J DIARRHOEAL DIS RES 1995 Mar 13(1):18- 28
0253-8768/95 $ 1.00+0.10

Diarrhoeal Diseases in the White Mountain Apaches: Epidemiologic Studies

MATHURAM SANTOSHAM, R. BRADLEY SACK, RAYMOND REID, ROBERT BLACK, JANNE CROLL, ROBERT YOLKEN, LAURIE AURELIAN, MARK WOLFF, EDWARD CHAN, STEVE GARRETT, AND JEAN FROEHLICH

Division of Geographic Medicine, Department of Medicine and Department of Pediatrics, School of Medicine, and Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21203, and the Indian Health Service Hospital, Whiteriver, AZ, USA

ABSTRACT

Acute diarrhoeal disease in children is known to be a major public health problem among native Americans living in reservations in the southwestern part of the United States. This study was undertaken to describe the epidemiology and causative agents of diarrhoea more completely, with the expectation that this information may help in the ultimate control of the disease in this population. Three interrelated epidemiologic studies were carried out in the White Mountain Apache Tribe, Whiteriver, Arizona, during 1981-1985: a three-year longitudinal study on a cohort of 112 newborns, a longitudinal two-year study in a cohort of 200 families, and a case-control study on 1,072 children with diarrhoea attending a medical facility. Both epidemiologic and microbiological patterns of diarrhoeal disease were found to be very similar to those seen in developing countries, indicating the need for basic improvements in sanitation and hygiene in this population.

Key words: Diarrhoeal diseases; Diarrhoea, Acute; Epidemiology

INTRODUCTION

Acute diarrhoeal disease continues to be one of the major causes of morbidity and mortality in the developing world, particularly among infants and children (1). In industrialized countries such as the United States, however, diarrhoeal diseases, even in small children, are not generally considered to be major public health problems. Nonetheless, approximately 400-500 deaths are estimated to occur each year in the United States due to acute diarrhoeal disease (2). There are populations within the United States that are known to have unusually high rates of acute diarrhoeal disease in children. Among these populations are the native Americans living in reservations in the southwestern part of the country. We have previously studied diarrhoeal diseases in the White Mountain Apache reservation in Arizona, and have documented the importance of diarrhoeal illnesses there (3). We undertook the present studies to describe the epidemiology, clinical features, and causative agents of diarrhoea more completely, with the expectation that this information may help in the ultimate control of the disease in these populations.

During 1981-1985, we carried out three interrelated epidemiologic studies on diarrhoeal diseases in the White Mountain Apache Tribe: (i) a three-year longitudinal study on a cohort of newborns, (ii) a two-year longitudinal cohort study of families, (iii) a case-control study on children with and without diarrhoea, as well as clinical studies (4). These studies document the importance of diarrhoeal diseases in this American population.

MATERIALS AND METHODS

Study area

The White Mountain Apaches, descendants of the southern Athapaskans, reside on the Fort Apache reservation, located on 1.7 million acres of land in the White Mountain area of east-central Arizona (Figure 1).

Correspondence and reprint requests should be addressed to:
Prof. R Bradley Sack, Department of International Health, Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205, USA

The land is mountainous and timbered, at an elevation of about 5,000 feet above sea level, and the area has a temperate climate. The size of the population was approximately 10,000, of which approximately 7,000 live in and around the largest city Whiteriver, which is the centre of the tribal government. Approximately half of the population is less than 15 years of age, and about 20% is less than 5 years of age. The Apache language is commonly spoken, and traditional ceremonies and customs are widely practised. Breastfeeding is not widely practised beyond the first few months of life. Medical care is provided free of charge to the population at the only hospital located on the reservation at Whiteriver, and at the Cibeque Clinic which is 50 miles from Whiteriver.

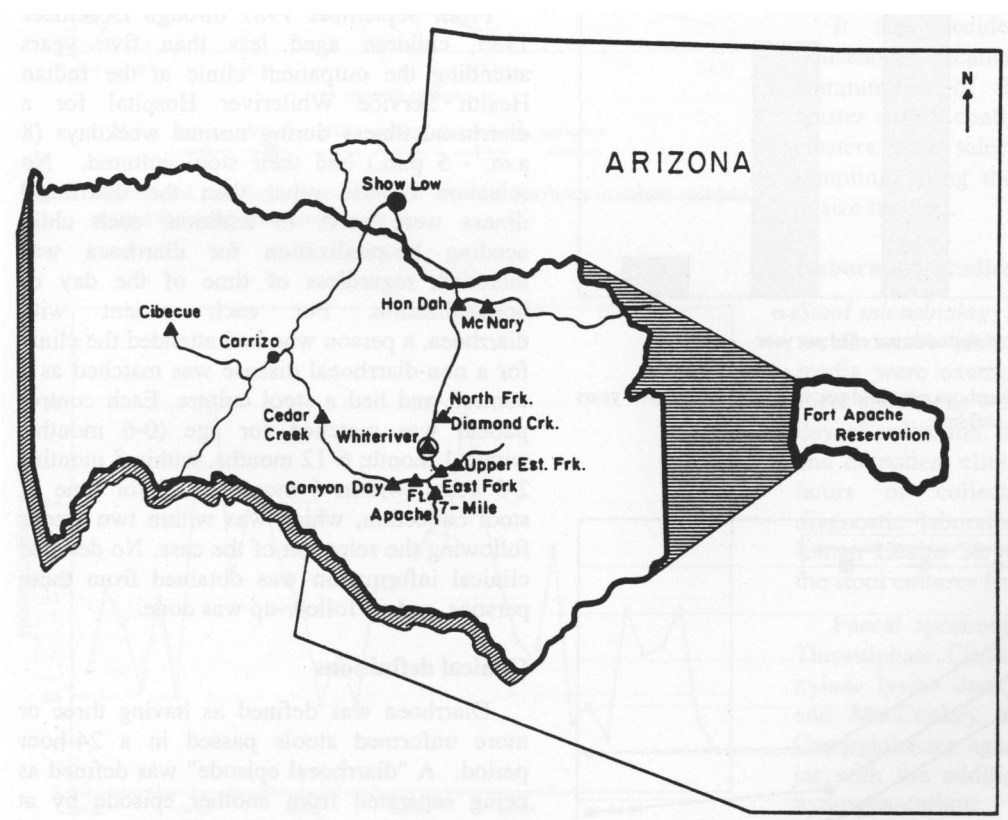

Fig. 1. Map of Arizona showing the White Mountain Apache Reservation

Study patients

The patients enrolled in the study were primarily White Mountain Apaches who lived on the Fort Apache Indian Reservation, Arizona. Written, informed consents were obtained from adults and from the parents or guardians of the study children. All protocols were approved by the Joint Committee on Clinical Investigation at the Johns Hopkins University and by the Tribal Council of the White Mountain Apache Tribe and by the Indian Health Service.

