STUDIES ON ESCHERICHIA COLI SEROTYPES IN ANIMALS*

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The coli group of organisms has long been recognized as normal inhabitants of the intestinal tract of man and animals and their presence in food and water has been universally used as index of fecal contamination.

During the past few years, a number of European investigators (1, 2, 3) reported the association of *Escherichia coli* serotypes with outbreaks of infant diarrhea and gastro-enteritis (infantile coli-enteritis) in young children. Essentially similar epidemiologic and serologic findings were likewise reported by various American investigators (4, 5, 6, 7, 8, 9) since the discovery of the first *E. coli* serotype by Bray (10) in 1945 and the second serotype by Giles, *et. al.* (11) in 1947. In confirmation of these reports, feeding experiments on adult human volunteers and infants have been described by Ferguson, (12) Neter, (13) and June (14). The transmissibility and reproducibility of the disease in animals have also been successfully demonstrated and confirmed by Namioka (15) and Dunne, *et. al.* (16). As a result of these pioneering experiments in man and animals, it is now universally recognized that certain "enteropathogenic *E. coli* cause epidemic gastro-coli-enteritis in infants and young children. With the development of serologic technic by Kauffman (17) and the improvement of methods of isolation and identification by Ewing (18), more *E. coli* serotypes have been reported in recent years not only in young children and animals but also from sources like soil and vegetables.

While marked progress has been made abroad in the study of *Escherichia coli* serotypes, very limited information has been

* Supported by a research grant from the University of the Philippines.

reported locally. The first report on the existence of enteropathogenic *E. coli* (026:B6, 055:B5, 0111:B4 and 0127:B8) associated with diarrhea-enteritis in infants in the Philippines were made by Aragon, *et. al.* (19) and Guerrero, *et. al.* (20) in separate investigations carried out in the Philippine General Hospital and San Lazaro Hospital, respectively. In addition, Guerrero points out that A.P. de Roda has isolated eleven *E. coli* serotypes in the City of Manila. The occurrence, however, of enteropathogenic *E. coli* in animals has never been reported locally. The isolation of pathogenic *E. coli* from cases of "calf scours" or calf diarrhea was previously reported by Smith (21) in 1927 and by Lovell (22) in 1937. These reports were later confirmed by Orskov (23) in 1951 and in 1955, Glanta (24) successfully isolated *E. coli* serotype 026:B6 from calves with "white scours."

The present study was carried out to determine whether four of the most common enteropathogenic *Escherichia coli* serotypes (026:B6, 055:B5, 0111:B4 and 0127:B8) are present in the feces of animals closely associated with man.

MATERIALS AND METHODS

The present study was conducted in the Department of Medical Microbiology, Institute of Hygiene, University of the Philippines, during the period from August 1957 to February 1959.

Animals examined:

The 856 animals included in this study consisted of 368 rats, 232 dogs and 256 pigs. The rats (*R. rattus, R. norvegicus* and *Mus musculus*) were obtained from the Bureau of Quarantine and the Section of Insect and Vermin Control of the Manila Health Department. A number of rats caught in the neighboring provinces of Batangas, Bulacan and Rizal and which were used in a separate investigation were also included in this study. All the live rats were killed with ether and were immediately autopsied in the laboratory. The intestines of each rat were isolated with forceps and were examined for any gastrointestinal disorder prior to the collection of the specimen. The consistency of the intestinal contents was examined and a small portion was streaked on a differential plating media with a sterile inoculating loop. Dead rats were similarly examined.

The dogs used in this study included those found free of diarrhea and which were confined in the Pasay and Manila city pounds and SPCA (Society for the Prevention of Cruelty to Animals) compound. Dogs brought to the Veterinary Clinic of Dr. Nicanor Carlos in the district of Malate for rabies vaccination were also included. Clinical materials from these animals were collected with the use of sterile rectal swabs moistened with sterile normal saline and placed in individual sterile test tubes. The swabs were brought immediately to the laboratory and inoculated in the plating media. All the rectal swabs from pigs were collected at the Manila city abattoir before the pigs were butchered.

E. coli typing sera:

The anti "OB" and anti "O" specific typing sera employed in this study were prepared by immunizing healthy rabbits with pathogenic *E. coli* serotypes 055:B5, 0111:B4, 0127:B8 and 026:B6 obtained from the Communicable Disease Center, United States Public Health Service. The technic used in the preparation of the typing sera was based on the method described by Ewing (25).

