FINAL REPORT OF COMMUNICABLE DISEASES RESEARCH AND CONTROL PROJECT

(JAPAN-KENYA MEDICAL COOPERATION)

MARCH, 1985

JAPAN INTERNATIONAL COOPERATION AGENCY
(JICA)

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P R E F A C E

In response to the Government of Kenya, the Japan international Cooperation Agency(JICA) dispatched the implementation survey team which signed the Records of Discussion on the 7th of March, 1979, extending the technical cooperation for another five years.
The objectives of the Project were to conduct basic and preventive medical research activities on the prevailing infectious diseases, mainly diarrhea-oriented, in the fields of bacteriology, virology, and parasitology in the country, to apply the research works to the predesignated model areas in which any preventive measures could be pursued for better solution, and thus eventually to contribute to the improvement of people's welfare.

In Kenya it was by far indispensable to conduct basic medical research activities for the benefit of people's welfare. However, the shortage of manpower and malfacilitated lab conditions hampered from implementing the technical cooperation for the first and second year. Owing to the drastic efforts of the experts from Kenyan and Japanese sides, the basic medical research activities have gradually been activated to function and the knowledge and techniques have well been transferred to Kenyan specialits, who would be expected to promote the medical research activities including prevention and control by themselves.

This final report is the compilation of the scientific papers as the results of joint research activities during the cooperation period. I hope this report would be useful for the further progress of our medical cooperation in the future and at the same time, serve to strengthen the friendly relations between the two countries.

I avail myself of this opportunity to express my deep appreciation to Dr. Yoshiki AOKI, Former Team Leader, Professor of parasitology, School of Medicine, Nagasaki University, and others concerned for submitting the scientific papers to make this publication possible.

SHOSUKE SUENAGA
EXECUTIVE DIRECTOR,
JAPAN INTERNATIONAL
COOPERATION AGENCY

FOREWORD

Communicable diseases account for a considerable morbidity and mortality in developing countries, including Kenya. At the top of the list in the country are such diseases as diarrhoea, acute respiratory infection, malaria and intestinal worms. It was fitting therefore that technical cooperation between Kenya and Japan in medical research identified communicable diseases as the main theme.

Since the establishment of the Communicable Diseases Research and Control Project (CDRCP) under the auspices of the Kenya Medical Research Institute, we have witnessed considerable growth of research interest in diarrhoeal diseases, acute respiratory disease and schistosomiasis. Based on research conducted on these diseases, Kenyan counterparts have been trained both locally and in Japan. This effort has been greatly valued.

In the final analysis research must benefit the consumer who in our situation is the rural dweller. It has been gratifying to note that most of the research conducted by the CDRCP has been rural-based and has been of practical value through various interventions that have come about through research results.

The CDRCP lasted only five years. It, however, needs to be remembered that often it takes a long time for research to produce utilisable results. With this in mind we hope that future technical cooperation in medical research will be for longer than five years.

Finally I would wish to thank Japanese experts and Kenyan scientists who in one way or another were associated with the CDRCP for the commendable job they performed within a relatively short period.

PROF. M. MUGAMBI
M.B.Ch.B., Ph.D., Dip.Cardiol., FICA
DIRECTOR

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3.1.1.1

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR SCREENING ROTAVIRUS IN FAECES

Y. MAKINO[1*], L.N. MUTANDA[2], P.M. TUKEI[3] and E.O. LICHENGA[3]

[1]Department of Virology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[2] Medical Research Center, Kenya Medical Research Institute, Nairobi, Kenya

[3] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

The detection of rotavirus by ELISA in faecal specimens is described. The gamma globulin fraction of rabbit antiserum to Nebraska calf diarrhoea virus (NCDV) was used for coating polyvinyl microtiter plates. The gamma globulin fraction of rabbit antiserum to human rotavirus (HRV), labelled with horse-radish peroxidase (PO-anti HRV) was used as the conjugate.

The results of comparing this method with other ELISA methods and the indirect immunofluoresence (IIF) test, show that this method is as sensitive as the others certainly more sensitive than the IIF. It was found necessary to absorb the conjugate with human alimentary bacterial flora to remove false positive reactions.

ELISA is simple to perform, relatively cheap and can be used to test large numbers of specimens. These advantages make it suitable for the screening for rotavirus in developing countries where acute diarrhoea disease is a major public health problem.

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* Present Adress: Department of Virology, School of Medicine, Ryukyu University, Okinaea, Japan.

