frequency of detecting group C rotavirus may be due to the following reasons.

A sensitive test such as the ELISA test is not available for the detection of group C rotavirus. Among the 152 samples examined in this study, 46 (30%) were found to be positive for group A rotavirus by ELISA, but only 28 (18.4%) of these clearly showed a characteristic pattern of group A rotavirus by PAGE. Because of the lower sensitivity, PAGE missed nearly half of the group A rotaviruses detected by ELISA. Therefore, a more sensitive test, such as ELISA, is urgently needed for the detection of group C rotavirus.

Mixed infections between group A and group C rotavirus could be mistaken for group A rotavirus only. Since samples with such mixed infections are also positive by the ELISA test for group A rotavirus, a positive result could be taken as group A rotaviral infection only. In this study, six of the nine samples containing group C rotavirus were also contained group A rotavirus and were positive for group A rotavirus by ELISA. Even when the samples are analysed by PAGE, they could still be taken as group A rotavirus only, since mixed infections between two or more group A rotaviruses are very common (Lourenco et al. 1981, Spencer et al. 1983b). In the present investigation, the group C rotavirus in the mixed infections could be recognized only after comparison with other group A and C rotaviruses run on the same gel. Careful checking and comparison of mixed electrophoretotypes is hence essential to ensure that no other groups of rotaviruses are present.

In the present study, a non-group A and C rotavirus was also detected. This virus showed a pattern similar to that of group B rotavirus but differed in the mobilities of segments 7 and 8 from the group B rotaviruses reported to date (Bridger et al. 1982, Chasey and Banks 1984, Hung et al. 1984). Therefore, it may belong to a new group

of rotavirus yet to be classified.

5.5 CONCLUSIONS

1. The pattern of shedding of group A rotavirus in the other five New Zealand piggeries is similar to that observed at Massey Piggery. Piglets aged 15-20 and 30-35 days shed group A rotavirus with prevalence rates of 30 and 60%, respectively. Piglets aged 70-90 days did not shed the virus.

2. The ELISA test detected group A rotavirus in 46 (30%) of 152 faecal samples, while PAGE showed characteristic RNA patterns of group A rotavirus in only 28 (18.4%) of these samples. Those samples which showed RNA patterns of group A rotavirus by PAGE were all positive by ELISA. The results confirm the high sensitivity of the ELISA test and the low sensitivity of the PAGE technique as discussed in chapter two.

3. Variations of group A rotavirus in electrophoretic patterns are detected by PAGE. Among the six piggeries studied, no common electrophoretype was detected, although the distance between some of the piggeries was as short as 30 Km. This is probably due to the pig herds being relatively closed with minimal trafficking between them.

4. More than one electrophoretype of group A rotavirus was found to be circulating in three of the five piggeries.

5. Group A rotavirus detected at Massey Piggery appeared to be relatively stable, with a single electrophoretype detected for two consecutive years. A different electrophoretype was detected in the third year of the study. This may have arisen by mutations and/or reassortment.

6. A group B-like rotavirus was detected by PAGE in one of the 152 samples. Its RNA pattern was similar to that of group B rotavirus already reported. There were slight differences in the mobility of segments 7 and 8. Therefore, confirmation of this virus by serology is required.

7. A RNA pattern typical of group C rotavirus was detected in nine (6%) of the 152 samples from four of the six piggeries. This was the second most frequent pattern detected after group A rotavirus. This suggests

that infection with group C rotavirus may be as widespread as that with group A rotavirus, even though the rate of detection of group C rotavirus is low.

8. The low rate of detection of group C rotavirus is due to the low sensitivity of the PAGE technique and the presence of mixed infection between group A and C rotaviruses. Thus, a more sensitive test such as ELISA is urgently needed for diagnosis and epidemiological investigation of infection by group C rotavirus. Careful checking and comparison of mixed electrophoretotypes is also essential to ensure that, if other groups of rotaviruses are present in samples, they will not be overlooked.