Method of isolation and identification:

The method employed in the isolation and identification of *E. coli* serotypes from the animals was based on the technique prescribed by Ewing and Edwards (18, 26) with some modifications. All fecal samples and rectal swabs from the different animals were inoculated into eosin methylene blue (EMB) agar, MacConkey agar and occasionally blood agar plates. This was done by surface streaking and swabbing. After 16-20 hours incubation at 37°C. The inoculated plates were examined for the presence of *Escherichia coli* colonies. Typical *E. coli* colonies on EMB agar are smooth, round and low convex with entire edge 1-2 mm. in diameter and with characteristic greenish metallic lustre. They are red in color in MacConkey and grayish white in blood agar, generally producing no hemolysis. With the use of a sterile inoculating needle, several typical *E. coli* colonies about 10-15 in number were picked from the different parts of the plating media and each was transferred into nutrient agar slants. The slants were incubated at 37°C and after 20 hours.

a small portion of the surface growth from each slant was obtained with a sterile inoculating loop and tested by slide agglutination for preliminary screening. The test was accomplished by using pooled anti "OB" typing sera (Anti "OB" sera against 026:B6, 055:B5, 0127:B8 and 0111:B4). If agglutination occurs, the test was repeated by using single anti "OB" specific typing sera. Again, if agglutination is observed, with any one of the four single anti "OB" typing sera, the growth was harvested with normal saline and heated at 100°C in a water bath for a period of one hour, to destroy the "B" component of the antigen. The heat killed antigen was then centrifuged for 30 minutes at 2,500 rpm and washed with normal saline. A thick suspension of the heated antigen was then prepared and retested by slide agglutination using single anti "OB" specific typing serum as above. A positive slide agglutination test with the individual anti "OB" typing sera is indicative of the presence of any one of the four *E. coli* serotypes. Confirmation as to the presence of the "O" component of the antigen was done by using single anti "O" specific typing sera against any of the four serotypes. Control tests were also made for each specimen to rule out false positive reaction. Other *E. coli* serotypes were not determined due to the lack of specific typing sera. No attempt was made to isolate other enteric pathogens and saprophytic bacteria. The physiological and morphological characteristics of the isolates were also studied to confirm the serologic findings.

RESULTS

The *Escherichia coli* serotypes isolated from diarrhea-free animals are shown in the table below. Of the 856 animals examined, 11.8 percent yielded pathogenic *E. coli* serotypes. From the table, it can be seen that *Escherichia coli* serotypes 0127:B8 is the predominating type in rats and dogs, while in swine, 055:B5 is the most frequently isolated serotype. Of the three species of animals examined, rats yielded the highest percentage of *E. coli* serotypes.

From the results obtained, it is evident that pathogenic *E. coli* serotypes isolated locally from children with diarrhea are also found in the feces of diarrhea-free animals. These results confirm the findings of Sakazaki and Namioka (27) in Japan

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E. coli serotype	Rats and Mice			Swine			Dogs		
	No. examined	No. +	% +	No. examined	No. +	% +	No. examined	No. +	% +
026:B6	368	11	3.0	256	4	1.6	232	5	2.2
055:B5	368	12	3.3	256	10	3.9	232	6	2.6
0111:B4	368	9	2.4	256	8	3.1	232	3	1.3
0127:B8	368	16	4.3	256	7	2.7	232	10	4.3

who isolated pathogenic *E. coli* serotype 025 from dogs, swine and cattle, serotypes 055, 0126, and 0112 from dogs and cattle, and serotype 0124 from dogs and horses, except that they failed to isolated 0127:B8 and 0111:B4 in the animals they examined.

It is a well known fact that animals transmit a number of bacterial, viral and parasitic infections to man. According to Hull (28) there are forty diseases that are transmitted from animals to man, eight of which are primarily of human origin. In the Philippines, records show that rats, dogs and pigs share with man in the transmission of a number of bacterial, viral and parasitic infections. Since pathogenic *E. coli* serotypes has been established in infants in the Philippines, and since these organisms could be isolated from the feces of normal rats, dogs and pigs, it is not improbable that animals acquire the organisms from man or *vice versa* and therefore may serve as carriers and possibly reservoirs of pathogenic *E. coli* serotypes. It is not to be inferred, however, that animals are the chief sources of infection. U.S. Army laboratory personnel stationed in Japan (29) found that pathogenic *E. coli* were isolated in 8.3 per cent of 3,620 soil samples and 3.9 per cent of 4,077 vegetables examined.

Although a high percentage of pathogenic *E. coli* was isolated from these animals, it is not justifiable to draw definite conclusions as to whether animals are actually carriers of pathogenic *E. coli*. Further studies on the association of pathogenic *E. coli* serotypes in animals and man will probably be fruitful in clarifying the role of animals as pathogenic *E. coli* carriers.

SUMMARY

A total of 856 animals were examined for the presence of enteropathogenic *E. coli* serotypes. About 11.8 per cent of the animals yielded pathogenic *E. coli* of the following serotypes: 055:B5, 026:B6, 0127:B8 and 0111:B4.

ACKNOWLEDGMENTS

We would like to express our gratitude to Dr. Nicanor Carlos of the City Pound; Dr. Dominador Rubio of the Manila City Abattoir; Dr. Atanacio Montaire of the SPCA; Dr. Jesus Azurin of the Bureau of Quarantine; and Mr. Irineo Montemajor of the Section of Insect & Vermin Control, Manila Health Deaprtment, for their kind cooperation in providing the animals used in this study.

Our thanks are also due to Dr. Victor Valenzuela, Head Department of Biostatistics, Institute of Hygiene, U.P., for his invaluable suggestions in the preparation of the manuscript.

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