ニエリおよびモンバサ地区の小児のウイルス学的サーベイランス，糞便中のロタウイルス検出の月別推移

牧野芳太[1]，　松本一郎[2]，　千葉靖男[3]，
O.A. MOHAMMED[4]，P.O. OGAJA[4]，A.M. KIBUE[4]，J.M. MULI[4]，
and G.W. NAKITARE[4]

1　長崎大学熱帯医学研究所ウイルス学部門
（現：琉球大学医学部ウイルス学教室）
2　岩手医科大学医学部細菌学教室
3　札幌医科大学小児科学教室
4　Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

糞便中のロタウイルス検出のための免疫酵素測定法（ＥＬＩＳＡ）について検討した。ポリビニール製プレートに抗ＮＣＤＶγ-グロブリン（ウサギ）を固相化したものを用いた。標識抗体として抗ＨＲＶγ-グロブリン（ウサギ）をペルオキシダーゼで標識したものを用いた。このＥＬＩＳＡ法の感度は他のＥＬＩＳＡ（ＷＨＯのＥＬＩＳＡ，市販のロタザイムキット）とほぼ同じ感度であり，間接螢光抗体法より高い感度を示した。標識抗体を腸内細菌の抗原で吸収することにより非特異反応をおさえることができた。ＥＬＩＳＡ法は安価でしかも検査法が簡単であるので，下痢症が公衆衛生上重要な問題となっている開発途上国でのウイルスのスクリーニングには適した方法である。

East African Medical Journal
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3.1.1.2

VIROLOGICAL SURVEY OF CHILDREN IN NYERI AND MOMBASA MONTHLY SURVEY OF ROTAVIRUS IN FAECES

Y. MAKINO[1]*, I. MATSUMOTO[2], Y. CHIBA[3], O.A. MOHAMMED[4], P.O. OGAJA[4], A.M. KIBUE[4], J.M. MULI[4] and G.W. NAKITARE[4]

[1] Department of Virology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[2] Department of Bacteriology, School of Medicine, Iwate Medical University, Morioka, Japan

[3] Department of Pediatrics, Sapporo Medical College, Sapporo, Japan

[4] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

Detection of rotaviruses in the faeces of infants with gastroenteritis (G/E) and reference cases of non-G/E (5-years-old) were carried out in Nyeri (from September 1981 to September 1982) and in Mombasa (from July 1981 to September 1982), Kenya, using commercial enzyme-linked immunosorbent assay (ELISA) kit and ELISA provided by the authors. Total of 55(12.2%) out of 454 specimens in Nyeri and 151(20.4%) out of 741 specimens in Mombasa were positive for rotaviruses. In Nyeri, the highlands with rather cool environment, rotaviruses were detected every month except in July. The highest positive rate was observed in January 1982 (34.3%) followed by September 1981 (21.4%) of the specimens diagnosed as G/E. While in Mombasa, being at sea-level with a warm climate, rotaviruses were detected every month. The highest rate was observed in July (51.5%) followed by January (29.5%) of the specimens diagnosed as G/E. It was observed that rotaviruses were more commonly detected during dry seasons. Infants under 6-months-old were most commonly infected with this virus.

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* Present Adress: Department of Virology, School of Medicine, Ryukyu University, Okinawa, Japan.

糞便中のロタウイルスのスクリーニングのための免疫抗素測定法

牧野芳太[1]，　L.N. MUTANDA[2]，P.M. TUKEI[3]and
E.O. LICHENGA[3]

1　長崎大学熱帯医学研究所ウイルス学部門
（現：琉球大学医学部ウイルス学教室）
2　Medical Research Center, Kenya Medical Research Institute, Nairobi, Kenya
3　Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

５才以下の乳幼児の胃腸炎および対照患者の糞便検体からロタウイルスの検出をニエリ地区（１９８１．９～１９８２．９）およびモンバサ地区（１９８１．７～１９８２．９）でＥＬＩＳＡを用いて行なった。高地で比較的涼しいニエリ地区では，７月を除いて毎月ロタウイルスが検出された。１９８１．９および１９８２．１月に高い検出率を示した（各々胃腸炎患者の２１.４％および３４.３％）。一方低地で暑いモンバサ地区ではロタウイルスは毎月検出され，特に１９８２．１および６月に多く検出された（各々胃腸炎患者の２９.５％および５１.５％）。調査期間中，ニエリ地区では５５／４５４（１２.２％），モンバサ地区では１５１／７４１（２０.４％）が陽性を示した。ロタウイルスは乾期に多く検出される傾向が見られた。生後６カ月未満の乳児で最も検出率が高かった。

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3.1.1.3

VIROLOGICAL SURVEY OF ENTEROVIRUS AND ROTAVIRUS INFECTIONS IN KENYAN CHILDREN

I.MATSUMOTO[1], Y. MAKINO[2*], O.A. MOHAMMED[3], P.O. OGAJA[3] and J.M. MULI[3]

[1] Department of Bacteriology, School of Medicine, Iwate Medical University, Morioka, Japan

[2] Department of Virology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[3] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

* Present Adress: Department of Virology, School of Medicine, Ryukyu University, Okinawa, Japan

The results of virological survey of enterovirus and rotavirus infections in Mombasa and in Nyeri, Kenya, are reported. The incidence of entervirus infections increases at a climate of high temperature and high humidity. There was no dominant serotype among the isolates during the surveyed period, though Coxsackievirus B5 was isolated almost every month only in Mombasa. The incidence of rotavirus infection increased in dry season, and no regional difference between Mombasa and Nyeri were found.