9. Mixed infection between group A and C rotavirus is common. Of the nine samples containing group C rotavirus, six also contained group A rotavirus.

10. No common electrophoretotypes of group C rotavirus were detected among these piggeries. More than one electrophoretotype of group C rotavirus was detected in at least one of these piggeries.

11. Eight of the samples containing group C rotavirus were collected from pigs aged 30-35 days. One was collected from two to four day old piglets with diarrhoea. An association between infection by group C rotavirus and the diarrhoea in two to four day old piglets is indicated, while any possible association of infection by group C rotavirus with diarrhoea in pigs aged 30-35 days is not known.

12. The epidemiology of rotaviral infection in pigs is complicated since not only different electrophoretotypes of group A rotaviruses, but also different groups of rotaviruses are circulating in one piggery at the same time.

Chapter Six

GENERAL DISCUSSION

Throughout the world, group A rotavirus is still a major cause of non-bacterial gastroenteritis in infants, children and the young of other mammalian and avian species. In pigs, infection by group A rotavirus has been associated with two common diarrhoeal syndromes, referred to as milk scours in sucking piglets, and post-weaning diarrhoea (PWD) in recently weaned pigs. In the present study, infection by rotavirus was closely associated with the syndrome of milk scours in sucking piglets. Thus, in both the cross-sectional and cohort studies, sucking piglets with diarrhoea were shedding rotavirus, while diarrhoea was not observed in those which did not shed rotavirus. Diarrhoea usually occurred in sucking piglets soon after rotaviral shedding was first detected. However, there was not an obvious association between rotaviral shedding and PWD. Most of the piglets with PWD which were investigated in the present study did not shed group A rotavirus at that time. PWD occurred in these piglets soon after the aerobic faecal flora changed to one in which haemolytic E.coli predominated. These observations support those of Lecce et al. (1982) and Tzipori et al. (1980b). These authors also found that piglets shed potentially pathogenic E.coli soon after weaning and that this coincided with the occurrence of PWD.

In the present study, an interesting observation was made in the third cohort of two litters of piglets described in chapter four. None of the 18 piglets shed group A rotavirus or developed diarrhoea before weaning. However, all these piglets shed rotavirus within five days after weaning, and nine of these animals developed mild diarrhoea lasting for one to two days only. Four of the 18 piglets shed haemolytic E.coli two to three days after weaning and another five shed this bacterium 18 to 21 days after weaning. None of them showed a predominance of haemolytic E.coli in their aerobic faecal flora or developed diarrhoea in relation to the infection. This observation was similar to that made by Lecce et al. (1982). These authors also reported that weaner pigs with rotaviral infection only developed mild diarrhoea while those infected with both rotavirus and pathogenic E.coli developed severe diarrhoea. Furthermore, severe diarrhoea could not be induced in pigs by artificial inoculation of pathogenic E.coli unless the pigs were inoculated with the pathogenic E.coli during a mild

rotavirus-associated diarrhoea. From these results, the authors postulated that rotavirus damaged the epithelium of the small intestines, therefore altering the luminal environment to one that favoured colonization by pathogenic E.coli. The results from the present study support this postulate. Piglets in the first two cohorts shed rotavirus before or at the time of weaning and therefore their intestinal epithelium would have been damaged. The damaged epithelium or a changed intestinal environment in these piglets favoured the colonization by haemolytic E.coli which resulted in the PWD observed in them. Piglets in the third cohort did not shed group A rotavirus before weaning. Presumably, the intestinal epithelium in these pigs was not damaged, and thus did not favour the colonization by haemolytic E.coli. Haemolytic E.coli was only detected sporadically and severe PWD, comparable to that seen in the other two cohorts of piglets, was not observed. It is therefore probable that group A rotaviral infection was indirectly involved in the PWD observed.

Nevertheless, because infection by group A rotavirus is closely associated with milk scours and may also exacerbate PWD, its control in pigs would be beneficial to the pig industry. Any control program must be based on a sound knowledge of the epidemiology of the particular infection or disease.