Cohort study on the newborns

One hundred twenty children were enrolled by contacting the mothers within 48 hours after birth, from March 1981 through April 1983. All births occurred in the hospital. No specific exclusion criteria were used. A total of 112 children made up the final cohort. From the time of enrollment of each newborn till 31 December 1984, weekly visits by female Apache field workers were made to the homes of the study children to interview the mothers or caregivers, to detect the occurrence of diarrheal illnesses and, if present, to obtain relevant clinical information, which included: time of onset, and duration of diarrhea, the presence of vomiting and blood in the stool, fever, and respiratory symptoms. Children with mild diarrhea were treated at home with oral rehydration fluids given to the caretakers by the field workers. If the diarrhea was considered to be of greater severity, the mother was referred to the clinic at the Indian Health Service Hospital, where treatment was available at all times.

Although the initial enrollment included 120 newborns, only 112 (53 males and 59 females) were followed sufficiently long period to be included in the analysis. Eight children withdrew from the study during their first few months after enrollment, and their data were not included. A child had to be enrolled for at least 16 months to be included in the study. Fifteen of the 112 children were enrolled during the last three months of 1981 (data are included only from 1 January 1982, when the infants were several months old), 51 in 1982, and 46 in the first 4 months of 1983. Weekly diarrhoea surveillance began from the time of enrollment and continued through December 1984 (or until a child reached 3 years of age).

A total of 247.6 child-years of observations was made. The mean number of years each child was followed up was 2.21 (range 1.42-2.98 years).

Stool cultures from either whole stool or rectal swabs were taken at the time of the home visit: (i) whenever the child was having diarrhoea at the time of the visit, or (ii) on a routine basis, approximately every two months during the study when the child was not having diarrhoea.

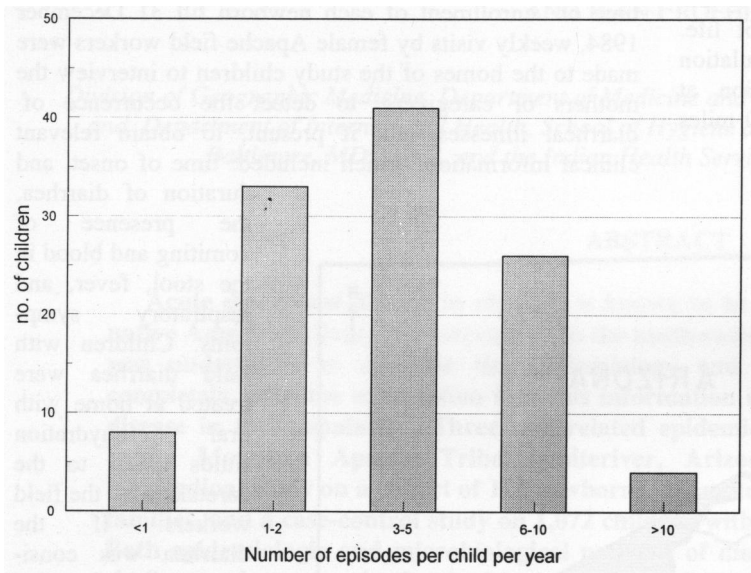

Fig. 2. Number of episodes of diarrhoea per child per year during the first 3 years of life (n=112) in cohort children

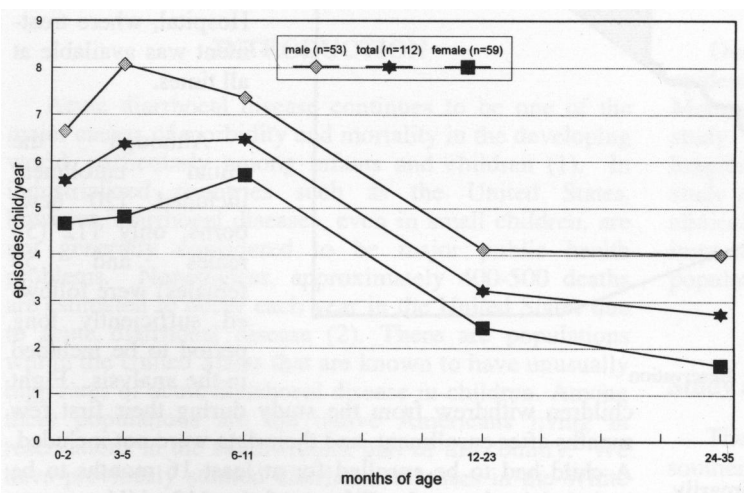

Fig. 3. Diarrhoea attack rates, by age and sex in cohort children

Family cohort study

A randomly selected cluster sample of 200 families, (8 families per cluster x 25 clusters) with a total population of 1,180 persons, all living in the Whiteriver area, was chosen for the study. This represented approximately a 15% sample of the total population of Whiteriver. They were enrolled in the later part of 1981, and surveillance began in January 1982 and continued for 24 months, ending on 31 December 1983. Weekly home visits were made by female Apache field workers to enquire about the occurrence of diarrhoeal illness in each member of the family, and information regarding any diarrhoeal episodes was obtained, as in the cohort study.

Stool cultures were obtained whenever the person was having diarrhoea at the time of home visit. No routine stool samples were taken as part of this study.

Case-control study

From September 1981 through December 1985, children aged less than five years attending the outpatient clinic at the Indian Health Service Whiteriver Hospital for a diarrhoeal illness during normal weekdays (8 a.m. - 5 p.m.) had their stool cultured. No selection criteria other than the diarrhoeal illness were used. In addition, each child needing hospitalization for diarrhoea was included, regardless of time of the day of hospitalization. For each patient with diarrhoea, a person who had attended the clinic for a non-diarrhoeal disease was matched as a control, and had a stool culture. Each control patient was matched for age (0-6 months, within 1 month; 6-12 months, within 3 months; 2-5 years, within 6 months) and for time of stool collection, which was within two weeks following the selection of the case. No detailed clinical information was obtained from these persons, and no follow-up was done.