Rotavirus is a major cause of infantile gastroenteritis, and is exclusively prevalent during colder months in temperate zones. However the situation in tropical or subtropical zones is not yet clear. On the other hand, enteroviruses are considered as one of the causes of gastroenteritis during summer in temperate zones as well as the causes of poliomyelitis, aseptic meningitis, herpangina, etc. Enteroviruses are considered as one of the causes of gastroenteritis in Kenya also.

This paper describes the results of virological and epidemiological survey of enterovirus and rotavirus infections in Kenyan children.

These surveys were performed in Mombasa and in Nyeri. Mombasa is the largest port in East Africa and its climate is tropical. The maximum mean monthly temperature is over 30C from November to April. Nyeri is a highland town close to the equator in central Kenya. The minimum mean monthly temperature falls to under 10C in January, and the maximum mean monthly temperature does not exceed 30C. In both areas there are two rainy seasons annually, the long rains in April and May and short rains in October and November.

Stool specimens were collected in the under fives from patients with gastroenteritis and, from a control group, with other diseases.

Stool specimens were suspended at 5 - 10% in phosphate buffered saline and were centrifuged at 2,000g for 20 minutes. The supernatants were harvested and stored at -20C or -80C until used.

For the isolation of enteroviruses, secondary baboon monkey kidney cells and MA-104 cells were used. The CPE agents were identified serotypes by microneutralization test using Schmidt's antisera pools and single antisera for enteroviruses. These antisera were kindly supplied by Dr. Minoru Hara, NIH, Japan.

Detection of rotavirus antigen was carried out by enzyme-linked immunosorbent assay using commercial kit (Rotazyme, Abbott Lab., USA).

The isolation of enteroviruses has been done on the specimens collected from June 1981 to March 1982 in Mombasa and from September 1981 to March 1982 in Nyeri (Fig. 1). Enteroviruses were isolated in 91 (19.0%) out of 480 children in Mombasa and in 24 (9.6%) out of 250 children in Nyeri. A clear regional difference in frequency of enterovirus infections was observed ($x^2 = 10.4$, $P < 0.005$). The association of gastroenteritis and enterovirus could not be found from the results compared with isolation rates of enterovirus from patients with gastroenteritis and with other diseases. Enteroviruses were isolated every month in both areas except in September in Nyeri. The highest positive rate was observed in November in both areas (32.4% in Mombasa and 30.3% in Nyeri). The incidence of

enterovirus infections was higher in the rainy than in the dry season.

Serotype identification of isolates was attempted from the strains that were collected until December 1981 (table 1). Polioviruses were isolated from only 9 (1.9%) out of 483 children. This was much lower rate than in the previous report[1]. This may indicate the effect of poliovirus vaccination.

The enteroviruses isolated consisted of various serotypes with a few strains, and could not be observed dominant serotype. However Coxsackievirus B5 was isolated almost every month in Mombasa during the survey period.

The detection of rotavirus antigen has been done on the specimens collected from June 1981 to April 1982 in Mombasa and from September 1981 to April 1982 in Nyeri (Fig. 2). Rotavirus was detected in 124 (21.7%) out of 572 patients with gastroenteritis, but in only 10 (4.4%) out of 247 control patients. The association of gastroenteritis and rotavirus was recognized ($x^2 = 39.2$, $P < 0.0001$). A regional difference in detection rates was not found between the two areas (22.0% out of 364 patients with gastroenteritis in Mombasa and 21.2% out of 208 patients with gastroenteritis in Nyeri). The highest detection rate of rotavirus from patients with gastroenteritis was observed in January (38.5%) followed by September (29.4%). The incidence of rotavirus infection had a tendency to increase in the dry season. This data is in agreement with previous reports[2-4].