Investigations of the epidemiology of porcine group A rotaviral infection have been conducted in many parts of the world (Askaa et al. 1983, Debouck and Pensaert 1983, Liprandi et al. 1987, Utrera et al. 1984). However, the information gathered from these studies is limited because pooled faeces from litters of pigs rather than from individual piglets were examined, and samples were collected weekly rather than daily. In some of the studies, only faeces from diarrhoeic pigs were investigated. In the present study, the epidemiology of group A rotaviral infection in pigs was investigated more thoroughly by examining individual piglets daily to determine the patterns of shedding and transmission of group A rotavirus, the types of rotaviruses involved and the influence of maternally-derived antibody on resistance to natural infection.

The pattern of shedding of group A rotavirus was investigated at Massey Piggery initially by a cross-sectional survey in all age groups of pigs, and then by following three cohorts of piglets from birth to either two months of age or to the time of slaughter. The cross-sectional study revealed that 47 (9%) out of 521 pigs in the

piggery were shedding rotavirus at the time of sampling. Only sucking (under four or five weeks of age) and weaner (from weaning to two months old) pigs shed group A rotavirus. Rotavirus was not detected in fatter pigs (from two months of age to slaughter) or adult pigs (sows and boars). During the sucking period, very young piglets (less than one week old) did not shed group A rotavirus. Piglets began to shed the virus at two weeks of age. The prevalence rates of shedding increased with age, and reached a peak at five weeks of age, just before or at the time of weaning.

The cohort studies clearly show that all the pigs became infected with group A rotavirus before they were 40 days old. The age at which group A rotavirus was first detected from them ranged from 13 to 39 days. The duration of shedding in individual piglets was about eight days, ranging from one to 12 days. A proportion of these piglets shed group A rotavirus for a second time one to two weeks after the first shedding. All pigs ceased shedding the virus by two months of age. No rotaviral antigen was detected in the faeces of piglets from two months of age to the time of slaughter. Sows did not shed group A rotavirus throughout the period of farrowing. These results confirm those obtained from the cross-sectional study.

The pattern of shedding of group A rotavirus in sucking and weaner piglets observed in this present study is generally in agreement with that observed by most other workers (Askas et al. 1983, Debouck and Pensaert 1983, Utrera et al. 1984). However, the failure to detect group A rotavirus in older pigs, including fatter pigs and sows, in the present investigation is in contrast to some other reports. Group A rotavirus has been detected in fatter pigs under natural conditions (Grom et al. 1984) and has been established by experimental inoculation (Bernard et al. 1984). Group A rotavirus has also been recovered occasionally from gilts and sows with or without diarrhoea (Corthier et al. 1980, McNulty et al. 1976c) and from sows before and after parturition (Benfield et al. 1982). The discrepancy between the results obtained from the present study and those from other studies could be due to a number of reasons which have been discussed in detail in chapters three and four. The most significant factors, contributing to the differences in results obtained in the various studies, are probably the immune status of older pigs and the virulence of the particular virus strains.