Clinical definitions

Diarrhoea was defined as having three or more unformed stools passed in a 24-hour period. A "diarrhoeal episode" was defined as being separated from another episode by at least 2 diarrhoea-free days. The presence of blood in the stool was defined by the gross appearance of the stool, as indicated by the mother or noted by study personnel. A non-diarrhoeal period, used for defining the period of collection of normal stool samples, was one in which no diarrhoea was noted either two days before or two days after the collection of the specimen.

Fever was defined according to the mothers' assessment, unless this was documented to be >101 °F at the clinical facility. Respiratory symptoms were those

described by the mother, and/or noted by the visiting field staff.

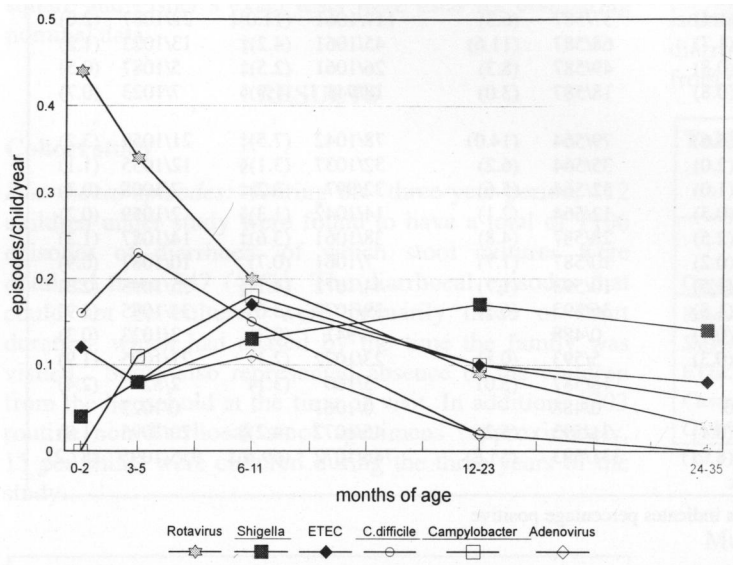

Fig. 4 Age-related, aetiologic specific, attack rates for diarrhoea in cohort children, plotted for major enteropathogens

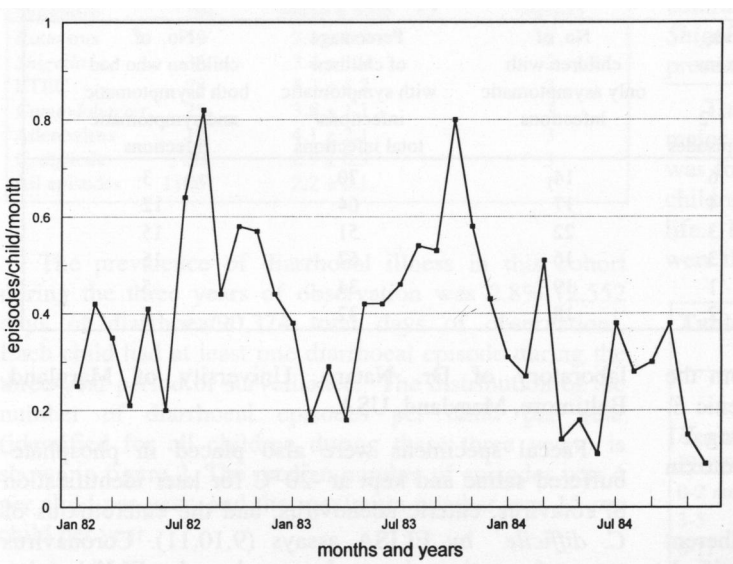

Fig. 5. Seasonal attack rates for diarrhoea in cohort children

Sample size calculations

The birth cohort sample size was based on the ability to identify 10% of diarrhoeal episodes due to specific aetiologic agents, assuming an attack rate of 3 episodes per child per year. Additional children were added to make up for possible drop-outs. The sample size was estimated at 100 newborns.

The surveillance population was chosen to include appropriate numbers of both children of less than 5 years and persons of more than 5 years. The sample size for children of less than 5 years was made also assuming 3 diarrhoeal episodes per child per year. We wished to detect agents responsible for 10% of all diarrhoeas, or 0.3 episodes/year with limits of 0.1 episodes per year. The number required was calculated to be 84 children. This was increased to 100 to account for possible drop-outs. The persons aged over 5 years were assumed to have 1 episode per person per year. We wished to detect agents responsible for 10% of all diarrhoeas, or 0.1 episode/year with limits of 0.03 episodes/year. The sample size was estimated to be 480.

It was decided to enroll clusters of households scattered throughout the 11 communities of the Whiteriver area, each cluster with 8 contiguous households. The 25 clusters were selected by systematic random sampling, using the probability proportionate to size method.

Laboratory studies

Stool microbiology: Whole stool specimens or rectal swabs placed in Cary-Blair transport media were examined for enteric pathogens. Field specimens were cultured within the same day of collection, and those from the hospital and outpatient clinic were cultured within 1-2 hours of collection. A special enteric diagnostic laboratory was established in the Indian Health Service Hospital to process all the stool cultures from the ongoing studies.

Faecal specimens were initially plated on Thiosulphate Citrate Bile Salt Agar (TCBS), xylose lysine deoxycholate (XLD), Hektoen, and MacConkey agar plates at 37 °C, and Campylobacter agar at 42 °C in an anaerobic jar with the addition of a Campy gas-pack, hydrogen-carbon dioxide generator. Specimens were also inoculated into Selenite F broth and Gram-negative broth overnight, and subcultured the following day onto XLD and Hekteon agar plates. All plates were examined for suspected enteropathogens after overnight incubation, except the Campylobacter agar, which was examined at 48 hours. The MacConkey plate was re-incubated at 25 °C for another 24 hours for isolation of *Yersinia*. Identification of *Shigella, Salmonella, Campylobacter jejuni*, vibrios, *Aeromonas*, and *Yersinia* was done by standard methods (5).