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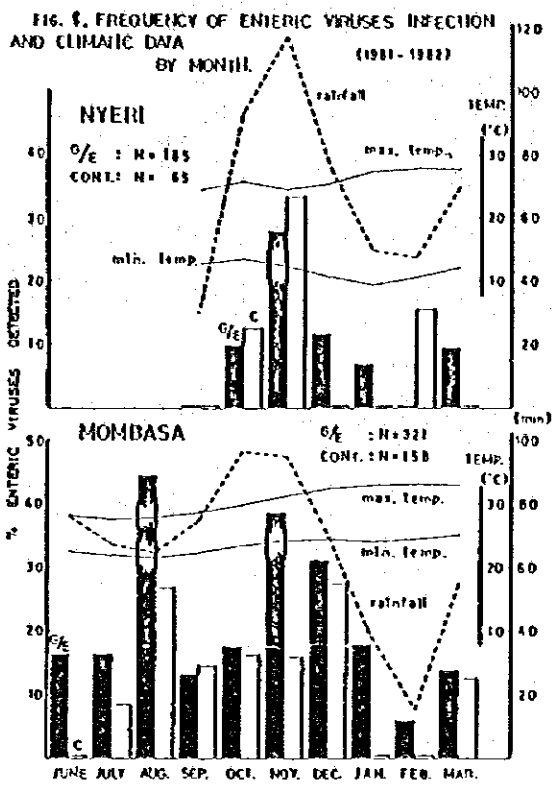

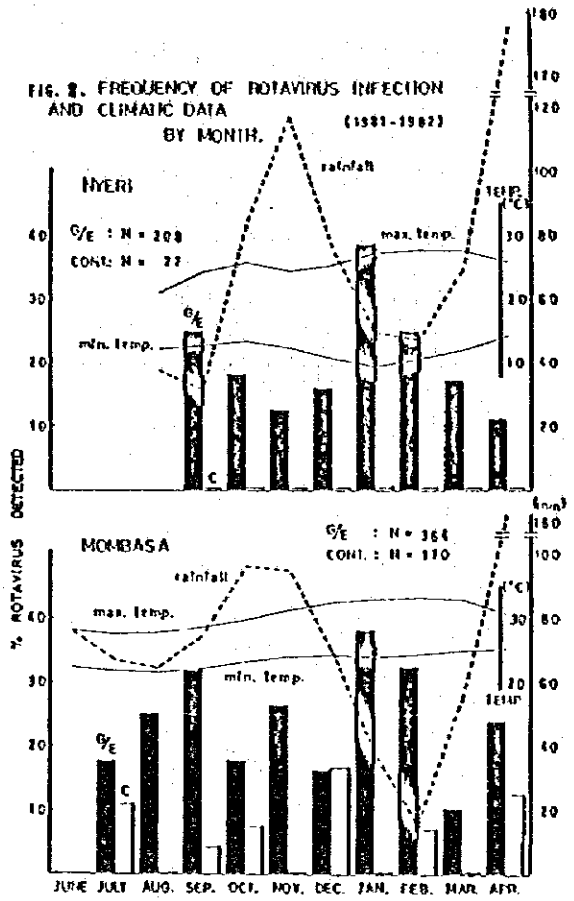

Table 1 Serotypes of isolates from fecal samples (1981)

Area	MOMBASA								NYERI				
Month	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Sept.	Oct.	Nov.	Dec.	Total
No. of samples	22	36	32	58	84	71	53	357	18	43	33	32	126
No. of positives	2	4	9	8	13	23	17	76	2	4	10	3	19
positive percent	9.1	11.1	28.1	13.8	15.5	32.4	32.1	21.3	11.1	9.3	30.3	9.4	15.1
Polio 1			1					1					
" 2			1	1		1		3	1		1		2
" 3			2	1				3					
Cox. A7						1*		1					
" A9					1		1	2					
" B1		1						1					
" B4						1		1					
" B5	1		1	1	3	3	3	12	1				1
" B6			1*		1	1	1	4					
ECHO 1		1				1		2					
" 2		1	1			1		3					
" 3										1			1
" 4										1			1
" 5				1				1					
" 6				1				1					
" 7							1	1					
" 9		1						1					
" 11						1		1					
" 15					1			1					
" 20						1		1					
" 25											1		1
" 30						2		2					
" 31				1				1					
" 33							1	1					
Unidentified	1			2	6	10	10	29	1	2	8	3	13
Adeno-like			2		1			3					

* a dual infection with rotavirus

ケニア国の小児におけるenterovirus及びrotavirus感染のウイルス学的サーベイランス

松本一郎[1]，牧野芳太[2]，O.A. MOHAMMED[3]，
P.O. OGAJA[3] and J.M. MULI[3]

1 岩手医科大学医学部細菌学教室
2 長崎大学熱帯医学研究所ウイルス学部門
（現：琉球大学医学部ウイルス学部門）
3 Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