The failure to detect group A rotavirus in adult pigs in the

present study indicates that adult pigs, especially sows, do not act as carriers for rotaviral infection in young piglets as previously suggested by others. After detecting rotavirus from sows before and after parturition, Benfield et al. (1982) suggested that adult pigs are of primary importance in initiating rotaviral infection in piglets. Lecce et al. (1978) also concluded that there was a postpartum transmission of a small amount of virus from the dam to the young. Based on the findings of the present study, this now appears unlikely. By 40 days of age, all the piglets shed group A rotavirus at least once. None of the pigs investigated in this study shed rotavirus when they were over two months of age. Rotaviral antigen was not detected in any of the sows (11) during the period of investigation. This was despite the fact that faeces from these sows were also collected daily and tested for group A rotavirus from one week prior to farrowing until the weaning of their piglets. Furthermore, rotaviral antigen was not detected in the environment where old pigs (fattener and sow houses) were housed. The pattern of shedding of group A rotavirus in pigs was also studied by Debouck and Pensaert (1983) and they found that faeces from all sows investigated were always free from rotavirus and that shedding of rotavirus in pigs two weeks after weaning was an exception. These results, together with the results of this present study, strongly suggest that adult pigs do not normally shed group A rotavirus and therefore are not a reservoir for group A rotaviral infection. The occasional detection of group A rotavirus from gilts and sows, especially during the time of farrowing (Benfield et al. 1982, Corthier et al. 1980, Grom et al. 1984) might have been due to these sows being transiently infected with rotavirus from the contaminated environment of the farrowing unit. This is supported by the findings of the present study in which rotavirus was readily detected in the environment of the farrowing unit. Furthermore, subclinical infection as shown by the increase of serum neutralizing antibodies during the period of farrowing was demonstrated in two gilts. The stress associated with moving sows into the farrowing house may also result in immunosuppression and this render them more susceptible to infection.

A study (not reported in detail in this thesis), which aimed to clarify whether or not sows are carriers of rotavirus, was carried out. Two pregnant sows were thoroughly cleaned and then transferred to a quarantine unit where pigs had not been previously housed. Piglets born of the two sows did not shed group A rotavirus before weaning. Rotaviral antigen was not detected in the sows during the period of investigation. Unfortunately, these piglets shed the virus a week after

weaning. However, in this study, the transmission of rotavirus by the author could not be ruled out. Further experiments of this kind should be carried out to confirm or deny the hypothesis that sows are not carriers of rotaviral infection. However, they are extremely difficult experiments because rotaviral infection is ubiquitous and cross transmission of rotavirus between species may occur.

In man, Ryder et al. (1986) also reported that adults were not the important reservoir for infection since the rates of rotaviral infection in children were the same regardless of whether or not their parents had concurrent infection with rotavirus. However, rates of rotaviral infection were higher in parents of children with rotaviral infection than in those of children without such infection. It appears that infected children are the most important source of rotaviral infection.

The natural transmission of rotavirus from piglet to piglet and from litter to litter was observed in this present study. In each of the cohorts, usually one or two piglets began to shed group A rotavirus first, then infection spread to other piglets within the same litter and subsequently to piglets in other litters. A management system of continuous farrowing in a piggery provides a constant supply of susceptible young piglets and hence ensures the maintenance of rotaviral infection.

A contaminated environment has been reported to be an important source of rotaviral infection in pigs (Lecce et al. 1982, Woode and Bohl 1981). However, no previous attempts have been made to detect rotavirus in the environments of a piggery. In the present study, rotaviral antigen was demonstrated in dust and dry faeces from the farrowing and weaner houses and also from a disused weaner house which had been free of pigs for three months. Rotavirus was only detected in the environments where young piglets were kept. Rotaviral antigen was not detected in houses where fatter pigs and sows were kept. Furthermore, porcine rotavirus can survive in faeces at ambient temperature for a long period (more than four months).

The results from this study suggest that rotaviral infection is maintained in a piggery by continuous transmission from piglet to piglet and from litter to litter. The environment of the piggery, especially those places where young piglets are housed, also plays an important role in maintaining the cycle of rotaviral infection. Adult pigs, including sows, do not act as carriers, and therefore are not a source

of infection for young piglets.