Table I. Organisms detected in stools of study populations during diarrhoeal and non-diarrhoeal periods*

Organism	Birth cohort study: Diarrhoeal episodes		Birth cohort study: Non-diarrhoeal period		Family surveillance: Diarrhoeal episodes		Case-control study: Cases		Case-control study: Controls	
Rotavirus	39/486†	(8.0)	18/1620	(1.1)	37/587	(6.3)	117/1061	(11.0)‡	22/1087	(2.0)
Shigella (all)	36/485†	(7.4)	28/1638	(1.7)	68/587	(11.6)	45/1061	(4.2)‡	13/1023	(1.2)
S. flexneri	17/485†	(3.5)	14/1638	(0.8)	49/587	(8.3)	26/1061	(2.5)‡	5/1087	(0.5)
S. sonnei	19/485†	(3.9)	14/1638	(0.8)	18/587	(3.0)	18/941	(1.9)§	7/1023	(0.7)
Enterotoxigenic *E. coli* (all types)	28/534	(5.2)	60/1650	(3.6)	79/564	(14.0)	78/1042	(7.5)‡	21/1059	(3.2)
LT only	0/534†	0	33/1650	(2.0)	35/564	(6.2)	32/1037	(3.1)§	12/1055	(1.1)
ST only	6/506	(1.1)	18/1650	(1.0)	32/564	(5.6)	32/997	(3.2)‡	7/1008	(0.7)
LT/ST	22/506†	(4.3)	9/1650	(0.5)	12/564	(2.1)	14/1042	(1.3)§	2/1059	(0.2)
Campylobacter	25/489†	(5.1)	34/1338	(2.5)	28/587	(4.8)	38/1061	(3.6)‡	14/1087	(1.3)
Salmonella	2/483	(0.4)	3/1638	(0.2)	10/587	(1.7)	7/1061	(0.7)	10/1087	(0.9)
Adenovirus	12/492	(2.4)	25/1703	(1.5)	10/593	(1.7)	44/1072	(4.1)§	25/1095	(2.3)
C. difficile	20/492†	(4.1)	31/1703	(1.8)	36/593	(6.1)	39/1072	(3.6)	34/1095	(1.9)
Vibrio species	0/443	0	2/1488	(0.1)	0/488		3/941	(0.3)	3/1023	(0.3)
Aeromonas	3/486	(0.6)	5/1703	(0.3)	5/593	(0.8)	23/1072	(2.1)	21/1095	(1.9)
Coronavirus	1/60	(1.6)	N.D.		18/587	(3.0)	5/160	(3.1)	2/82	(2.4)
Yersinia	0/443	0	0/1703	0	0/488		0/1061		0/1023	
Mixed infections	39/547†	(7.1)	7/1703	(0.4)	31/593	(5.2)	45/1072	(4.2)§	20/1095	(1.8)
No enteric pathogens detected	393/547†	(71.8)	1416/1705	(83.1)	331/593	(55.8)	746/1072	(69.6)‡	955/1095	(87.2)

* Number of specimens positive/total examined; parentheses indicates percentage positive
† p = <.05 between diarrhoeal and non-diarrhoeal periods
‡ p = <.001 between cases and controls
§ p = <0.05 between cases and controls

Table II. Detection of aetiologic agents in 112 birth cohort children during symptomatic and asymptomatic periods

Aetiologic agent	No. of children with documented infections (%)		No. of children with symptomatic infections: Total	1 Episode	2 Episodes	No. of children with only asymptomatic infections	Percentage of children with symptomatic infections/ total infections	No. of children who had both asymptomatic and symptomatic infections
Rotavirus	47	(42)	33	27	6	14	70	3
Shigella	48	(43)	31	27	4	17	64	12
ETEC	45	(40)	23	20	3	22	51	15
Campylobacter	37	(33)	21	18	3	16	57	15
Adenovirus	29	(26)	10	9	1	19	34	5
C. difficile	29	(26)	11	9	2	18	37	5

Five lactose-positive colonies were picked from the MacConkey plates for identification of enterotoxigenic *E. coli*; heat-labile enterotoxin (LT) was identified using Y1 adrenal tissue culture cells (6) and heat-stable enterotoxin (ST) by the infant mouse assay (7).

Enteropathogenic serotypes and enteroadherent (localized) strains of *E. coli* were not routinely identified; only strains isolated from patients from whom no other enteric pathogen was identified were selected for identification. Enteroinvasive strains of *E. coli* were not identified, nor were the more recently recognized diarrhoeagenic types of *E. coli* (other enteroadherent and enterohaemorrhagic strains). Serotyping of selected strains of *E. coli* was done by Dr. Fritz Orskov at the WHO reference laboratory in Copenhagen, Denmark. The selected strains of *E. coli* were also tested for localized adherence using the EAF probe (8) in the laboratory of Dr. Nataro, University of Maryland, Baltimore, Maryland, USA.

Faecal specimens were also placed in phosphate-buffered saline and kept at -20 °C for later identification of rotavirus, enteric adenovirus, and the enterotoxins of *C. difficile* by ELISA assays (9,10,11). Coronavirus assays for antigen in stool were done by ELISA using antisera prepared in guinea pigs by immunization with coronavirus 229E.

During the first year of the study, whole stools were also examined for ova and parasites by direct microscopy, and samples were preserved in polyvinyl alcohol (PVA) and merthiolate-iodine-formaldehyde (MIF). Because of the very low yield of positive identifications of *Giardia lamblia* and *Entamoeba histolytica*, this was not continued; *Cryptosporidium parvum* was not looked for.

Statistical analyses

Data management was done using dBASE IV; SAS/PC, version 6.04 was used for data analysis. Chi-square and Fisher's exact tests were used for comparing nominal data.

RESULTS

Cohort study

Diarrhoeal episodes: During the three-year period, 112 children under study were found to have a total of 1106 episodes of diarrhoea, of which stool cultures were obtained from 547 (49%). The diarrhoeal episodes that could not be cultured were primarily those of short duration, which had ceased by the time the family was visited. Some also represented absence of the children from the household at the time of visit. In addition, 1703 routine non-diarrhoeal stool specimens (approximately, 15 per child) were cultured during the three years of the study.

Table III. Duration of diarrhoeal disease episodes in birth-cohort children according to major aetiologic agents

		Duration (days)	
Organism	No.	Mean ± 95% CI	Median
Rotavirus	39	3.1 ± 0.9	2
Shigella	36	3.4 ± 1.0	2
ETEC	28	3.7 ± 1.2	3
Campylobacter	25	3.8 ± 1.1	3
Adenovirus	12	4.1 ± 2.1	3
C. difficile	20	2.9 ± 0.9	3
All episodes	1106	2.2 ± 0.1	1

The prevalence of diarrhoeal illness in this cohort during the three years of observation was 2.8% (2,552 days of diarrhoea/90,374 total days of observation). Each child had at least one diarrhoeal episode during the three-year period of surveillance. The distribution of the number of diarrhoeal episodes per child per year (identified for all children during these three years) is shown in figure 2. The median number of episodes was 4 per child per year, and the maximum number was 15 per child per year.