ケニア国，モンバサとニエリにおいてenterovirusとrotavirus感染のサーベイランスを行ったので，その結果について報告する。Enterovirus感染は高温多湿の季節に増加した。調査中，特に流行を示した血清型はみられなかったが，Coxsackievirus B5はモンバサでのみ，毎月のように分離された。Rotavirus感染は乾期に多く，モンバサとニエリの間に地域差はみられなかった。

Rotavirusは乳幼児胃腸炎の主要病原体であり，温帯では殆んど冬期に流行する。しかし，熱帯あるいは亜熱帯での状況は未だ明らかにされていない。一方，enterovirusはポリオ，無菌性髄膜炎，ヘルプアンギーナなどの病原体であるのと同時に，温帯では夏期の胃腸炎の原因の一つと考えられている。ケニアにおいても同様に胃腸炎の原因となっているであろう。

本報告では，ケニアの小児のenterovirusとrotavirus感染のウイルス学的，疫学的調査結果を述べる。

調査はモンバサとニエリで実施されたが，モンバサは東アフリカ第一の港町で，熱帯性気候である。平均月別最高気温は11月から4月までは30°Cを越す。ニエリは赤道に近い高地の町で，平均月別最低気温が1月には10°C以下になり，最高気温は30°Cを越すことはない。両地とも雨期が2回あり，大雨期が4～5月，小雨期は10～11月である。

糞便は5才以下の胃腸炎患者と，対照として，その他の疾患の患児より採取した。

糞便はPBSで5～10%溶液にし，2,000g，20分遠心，その上清をテストまで−20°Cあるいは−80°Cに保存した。

Enterovirusの分離はsecondary baboon monkey kidneyとMA-104細胞を用いた。CPE agentはSchmidtの抗enterovirusプール血清と各型の抗enterovirus血

清を用いてmicro 中和試験で血清型別を行った。これらの血清は日本の予研，原 稔博士の御好意により分与を受けた。

Rotavirus 抗原の検出は市販のキット（ Rotazyme, Abbott Lab, USA)を用い，ＥＬＩＳＡ法で行った。

Enterovirusの分離は，モンバサでは１９８１年６月から１９８２年３月まで，またニエリでは１９８１年９月から１９８２年３月までに採取した糞便について実施した（ Fig. 1)。Enterovirusは，モンバサでは４８０名の小児中９１名（１9.0％）から分離され，ニエリでは２５０名中２４名（ 9.6％）から分離された。Enterovirus感染の頻度に明らかな地域差がみられた（ X^2＝１0.4, Ｐ＜0.0０５）。胃腸炎とEnterovirusの関連は，胃腸炎患者群と対照群からのウイルス分離率の比較からでは認められなかった。Enterovirusはニエリの９月を除き，毎月分離された。最も分離率が高かったのは，両地ともに，１１月であった（モンバサ３2.4％ ニエリ３0.3％）。Enterovirus感染は乾期よりも雨期に多かった。

分離株の血清型別は１９８１年１２月までに採取された検体から分離されたものについて試みた。（ Table 1)。Poliovirus は４８３名中９名（ 1.9％）に検出されたのみであった。これは先の報告よりも低値であった。[1] これはpoliovaccine の効果を示すのかもしれない。

分離株の血清型は多岐にわたり，数株ずつ分離され，主流の型はみられなかった。しかし，Coxsackievirus Ｂ５は，調査中，モンバサでのみ毎月の様に分離された。

Rotavirus 抗原の検出は，モンバサでは１９８１年６月から１９８２年４月まで，またニエリでは１９８１年９月から１９８２年４月までの検体につき実施した（ Fig. 2)。Rotavirus は胃腸炎の５７２名中１２４名（２1.7％）に検出されたが，対照群では２４７名中わずか１０名（ 4.4％）に検出されたのみであった。ここにRotavirusと胃腸炎との関連が認められた（ X^2＝３9.2, Ｐ＜0.０００１)。両地での地域差は認められなかった（モンバサの胃腸炎患者３６４名中２2.0％に検出，ニエリでは２０８名中の２1.2％に検出）。胃腸炎患者からのrotavirus 検出率は１月が最も高く（３8.5％）次いで９月（２9.4％）であった。Rotavirus の感染は乾期に増加する傾向がみられた。この成績は先の報告と一致する[2~4]。

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3.1.1.4

ROTAVIRUS INFECTION OF YOUNG CHILDREN IN TWO DISTRICTS OF KENYA FROM 1982 TO 1983 AS ANALYZED BY ELECTROPHORESIS OF GENOMIC RNA