The protective effects of maternally-derived antirotaviral antibody against rotaviral infections have not been fully elucidated. In man, rotaviral infection occurs mostly in young children between six and 24 months of age but very rarely in those less than six months of age (see section 1.3). However, investigations of the protective effects of antirotaviral antibody in colostrum have produced conflicting results. In some studies, breast-fed babies were found to be much less frequently infected by rotavirus than were bottle-fed babies (Chrystie et al. 1975, Totterdell et al. 1976). Others (Weinberg et al. 1984) failed to observe any significant differences between breast- and bottle-fed babies in either the numbers of those infected with rotavirus or the average age at infection. Furthermore no correlation was found between the levels of antirotaviral antibody in mothers' colostrum and milk and protection of their babies from rotaviral infections (Totterdell et al. 1982, 1983). In pigs, rotaviral infection occurs in a somewhat similar manner. Piglets aged between three and six weeks of age are mostly infected. Piglets less than ten days of age are rarely infected. This has also been attributed to the presence of maternally-derived antibodies (Bohl 1979, Debouck and Pensaert 1983, Utrera et al. 1984). All the piglets in the present study acquired antibody from their dams. The maternally-derived antibody declined to a low level at three to five weeks of age. This coincided with the onset of shedding of rotavirus as observed in both this study and by others (Bohl et al. 1978, Debouck and Pensaert 1983, Utrera et al. 1984). Therefore, maternally-derived antibody appears to be protective against natural infection by rotavirus in pigs during the first two weeks of life. However, a correlation between the levels of maternal antibody and the occurrence of rotaviral infection was not observed in individual litters of piglets in the present study. From the pattern of shedding of group A rotavirus in these piglets, it is suggested that young piglets are usually protected against rotaviral infection during the first one or two weeks of life. As the antibody in some piglets falls to low, unprotective levels, these piglets become infected from other infected animals or from the contaminated environment. These initially infected animals shed large amounts of rotavirus, thus amplifying the challenge dose, so that other piglets, including those with relatively high levels of antibody, become infected. This hypothesis is supported by the experiment carried out by Lecce et al. (1978) in which a steady build up of rotaviral infection, of increasing clinical severity, was observed in piglets, when they were weaned at one day of age and introduced continuously to a nursery

without it being further fumigated. The build up of the challenge dose of virus so that it exceeds the protective effects of antibody has also been reported in other species of animals (Conner and Darlington 1980, Fahey et al. 1981, Snodgrass et al. 1980).

The PAGE technique was also usefully employed in this study to investigate the epidemiology of porcine rotaviral infection in New Zealand. Not only were variations in RNA patterns noted among group A rotaviruses from pigs in different piggeries in New Zealand, but also non-group A rotaviruses (group B and C) were detected in the country for the first time. Variations in electrophoretic patterns have been widely detected among human group A rotaviruses (Gerna et al. 1987, Lourenco et al. 1981, Rodger et al. 1981). Heterogeneity of the segments of rotaviral RNA has also been described in porcine group A rotaviruses by De San Juan et al. (1986) and Liprandi et al. (1987). The latter authors reported that no common electrophoretotypes of group A rotavirus were detected in nine herds. In the present studies, group A rotaviruses detected from the six piggeries all showed different electrophoretic patterns.

Since the initial detection in the early 1980's of a rotavirus in pigs which was morphologically similar to, but antigenically distinct from, group A rotavirus (Bridger 1980), such viruses have been demonstrated in a number of other species of animals including man. These rotaviruses have been further characterized and classified into groups B, C, D, E, F and G on the basis of serological reactions and RNA analysis (Bridger 1987, Pedley et al. 1983, 1986). Among these, groups B, C and E have been found to infect pigs (Bridger 1980, Chasey et al. 1986, Saif et al. 1980). In the present study, rotaviruses with electrophoretic patterns resembling group B and C rotaviruses were detected by PAGE. Further identification of these viruses by serology is required.

In conclusion, rotaviral infections are important causes of milk scours in piglets, and are probably significant in exacerbating PWD. The epidemiology of rotaviral infections is complicated by a number of factors. These include the continuous transmission of virus from pig to pig and from litter to litter, the survival of the virus in the piggery environment, the incomplete protection afforded by maternally-derived antibody, and the simultaneous circulation of different strains and different groups of rotavirus in one piggery. Therefore, any programme aimed to control rotaviral infection and associated diseases in pigs

should take these factors into consideration. The information obtained in this study may also have applications in terms of control of rotaviral infection in man, especially in control of nosocomial infections in hospitals.