The diarrhoea attack rates were highly dependent on age (Figure 3). The peak rates occurred in the 4-12 months-age group, when they reached an average of 6.4 episodes per child per year. Significantly higher attack rates were seen among the male than the female infants at all ages.

Aetiologic agents: The aetiologic agents identified in diarrhoeal, as well as normal stools are shown in the first section of table I. An enteric pathogen was identified in 28% of diarrhoeal stool specimens and in 17% of normal stools. The most common diarrhoeal pathogens were: rotavirus, ETEC, *Shigella*, and *Campylobacter*. Other frequently identified pathogens were *C. difficile* and enteric adenoviruses. Most of these pathogens were isolated with significantly greater frequency in the diarrhoeal stools, compared to the non-diarrhoeal stools from the same infants.

Table IV. Clinical characteristics of diarrhoeal disease episodes in birth cohort children according to major aetiologic agents

		Percent positive			
Organism	N*	Blood in stool	Fever	Vomiting	Respiratory symptoms
Rotavirus	37	0	5	3	3
Shigella	32	9	6	9	3
ETEC	28	4	0	0	0
Campylobacter	23	9	4	9	0
C. difficile	12	8	8	0	17

* N = Number of episodes for particular organism for which data are available

Multiple infections with two (or more) enteropathogens were found in 39 (7.1%) of the diarrhoeal stools. In approximately 40% of the persons in whom either enteric adenovirus or *C. difficile* was identified, other enteric pathogens were also found. This contrasts to a rate of about 20% when rotavirus, ETEC, *Shigella*, or *Campylobacter* was isolated (data not presented).

The age-related, aetiology-specific attack rates for the major enteropathogens are shown in figure 4. Rotavirus was found to have the highest attack rate in the youngest children, and this decreased with age, till the third year of life. By way of contrast, ETEC and *Shigella* infections were the highest during the age of 7-24 months.

Table V. Diarrhoea surveillance in families: ages of population (1982-1983)

	Beginning of study (Jan 1982)		End of study (Dec 1983)	
Age of persons	No. of persons	Percentage of total	No. of persons	Percentage of total
0-2 months	24	2.0	0	0
3-5 "	11	0.9	0	0
6-11 "	25	2.1	2	0.2
12-23 "	48	4.0	23	1.9
24-35 "	47	3.9	49	4.1
36-47 "	28	2.4	50	4.2
48-59 "	32	2.7	47	3.9
5-9 years	117	9.9	127	10.7
10-19 "	304	25.8	305	25.6
20-39 "	319	27.0	347	29.1
40-59 "	155	13.1	158	13.3
>60 "	70	5.9	84	7.0
Total	1180	100	1192	100

The incidence of diarrhoea increased in the warm summer months (Figure 5). ETEC, *Shigella*, and *Campylobacter* were most commonly isolated during this time (Figure 6). Rotavirus, on the other hand, while primarily identified in the cold winter months, was also found throughout the year, including one peak that occurred in May of 1983. By way of contrast, isolation of *C. difficile* occurred at about the same frequency throughout the year.

Table VI. Ages of children with diarrhoea (cases) and matched controls

Age (month)	Case	(%)	Control	(%)
0 - 2	226	(19.7)	212	(18.4)
3 - 5	261	(22.9)	270	(23.5)
6 - 11	362	(31.7)	363	(31.6)
12 - 23	252	(22.1)	259	(22.6)
24 - 35	36	(3.2)	39	(3.4)
36 - 47	2	(0.2)	3	(0.3)
48 - 59	3	(0.3)	3	(0.3)
Total	1142		1149	

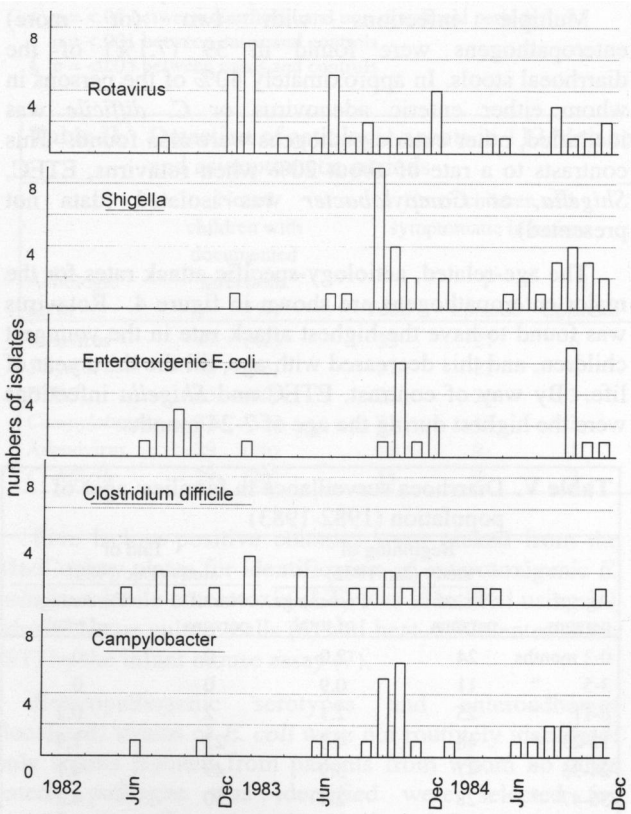

Fig. 6. Seasonal isolation of major enteropathogens in cohort children

Repeated infections with the same groups of aetiologic agents were found in approximately 4-5% of the infants. These data are summarized in table II. In some children, the organisms causing the second infection i.e. rotavirus could be identified sufficiently to be certain that both infections were caused by the same serotype. Infections with rotavirus and *Shigella* were most likely to be symptomatic (64% and 70% respectively). As also shown in table II, the three major enteropathogens (rotavirus, ETEC, and *Shigella*) were identified in stools of about 40% of all children during the study. *Campylobacter, C. difficile* and enteric adenovirus were identified in approximately 25-30% children.