Y. CHIBA[1], C. MIYAZAKI[2], Y. MAKINO[3*], L.N. MUTANDA[4], A. KIBUE[5], E.O. LICHENGA[5] and P.M. TUKEI[5]

[1] Department of Pediatrics, Sapporo Medical College, Sapporo, Japan

[2] Department of Virology, School of Medicine, Kyushu University, Fukuoka, Japan

[3] Departemnt of Virology, Institute for Tropical Medicine, Nagasaki university, Nagasaki, Japan

[4] Medical Research Center, Kenya Medical Research Institute, Nairobi, Kenya

[5] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

Employing techniques of polyacrylamide gel electrophoresis of viral RNA segments, we studied rotavirus strains and their relative contributions to rotavirus gastroenteritis epidemics in two major districts of Kenya. From early 1982 to the middle of 1983, 18 representative electropherotypes, including 6 short strains, were detected in 30 rotavirus specimens obtained from Nairobi, whereas 16, including 3 short strains, were detected in 70 virus specimens from coastal areas. With the exception of one strain, there were no identical electropherotypes between the two groups of rotaviruses obtained from these different districts. A change in predominant electropherotypes was observed in Mombasa in early 1983, and subsequently, newly occurring strains were detected in a small town along the coast when an apparent increase in gastroenteritis was observed in the district.

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* Present Adress: Department of Virology, School of Medicine, Ryukyu University, Okinawa, Japan

遺伝子ＲＮＡの電気泳動により調べた１９８２～１９８３年ケニア２地区の小児におけるロタウイルス感染

千葉靖男[1]，宮崎千明[2]，牧野芳太[3]，
L.N. MUTANDA[4], A. KIBUE[5], E.O. LICHENGA[5], and P.M. TUKEI[5]

1 札幌医科大学小児科学教室
2 九州大学医学部ウイルス学教室
3 長崎大学熱帯医学研究所ウイルス学部門
（現：琉球大学医学部ウイルス学教室）
4 Medical Research Center, Kenya Medical Research Institute, Nairobi, Kenya
5 Virus Research Center, Kenya Medical Research In titute, Nairobi, Kenya

ロタウイルス遺伝子ＲＮＡのポリアクリルアミドゲル電気泳動により，ケニヤの２大地区に存在するウイルス株と，胃腸炎の流行におけるそれらの相対的役割について調べた。１９８２年初期から１９８３年前半にかけ，ナイロビで得られた３０検体から１８の代表的泳動型が得られ，そのうち6パターンはSHORT タイプであった。一方，海岸地域の７０検体からは１６の泳動型が得られ，３パターンがSHORTタイプであった。これら２地区で得られた泳動型のうち，共通したものは１株だけであり，他は全て，異る型であった。１９８３年初期，モンバサ市内において優勢なロタウイルス株に変化があり，この新たに出現したロタウイルス株は，後に，海岸地域にある小集落の本症流行時に検出された。

Journal of Clinical Microbiology
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3.1.1.5

EPIDEMIOLOGY OF HUMAN ROTAVIRUS INFECTION IN COAST PROVINCE IN KENYA FROM 1981 TO 1983

C. MIYAZAKI[1], Y. MAKINO[2]*, I. MATSUMOTO[3], Y. CHIBA[4], H. TERASHIMA[4], S. SATO[2], P.O. OGAJA[5], C. ICHORO[5], J.M. MULI[5], A. KIBUE[5], G.W. NAKITARE[5] and P.M. TUKEI[5]

[1] Department of Virology, School of Medicine, Kyushu University, Fukuoka, Japan

[2] Department of Virology, Institute for Tropical Medicne, Nagasaki University, Nagasaki, Japan

[3] Departemnt of Bacteriology, School of Medicine, Iwate Medical University, Morioka, Japan

[4] Department of Pediatrics, Sapporo Medical College, Sapporo, Japan

[5] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

* Present Adress: Department of Virology, School of Medicine, Ryukyu University, Okinawa, Japan

During the period July 1981 to December 1983, a total of 1214 stool specimens were collected from children with gastroenteritis (G/E) in Mombasa. In Kilifi, 291 stool specimens were collected from November 1982. Sixty-three diarrhoeal stool specimens were collected in Mariakani.

In young children aged three years and under, 24.5% and 26.8% of G/E cases were rotavirus positive in Mombasa and Kilifi, respectively. Whereas, in Mariakani, only 9.5% were positive.. Highest detection rate of rotavirus was observed among age group less than one year. Monthly detection rate of rotavirus in G/E cases did not correlate with climatic data (temperature, relative humidity and total rainfall) in Mombasa.

From July 1982, we analysed genomic RNAs of rotavirus isolates by polyacrylamide gel electrophoresis. Generally the long type was predominant in Coast Province; however, we could observe the change of prevalent electropherotype from long type to short type at the end of the year 1983.