It is probably impractical to consider attempts to eradicate rotaviral infection within conventional systems of pig husbandry. However, rotaviral infection and related diseases could be reduced, or the onset of rotaviral infection could be delayed, by reducing the environmental contamination with rotavirus and/or raising the specific immune status of the pigs. To achieve this, the following recommendations could be made for commercial piggeries.

In terms of reducing the environmental contamination with rotavirus, emphasis should be put on management practices. Since rotavirus is transmitted from pig to pig and from litter to litter, the farrowing unit should be operated on an "all in and all out" system. After each batch of piglets is removed, the farrowing unit should be cleaned thoroughly including the removal of any dust and faeces. The whole unit should then be fumigated. This would ensure that any rotavirus which remains in dust or faecal material is inactivated. The study showed that rotavirus could still be detected in the dust collected from surfaces of the walls, and metal and wood fittings, and from faecal material left on the floor space between the farrowing pen and the wall. This clearly indicates that the routine procedures of cleaning and disinfection carried out in New Zealand piggeries are not adequate to completely remove rotaviral contamination.

In terms of raising the immune status of pigs against rotaviral infection, sows could be immunized before parturition if a satisfactory vaccine or vaccines were developed. Successful vaccination would need to achieve a high and uniform level of immunity in herds of pigs. The present study shows that maternally-derived antibody is protective against rotaviral infection, especially during the first one or two weeks of life. However, piglets which acquired low levels of antibody from dams could become infected with rotavirus earlier and shed large amounts of virus, which eventually overcome the protective effect of even high levels of antibody in other piglets of the same group.

Nevertheless, rotavirus-free herds could be established by deriving piglets by caesarean section and rearing them under strict quarantine if it was ever considered necessary or desirable.

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Appendix I

COMPOSITION OF BUFFERS AND SOLUTIONS**A. BUFFER AND SOLUTION USED FOR CELL CULTURE****1. Antibiotic-Trypsin-Versene (ATV)**

0.5 g Trypsin
 0.2 g Versene (EDTA Sequestric acid)
 8.0 g NaCl
 0.4 g KCl
 1.0 g Dextrose
 0.58 g NaHCO_3
 2×10^5 IU Penicillin
 100.0 mg Streptomycin
 0.02 g Penol red

Made up to 1 L with deionised distilled water. Sterilise by filtration.

2. Eagle's minimum essential medium (MEM)

10.0 g MEM power
 1.0 g NaHCO_3

Made up to 1 L with deionised distilled water. Sterilise by filtration.
 Stored at -4°C .

3. Penicillin, Streptomycin, Kanamycin (PSK)

10.0 g Streptomycin
 10.0 1-mega vial Penicillin
 10.0 g Kanamycin

Made up to 1 L PBS. Sterilise by filtration.

4. Phosphate Buffered Saline (PBS)

8.0 g NaCl
 0.2 g KCl
 1.15 g Na_2HPO_4
 0.2 g KH_2PO_4

Made up to 1 L with deionised water. Sterilise by autoclaving (15lbs for 15 minutes). Final pH 7.2-7.4.

5. Trypan Blue

0.2 gm Trypan blue powder
 100.0 ml PBS

Dispense in 1.8 mls.

B. BUFFER AND SOLUTION USED FOR ELISA**1. Carbonate-bicarbonate Buffer (0.05 M)**

1.59 g Na_2CO_3
 2.93 g NaHCO_3

Made up to 1 L deionised water. Final pH 9.6.

2. Citrate-phosphatase Buffer (0.1 M)

7.3 g Citric acid, H_2O
 11.86 g $\text{Na}_2\text{HPO}_4, 2\text{H}_2\text{O}$

Made up to 1 L with deionised water. Final pH 5.