Clinical characteristics of diarrhoeal illness: The illnesses were usually brief in duration, with a mean duration of 2.2 days and a median of 1 day. A frequency distribution of the duration of illness is given in figure 7. Only 8 episodes (0.7%) lasted 14 days or longer.

Clinical data are presented according to the specific pathogens identified in the diarrhoeal stools of cohort infants (Tables III and IV). There were no striking differences between clinical syndromes seen with the different enteropathogens. The median duration of the pathogen-related illnesses (2-3 days), however, was generally longer than that for all episodes (1 day). The presence of blood in the stools, fever, vomiting, and respiratory symptoms were infrequent in all of these relatively mild diarrhoeal illnesses.

Of the 112 cohort infants, 42 (37.5%) were hospitalized 64 times for diarrhoeal illnesses during the study period (one child was hospitalized 4 times; four were hospitalized 3 times, 12 were hospitalized twice, and the remaining 25 once only). Sixty-three percent hospitalization occurred when the children were less than 6 months of age, and another 28% when they were between the ages of 7 and 12 months. The attack rates (hospitalization per 100 child-years) for the different age groups were: 0-5 months 78; 6-11 months 33; 13-15 months 4.3. Forty-four (69%) of the 64 hospitalizations occurred between June and October. Rotavirus (18.8%) and ETEC (15.6%) were the most common enteropathogens identified at the time of hospitalization (4). Details of the clinical and microbiological studies done on these hospitalized children are presented elsewhere (4).

Family surveillance study

This study involved 1,180 persons, 200 families in 25 clusters, living in Whiteriver. The ages of the family members at the beginning and end of the study are shown in table V. At the beginning of the study, 18.2% of the population was less than 5 years of age. Twenty-eight children who were part of the birth cohort also belonged to families which were part of this surveillance. Weekly diarrhoeal surveillance was carried out for 24 months from January 1982 through December 1983. A total of 1,418 person years of observation was made.

Diarrhoeal episodes: A total of 924 diarrhoeal episodes was identified, of which 593 (64%) were cultured. The overall diarrhoeal prevalence in this population was

0.35% (1,836 days of diarrhoea out of 517,513 days of observation).

The diarrhoeal incidence, according to age and sex, is shown in figure 8. The peak incidence of 4.5 episodes per child per year was seen in the 4-6 months of life. Throughout the first year of life, boys had significantly higher attack rates of diarrhoea than girls. Rates were low during the period of adult life, about 0.3 episodes per person per year, but increased to over 1 episode per person per year in adults over 60 years of age.

Aetiologic agents: The most common organisms identified in diarrhoeal stools were ETEC and *Shigella* (Table 1). Aetiology-specific attack rates, according to age, were similar to those seen in the cohort children (data not presented). The highest rates for rotavirus were seen in the 4-6 months age group, whereas the highest rates for ETEC and *Campylobacter* were seen in the 7-12 months age group, and the highest rates for *Shigella* in the second and third years of life.

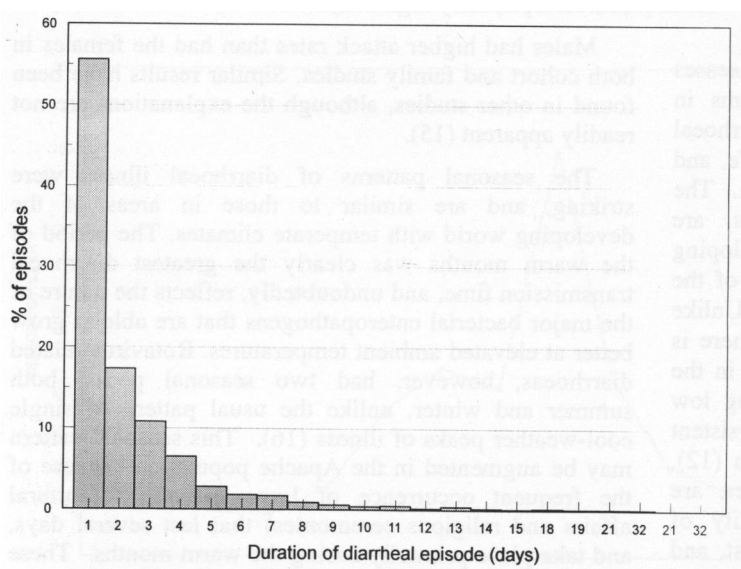

Fig. 7. Duration of diarrhoeal episodes in cohort children

The seasonal incidence of diarrhoeal illness, shown in Figure 9, also shows a marked late summer (September) peak, as did the isolation of enteropathogens (data not presented). *Shigella* and ETEC isolations peaked in the summer months, whereas rotavirus was found mostly in the cold season, although some cases occurred during summer as well.

Clinical Characteristics of Diarrhoeal Illness: The diarrhoeal episodes were generally of short duration, (mean 2.0 days and median 1 day), and there were only 5 (0.5%) episodes that lasted 14 days or more. The distribution of the lengths of illness is similar to that seen for the cohort children (data not presented).

There were 48 persons from this population who were hospitalized with diarrhoeal illness during this two-year period. The rates of hospitalization for diarrhoea (number of hospitalizations per 100 child-years) given by age were: 0-2 months 25; 3-5 months 44; 7-12 months 18; 13-24 months 3; 25-36 months 2. Clinical data from these hospitalized children are presented elsewhere (4).

Case-control study

A total of 1,155 pairs (diarrhoeal patients and control patients) was enrolled. Nearly all (96%) were under two years of age (Table 6). Because some of the cultures were not adequate or were inadvertently not obtained, the total number available for analysis is slightly less (1,072 diarrhoea patients, and 1,095 controls).

Aetiologic Agents: Rotavirus, ETEC, *Shigella*, and enteric adenovirus were the organisms most frequently identified (Table 1). The isolation rate in diarrhoeal patients was significantly higher than in matched controls for all agents, except *Salmonella, C. difficile*, Vibrios, *Aeromonas*, and coronavirus. Mixed infections in this group were seen in 4% patients and in about 2% of controls. Rotavirus was shown to be commonly associated with both adenovirus and *C. difficile* infections; of the 24 children with rotavirus detected in their diarrhoeal stools, 6 had adenovirus and 7 had *C. difficile* also identified in this stool.