We examined 238 stool specimens emplpying virological and bacteriological methods in July, September and November 1983. In six specimens (2.5%), we isolated not only rotavirus but also pathogenic bacteria, as follows: 4 were enterotoxigenic E. coli (heat stable toxin positive), one Salmonella B and one Campylobacter jejuni.

ケニア共和国コースト州におけるヒトロタウイルス感染の疫学
——１９８１年より１９８３年まで——

宮崎千明[1]，　牧野芳太[2]，　松本一郎[3]，　千葉靖男[4]
寺嶋秀幸[4]，　佐藤成大[3]，　P.O. OGAJA[5]，　C. ICHORO[5]，
JM. MULI[5]，　A. KIBUE[5]，　G.W. NAKITARE[5] and P.M. TUKEI[5]

1　九州大学医学部ウイルス学教室
2　長崎大学熱帯医学研究所ウイルス学部門
（現：琉球大学医学部ウイルス学教室）
3　岩手医科大学医学部細菌学教室
4　札幌医科大学小児科学教室
5　Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

１９８１年７月より，ケニア共和国コースト州において，小児のロタウイルス性下痢症の疫学的研究が行なわれた。

モンバサ市においては，８１年７月から，８３年１２月までに計１２１４検体，キリフィ地区では，８２年１１月から８３年１２月までに２９１検体の下痢便が，小児胃腸炎患者より集められた。また，マリアカニ地区では，８３年６月から１２月までに６３の下痢便検体が集められた。

３才以下の年令では，下痢症患者の中の，ロタウイルスの陽性率は，モンバサで２４.５％，キリフィにおいて２６.８％であったがマリアカニでは９.５％と低率であった。ロタウイルスの陽性率が最も高いのは一才未満の年令層で，約３０％であった。特に，６ケ月から１１ケ月児において最も多くの陽性検体が得られ，１才以降，陽性数，陽性率とも低下した。

ロタウイルスの毎月の陽性率と，気象データ（気温，相対湿度，雨量）との間に，明らかな相関は認められなかった。しかし，流行には波が認められ，その周期は６ないし９ケ月と考えられた。

１９８２年以降，我々はロタウイルス核酸（１１本に分節した複鎖RNS）の電気泳動パターンによるisolatesの分類を行なった。コースト地区においては，全体としてlong typeが多く（約７０％）検出されたが，８３年下四半期においては，short typeが優性となった。とくにキリフィでは，８３年１月より８月までlong typeしか検出できなかったが，その後short typeが出現し，１２月には流行の主体を占めるに至った。

一方，８３年７月，９月，１１月に，計２３８検体の下痢便が，細菌学的にも検査され，６検体（２.５％）にロタウイルスと，病原細菌の混合感染が認められた。うちわけは，ST（Heat stable toxin)産生大腸菌が４株，Salmonella B群が１株，Campylobacter jejuniが１株であった。

3.1.1.6

THE ELECTRON MICROSCOPICAL STUDY OF ACUTE GASTROENTERITIS IN CHILDHOOD

H. TERASHIMA[1], C. MIYAZAKI[2], S. SATO[3], P.O. OGAJA[4], C. ICHORO[4] and G.W. NAKITARE[4]

[1] Department of Pediatrics, Sapporo Medical College, Sapporo, Japan

[2] Department of Virology, School of Medicine, Kyushu University, Fukuoka, Japan

[3] Department of Bacteriology, School of Medcine, Iwate Medical University, Morioka, Japan

[4] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

In Kenya studies of rotavirus infection have been carried out for several years. As a result of such studies much valuable data have been compiled. From the information so far available a vaccination trial for rotavirus in the near future would seen indicated. Other than rotavirus infection, we also have some information on other viral causative agents of acute gastroenteritis in this country.

First, we tried to investigate the antibody titers against calicivirus in Kenya. We sent 50 serum specimens collected in Kenya to Japan to detect antibody against-calicivirus using the method of radioimmuno assay. 76% of serum specimens contained antibody to calicivirus, so we are confident that calicivirus as a possible causative agents of acute gastroenteritis in this country.

We started to examine diarrheal stool specimens of children by electron microscopy from the end of October 1983.

Over the first ten years, several virus groups have been shown to be associated with gastroenteritis. These are adenovirus, astrovirus, calicivirus, coronavirus, Norwalk agent, Otofuke agent and rotavirus.