3. Phosphate buffered saline (PBS) (0.02 M)

8.0 g NaCl
0.2 g KH_2PO_4
2.9 g Na_2HPO_4 (12 H_2O)
0.2 g KCl

Made up to 1 L with deionised water. Final pH 7.4.

4. PBS-Tween 20

0.5 ml Tween 20

Made up to 1 L with PBS.

5. PBS-Tween-1%BSA

1.0 g BSA

Made up to 100 ml with PBS-Tween 20.

6. Substrate solution

20 ml Citrate-phosphatase buffer
8.0 mg OPD
8.0 ml H_2O_2

Mix above reagents together immediately before use.

C. BUFFER AND SOLUTION USED FOR GEL Electrophoresis

1. 30% acrylamide (acryl) + 0.8% N, N'-methylene-bis-acrylamide (MBA)

30 g acryl
0.8 g MBA

Disolved and made up to 100 ml with distilled water.

2. Lower tris (1.5 M)

18.17 g Tris base

Made up to 100 ml with distilled water and adjust to pH 8.8.

3. Upper tris (0.5 M)

6.06 g Tris base

Made up to 100 ml with distilled water and adjust to pH 6.8.

4. 10% Acrylamide gel

20.15 ml H_2O
12.50 ml Lower tris
16.63 ml 30% acryl + 0.8% MBA
12.50 μl N,N,N',N'-tetramethylethylene-diamine (TEMED)
0.75 ml 2

5. 3% Stacking gel

12.40 ml H_2O
5.00 ml Upper tris
2.00 ml 30% acryl + 0.8% MBA
12.50 μl TEMED
0.65 ml 2

6. 1% Formaldehyde + 0.75 M KOH

4 ml 38% Formaldehyde

12 g KOH

Made up to 400 ml with distilled water.

7. Running buffer

12.0 g Tris base

57.6 g Glycine

Made up to 4 L with distilled water.

8. Sample buffer

7.5 ml Upper tris

2.5 ml Glycerol

10 mg Bromphenol blue

9. Silver nitrate (0.011 M)

0.37 g Silver nitrate

Dissolved and made up to 200 ml with distilled water.

10. Sodium acetate buffer with 1% sodium dodecyl sulfate (SDS)a. 0.1 M Acetic acid

6 g Glacial acetic acid

Made up to 1 L deionized water.

b. 0.1 M Sodium acetate

1.15 g Anhydrous sodium acetate

Made up to 140 ml with deionized water.

60 ml 0.1 M Acetic acid

140 ml 0.1 M Sodium acetate

2 g Sodium dodecyl sulfate

Mix the above and adjust to pH 5.0.

D. BUFFER AND SOLUTION USED FOR OTHER PURPOSE**1. Earle's balanced salt solution**

6.8 g NaCl

0.4 g KCl

0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.16 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 2.2 g NaHCO_3

1.0 g Dextrose

0.02 g Phenol red

0.2 g CaCl_2

Made up to 1 L with deionised distilled water. Sterilise by filtration.

2. Tris buffer (0.1 M)

14.04 g Tris HCl

1.34 g Tris base

Made up to 1 L with distilled water and adjust to pH 7.2.

Appendix II

PAPERS PUBLISHED

Papers published from this thesis:

- Fu, Z.F. (1987): Detection of an atypic (possibly group C) rotavirus in New Zealand pigs. N.Z. vet. J. 35:115-116.
- Fu, Z.F., Hampson, D.J. (1987): Group A rotavirus excretion patterns in naturally infected pigs. Res. Vet. Sci. 43:297-300.
- Fu, Z.F., Hampson, D.J. (1988): Natural transmission of group A rotavirus within a pig population. Res. Vet. Sci. In press.

Papers published from related work:

- Hampson, D.J., Fu, Z.F., Bettelheim, K.A., Wilson, M.W. (1988): Managemental influences on the selective proliferation of two strains of haemolytic Escherichia coli in weaned pigs. Epidemiol. Inf. 100:213-220.
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