The isolation rates of specific aetiologic agents, according to age, are shown in table 7. Rotaviruses were found more frequently in cases than controls at all age groups. *Shigella* and *Campylobacter* were found significantly more often only in children above 7 months of age. ETEC was found more often in all age groups except during the 4-6 months of age. Adenoviruses were found more often in cases only during the first 6 months of life. *C. difficile* was found more often in cases only during the 7-12 months of life.

A group of 111 ETEC isolated from children with diarrhoea was serotyped. The most common serotypes identified were: O27:H2O, 19 isolates (all ST-only positive); O25:H-, 14 isolates (11 LT-only and 4 ST/LT); O8:H-, 4 isolates (all LT-only); O159:H4, 4 isolates (all LT-only); and O9:H-, 5 isolates (3 LT-only and 2 ST-only). The remaining serotypes were of a wide variety, and there were no more than 3 of any other single serotype identified. Localized, enteroadherent *E. coli* were identified by EAF probe in 27 of 80 strains tested from children with diarrhoea; 14 of these 27 were of serogroup O111.

Table VII. Isolation of aetiologic agents in children with and without diarrhoea according to age group; case-control study

Aetiologic agent	Age (months) 0 - 2		3-5		6-11		12-23	
Rotavirus								
Cases	29/212*	(13.7%)	36/249*	(14.5%)	29/330*	(8.8%)	20/229*	(8.7%)
Controls	8/197	(4.1%)	7/258	(2.7%)	5/343	(1.5%)	2/241	(0.8%)
Shigella								
Cases	3/212	(1.4%)	2/249	(0.8%)	14/330*	(4.2%)	20/229*	(8.7%)
Controls	1/187	(0.5%)	0		4/343	(1.1%)	7/241	(2.9%)
ETEC								
Cases	24/209*	(11.5%)	5/240	(2.0%)	18/327*	(5.5%)	31/225*	(13.7%)
Controls	3/185	(1.6%)	4/243	(1.7%)	7/344	(2.0)	5/239	(2.5%)
Campylobacter								
Cases	3/212	(1.4%)	3/249	(1.2%)	19/330*	(5.8%)	12/229	(5.2%)
Controls	0		2/258	(0.8%)	6/343	(1.7%)	6/241	(2.5%)
Adenovirus								
Cases	11/217*	(5.1%)	18/250*	(7.2%)	13/333	(3.9%)	2/230	(0.8%)
Control	3/199	(1.3%)	7/259	(2.7%)	7/347	(2.0%)	5/242	(2.0%)
C. difficile								
Cases	7/217	(3.2%)	11/230	(4.4%)	19/333*	(5.7%)	2/230	(0.9%)
Controls	9/199	(4.5%)	11/259	(4.2%)	6/347	(1.7%)	5/243	(2.1%)

* = $p<0.05$, in comparison of cases and controls

DISCUSSION

This series of studies confirms that diarrhoeal diseases are important clinical and public health problems in Apache children living in Whiteriver, Arizona. Diarrhoeal episodes are frequent during the first 3 years of life, and often result in clinic visits and hospitalization. The aetiologic agents and epidemiologic patterns are essentially identical to those seen in the developing world; children were exposed to most, if not all, of the enteropathogens during their first 3 years of life. Unlike the situation in the developing world, however, there is essentially no mortality associated with diarrhoea in the Apaches; the diarrhoeal episodes are short, giving low prevalence rates; and there is a very low rate of persistent diarrhoea; and there is no significant malnutrition (12). The hospitalization rates, for diarrhoea, however, are high, probably because of the ready availability of medical services to the population at no extra cost, and the awareness of the population about the importance of prompt treatment for diarrhoea in infants.

Diarrhoeal attack rates were the highest (6-8 episodes per child per year) during the age of 4-12 months, and had decreased markedly by the age of 2 years. These high rates are comparable to those seen in Bangladesh (13) and Peru (14). During adult life, rates were low, but showed a definite increase after the age of 60 years. Relatively higher diarrhoeal attack rates in the elderly have also been seen in other US populations (2). Children in the birth cohort were found to have higher rates than those in the family surveillance study. This may reflect a difference in the intensity of the surveillance, although the visiting intervals were the same, or may have been due to the sub-set of children in the cohort study with exceptionally high attack rates.

Males had higher attack rates than had the females in both cohort and family studies. Similar results have been found in other studies, although the explanations are not readily apparent (15).

The seasonal patterns of diarrhoeal illness were striking, and are similar to those in areas of the developing world with temperate climates. The period of the warm months was clearly the greatest diarrhoeal transmission time, and undoubtedly, reflects the nature of the major bacterial enteropathogens that are able to grow better at elevated ambient temperatures. Rotavirus-related diarrhoeas, however, had two seasonal peaks, both summer and winter, unlike the usual pattern of single cool-weather peaks of illness (16). This seasonal pattern may be augmented in the Apache population because of the frequent occurrence of large gatherings (cultural affairs and religious ceremonies) that last several days, and take place primarily during the warm months. These events often take place along the banks of rivers, where adequate potable drinking water, sanitation facilities, and refrigeration are not readily available.

The water supply in the reservation, however, which comes primarily from the many rivers that run through it, is plentiful and of good quality, and is not thought to be a vehicle of transmission. Most homes have refrigeration, piped water of good quality, and indoor sanitary plumbing.

A study done in the same population, in which risk factors for acquiring rotavirus infections were identified, showed that living in a home with a septic tank or with poor environmental sanitation were risk factors for rotavirus diarrhoea (17).

