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SEROLOGICAL IDENTIFICATION OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM MONKEYS AFFECTED WITH COLIBACILLOSIS

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A biological products laboratory received monkeys from Ethiopia for the production of vaccine against the Heine-Medin disease. Four to six weeks since their arrival, colibacillosis was found in the animals. *E. coli* strains were isolated in an abundant and pure culture. As a result of serological studies 43.1% of the investigated strains were identified as belonging to serogroup O148, 16.7% to serogroup O157. The remaining serogroups occurred in small numbers. The isolated *E. coli* strains were pathogenic for white mice and slightly sensitive to neomycin alone. In the study attention is drawn to the role of serogroup O148 in the etiology of colibacillosis in monkeys.

In many countries, Poland including, monkeys serve as material for the production of vaccine against the Heine-Medin disease. The activities connected with the trapping of the animals followed by the prolonged time of transportation to their place of destiny evoke deep stresses which, among others, are manifested by a marked decrease of natural immunity to various diseases (10, 15). Enteric diseases caused by *Salmonella* and *Shigella* microorganisms appear frequently under these circumstances (1, 10, 12); yet reports indicating the occurrence of enteric diseases caused by *E. coli* are encountered rather infrequently (10, 12).

The aim of the present study was to investigate the etiological factor of diarrhoea in monkeys transported from Ethiopia to a biological products laboratory in Poland. For this purpose from the feces of the affected animals bacterial strains were isolated which were next subjected to biochemical and serological identification. The isolated strains were also studied in terms of their susceptibility to antibiotics and their pathogenicity for mice.

Material and Methods

The monkeys (*Cereophitecus aethiops*) came from Ethiopia. Prior to transportation to Poland, they had been examined by the tuberculin test for tuberculosis. At arrival at the biological products laboratory, they received two proventive doses of 3,000,000 units of debecillin, as well as polzomycin in drinking water for 5 days. The ages of the monkeys were estimated to be approximately 2 to 5 years. Four to six weeks since the arrival of the animals, there appeared diarrhoea. From the affected monkeys rectal swabs were inoculated on blood agar and on McConkey's medium. Single colonies were chosen from these media and cultured on nutrient agar. From each Petri dish 2 cultures were subcultured.

Biochemical studies. — The strains isolated on McConkey's medium, which looked like *E. coli*, were examined by Eijkman's test and after that on Kligler's medium, Christensen's medium, tryptophane broth. 10 per cent lactose broth, potassium nitrate broth and on Clark's medium. Only strains showing features typical of the *Escherichia* group were used for serological studies (19).

Serological studies. — In the isolated *E. coli* strains the O antigen was determined by a complete set of anti-O sera (O1-O157) according to the method described previously (2).

Antibiotic susceptibility. — The susceptibility of the isolated strains to antibiotics and sulphathiazole was determined by the use of paper discs. The standard discs were 7 in number (penicillin, streptomycin, chloromycetin, oxyterramycin, erythromycin, neomycin, sulphathiazole) and were produced by the Biological Products Laboratory, Warsaw. The studies were carried out in accordance with instructions received from that Laboratory.

The pathogenicity of the isolated strains of *E. coli* was determined on white mice of 18-20 g in body weight. They were inoculated with a 0.2-ml dose of 24-hour broth culture. The mice were observed for 7 days.

Results

The number of monkeys which had fallen ill was 44, that is about 10% of the total number of the received animals. They showed disturbances in the alimentary tract, most frequently in the form of acute diarrhoea. At the beginning the disease was catharrhal in form, but later it became haemorrhagic. The affected animals became sad and apathetic, they lost appetite and bristled their hair. The *E. coli* strains isolated from rectal swabs were abundant and pure cultures. Microorganisms of the *Salmonella* or *Shigella* genera were not found in any of the media used for isolation. Only in 2 cases single colonies were found besides *E. coli*. The affected monkeys were treated with chlorocide in a daily dose of 1 g administered up to recovery or death of the animal. Despite the treatment 13 monkeys died.