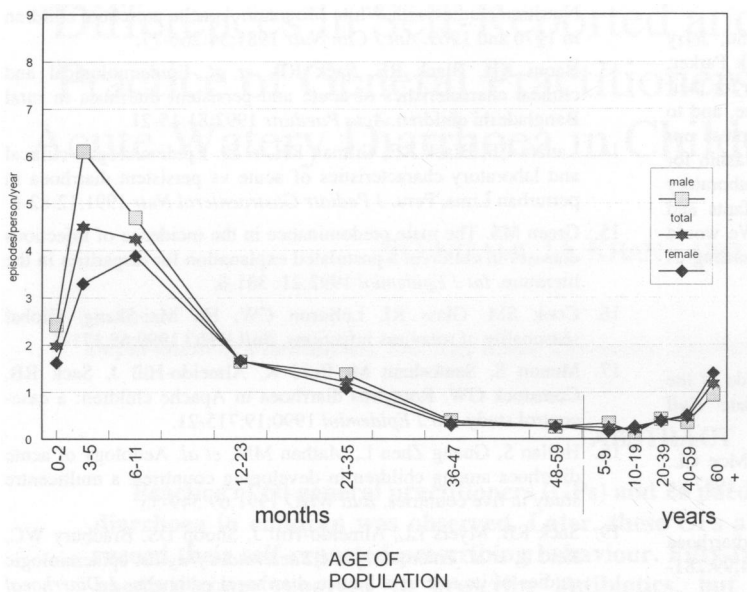

Fig. 8. Diarrhoea attack rates by age and sex in family surveillance population

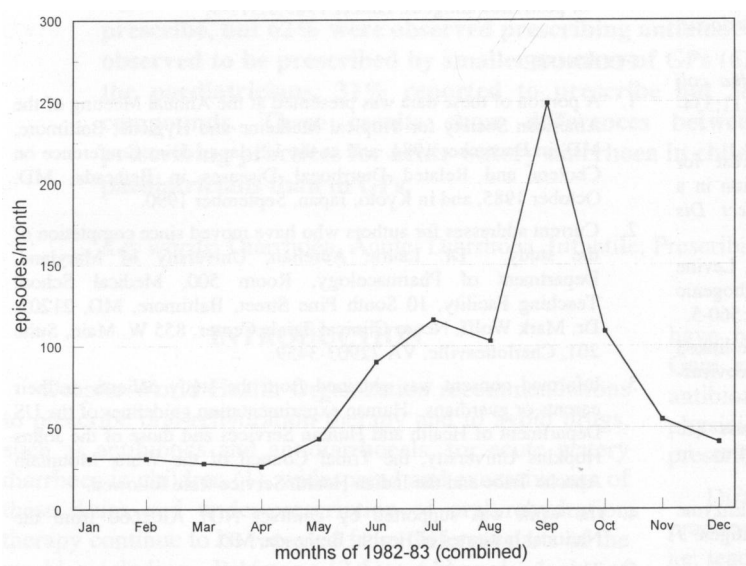

Fig. 9. Diarrhoeal episodes according to season in family surveillance population

The aetiologic agents of diarrhoea were essentially identical to those found throughout most of the developing world (18). The most commonly identified agents were rotavirus, *Shigella*, and enterotoxigenic *E. coli*. Since this study was done before 1985, there were enteric pathogens that were not yet recognized or established at the time, and therefore, were not routinely sought. Nonetheless, among the small numbers of *E. coli* tested, classical enteropathogenic serotypes, and enteroadherent properties were identified. A more recent study in this population on another newly-described enteropathogen enterotoxigenic *Bacteroides fragilis* (19) showed them to be epidemiologically related to episodes of acute diarrhoea in children over the age of one year. In other studies done on Apache children with diarrhoea with no known aetiologic agents identified in their stool, pestiviruses were found (20) along with group B rotaviruses (unpublished data). This information indicates that the rate of detection of known enteric pathogens was clearly lower during the study period than would now be the case.

The comparison of the isolation of enteric pathogens in children with and without diarrhoea generally corroborates similar findings in other parts of the world. Enteric adenovirus infections were found to be associated significantly with diarrhoea in the case-control study (only in children under 6 months of age), but not in the birth cohort study. *C. difficile*, on the other hand, was found to be associated with diarrhoea in the cohort study, but only during 6-11 months of age in the case-control study. There were too few of some pathogens: *Salmonella*, vibrios, coronavirus, to allow meaningful comparison. No *Y. enterocolitica* was isolated.

Association of these enteropathogens with age was also similar to that described in other studies in the developing world. The figures given in this study, however, are almost certainly under-estimates of the true rates, since only about half of the diarrhoeal episodes were cultured. This suggests that nearly all children were exposed to the major enteropathogens during their first three years of life.

Most episodes of diarrhoea were short (1-2 days) and clinically similar, regardless of the aetiologic agents identified, and it was impossible to predict the aetiology from the clinical syndrome. It was clear that diarrhoeal episodes caused by rotavirus were the most severe in that this agent caused the highest percentage of symptomatic illness and was most frequently found in children admitted to the hospital.

The patterns of diarrhoeal illness in this population are similar to those seen in the developing world where sanitation is sub-optimal, and potable water is scarce. In this community, sub-standard levels of hygiene and sanitation are probably the most important reasons for the high rates of diarrhoeal disease, since the water supply is clearly adequate. Major improvements in sanitation and hygiene are expected to exert a marked impact on the transmission of diarrhoeal agents in this population, as they have been historically in other parts of the United States.

ACKNOWLEDGMENTS

The authors would like to thank Carla Alchesay Nachu, Jerry Short, Stephen Foster, Bryson Roussey, Larry Croll, Dr. Jack Parker, Dr. Robert Betrando, Dr. Bert Attico, and Dr. Maurie Sievers for their help and guidance, to Mohammad Ali for computer assistance, and to Hanifur Rahman for secretarial help. We would like to express our gratitude to the Apache Field workers, especially Yolanda Nashio for conducting the interviews, and collecting the necesssary laboratory specimens. Our special thanks are due to the Apache infants and children and their families who participated in this study. We would also like to thank the White Mountain Apache Tribe for permitting us to conduct the study.

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FOOTNOTE

1. A portion of these data was presented at the Annual Meeting of the American Society for Tropical Medicine and Hygiene, Baltimore, MD, in December 1984, and at the US-Japan Joint Conference on Cholera and Related Diarrhoeal Diseases in Bethesda, MD, October 1985, and in Kyoto, Japan, September 1990.
2. Current addresses for authors who have moved since completion of the study: Dr. Laurie Aurelian, University of Maryland, Department of Pharmacology, Room 500, Medical School Teaching Facility, 10 South Pine Street, Baltimore, MD, 21202, Dr. Mark Wolff, Neuro Clinical Trials Center, 855 W. Main, Suite 201, Charlottesville, VA 22903-3459.
3. Informed consent was obtained from the study patients or their parents or guardians. Human experimentation guidelines of the US Department of Health and Human Services and those of the Johns Hopkins University, the Tribal Council of the White Mountain Apache Tribe and the Indian Health Service were followed.
4. The work was supported by contract NO1 AI02660 from the National Institutes of Health, Bethesda, MD.
5. The opinions expressed in this manuscript are not necessarily those of the Indian Health Service.