From the feces of all the affected monkeys 92 bacterial strains were isolated. Biochemical tests allowed 88 of the strains to be classified as belonging to the *Escherichia* genus, 1 being treated as atypical because of its lack of indole production. The results of serological studies of the 88 *E. coli* strains are presented in Table 1.

As follows from Table 1, 38 strains were identified as belonging to one O group, namely to O148 (43.1% of the total). Serogroup O157 ranked second in frequency of occurrence and included 14 strains, which constituted 16.7% of the studied strains. Strains of serogroup O98 were encountered 4 times, while the remaining groups were represented by 2 strains. Out of those serogroups O55, O25 and O119 are associated with

Table 1
Serogroups of *E. coli* strains
isolated from monkeys affected with colibacillosis

Serogroup	Number of strains	Percentage
O148	38	43.1
O157	14	16.7
O98	4	4.4
O9	2	2.2
O25	2	2.2
O30	2	2.2
O55	2	2.2
O119	2	2.2
O144	2	2.2
O145	2	2.2
unidentified	18	20.4

pathogenicity for man, and serogroups O9 and O30 are most frequently isolated from cattle.

The results of antibiotic susceptibility tests showed that the isolated *E. coli* strains were only weakly sensitive to neomycin and only 3 of them to chloromycetin. They were insensitive to all the other studied antibiotics and to sulphathiazole.

The *E. coli* strains isolated from the monkeys proved to be pathogenic for white mice. The latter died 3—5 days since infection, no matter whether the inoculated strains were serologically determined or not.

Discussion

From the performed studies it follows that in monkeys brought over from Ethiopia there appeared colibacillosis. This diagnosis was evidenced by the presence in the faeces of the affected animals of *E. coli* strains in pure and abundant culture, with a simultaneous absence of microorganisms of the *Salmonella* and *Shigella* genera which other authors (1, 10, 12) consider to be the cause of diseases in monkeys manifesting themselves in diarrhoea. The few *E. coli* strains isolated in such cases were treated by the authors (1, 10, 12) as merely accompanying flora. Serological studies of these strains allowed to classify them as associated with pathogenicity for man (O55, O119, O127).

The etiological role of *E. coli* seems to be brought out by the fact that 43 per cent of the isolated *E. coli* strains belonged to one serogroup, namely O148, which permits the supposition that it were strains belonging to this serogroup which played a role in evoking the disease. Such a conclusion is supported by data from literature (3, 5, 7, 8, 16) which indicate that, if a particular serotype is represented by such a high percentage, it usually plays an essential role in the disease in question.

As mentioned above, in this study almost one half of the strains

were identified as belonging to serogroup O148. The standard serotype O148:H28 was first described by Ørskova et al. (11) in 1972. The authors obtained it from the Salmonella Reference Laboratory, Central Public Health Laboratory, London, where it was isolated from soldiers stationed in barracks. Prior to that it was described by Rowe at al. (14) in 1970 as a pathogenic factor in a state which the authors called "travellers' diarrhoea". The presence of this serotype in almost one half of the affected monkeys indicates that colibacillosis in those animals was caused by the *E. coli* serotype originally associated with pathogenicity for man only. Reports of the recent years indicate, however, that this serotype is encountered in a number of animal species, as cattle, pigs, dogs, rabbits (9, 16, 17) though the occurrence is limited to sporadic isolated cases, not appearing in enzootic explosions.

Fourteen strains, i.e. 16.7% of the total, were identified as belonging to serogroup O157. The standard serotype O157:K88 was first described, similarly as in the case of O148:H28, in 1972. It was isolated from piglet diarrhoea (5, 18). This serotype has so far been encountered in the USA (6), Canada (7), Yugoslavia (13) and also in Poland (9).

In this study serogroup O98 was found in 4.4%. The group was reported upon by many authors both in this country (2, 9) and abroad with reference to pigs (8). Its presence was also established in industrial feed mixtures for pigs.

Out of the remaining determined serogroups occurring in small amounts (one strain per monkey) some were associated with pathogenicity for man (O55, O25, O119), some for cattle (O9, O30). Twenty per cent of the studied strains could not be serologically identified.

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