At the present time, human rotavirus is the best studied of all human gastroenteritis viruses and is clearly the major viral cause of gastroenteritis in children. But in recent years, direct examination of human faecal material has revealed numerous different small round virus-like particles,

The first of these small round virus-like particles to be detected was the Norwalk agent described by Kapikian et al in 1972. Apart from the Norwalk agent, only calicivirus and astrovirus have been established as etiological agents of acute gastroenteritis. Other many so-called small round viruses have not been clearly classified, because of their relative small sizes and lack of cell culture systems. We tried to demonstrate that in acute viral gastroenteritis in Kenya, other agents apart from rotavirus are also important.

MATERIALS AND METHODS

Stool specimens were collected from children with acute gastroenteritis in Coast Province, who were admitted to hospitals or came to out-patient clinics. All of the patients were under 10 years old. Immediate examination by Rotazyme was done and stool specimens were stored at -70°C until use. From these stored specimens we chose about 300 specimens which were negative by Rotazyme, which means these specimens are all negative of rotavirus infection. More than 2 grams of stool specimens was requied for examination by EM. Figure 1 shows the method of preparation for direct electron microscopy. Ten percent stool suspension in PBS was prepared and centrifuged at 8000g for 40 minutes. The supernatants were filtrated by filter paper No. 6 to remove debris and filtrates were ultracentrifuged at 100,000 g for 90 minutes by Beckman RP-40 rotor. Then, the supernatants were discarded and the pellets were kept over night in 3 or 4 drops of distilled water.

The pellet was resuspended by agitation. An equal volume of 2% phosphotungstic acid, pH 7.0 was added to the virus suspension and mixed. After 20 minutes, one drop of the mixture was layered on the carbon-coated collodion mesh and excess fluid was aspirated by filter paper. The specimen was then examined using JEM 100S electron microscope. The magnification used was between 25,000 to 40,000 and at least 10 squares of each grid mesh per specimen were examined.

RESULTS

Figure 2 shows the results of studies of Rotazyme negative faecal specimens from children with acute gastroenteritis. The details of the symptoms of acute gastroenteritis are not clear, but all the patients had diarrhea. Sixteen of the 300 children (5.3%) were excreting adenoviruses and nine children(3%) were excreting small round virus-like particles. In spite of their being negative for rotavirus by Rotazyme examination, rotaviruses were found in two specimens. One of these specimens was positive for both rotavirus and small round virus-like particles.

Of the about 300 specimens examined by Rotazyme and found negative for rotavirus, 25(8.3%) contained viruses or virus-like particles, other than rotavirus when examined by EM. Figure 3 shows an electron micrograph of the adenovirus particles. The white bar indicates 100 nm, and the diameter of these particles is approximately 75 to 80 nm.

Figure 4 shows adeno-associated viruses; the white bar indicates 100 nm. The diameter of these particles was 20 nm. We could find these adeno-associated viruses in only one specimen.

Figure 5 shows small round virus-like particles. These particles were observed as agglutinated mass. The surface structure of each particle is not clear but the surface is not smooth. Although the surface structure is not clear we can neverthless, find fine structures. The white bar indicates 50 nm, and the diameter of these particles is approximately 33 to 35 nm.

Figure 6 shows the same particle size as in the last electron micrograph, but the specimen is different from the former one. In this electron micrograph the surface structure is clearer than the other ones. We can see stain-filled surface hollows, and one hollow in the center is surrounded by other 6 hollows. In this specimen, particles existed almost separately.

DISCUSSION

We detected two types of viruses or viruse-like particles from stools of patients with acute gastroenteritis. One is adenoviruses and other one is small round virus-like particles. But it is not clear whether these two viruses or virus-like particles really play a role in acute gastroenteritis, because we have not excluded bacteriological causative agents and have not been able to get paired sera from patients. To confirm some virus or virus-like particles as causative agents, we must show the rise of antibody titer to these viruses in convalescent sera.

Adenoviruses are frequently observed in stools of patients with diarrhea as well as in faeces of asymptomatic persons. There is increasing evidence that adenoviruses play a significant role in gastroenteritis. Next to rotaviruses, they are the group most commonly found by EM. Murphy reported in 1981 that adenoviruses were found in 6% of faecal specimens of children with non-bacterial gastroenteritis throughtout the year in Australia. In our study 8% of specimens contained adenoviruses. It is generally said that F-group adenoviruses are a causative agents of acute gastroenteritis. We have not serotyped our isolates yet.

Many groups of the so-called small round viruses, have been reported by various investigators. In general small round viruses are the particles with diameter between 15 nm to 40 nm.

Figure 7 shows the scheme for grouping of small round faecal viruses as proposed by Appleton et al 1982. Other than these viruses several other particles have been reported. For examples, Otofuke agent group from Japan, mini-rotaviruses from Canada, so-called SRV from Norway. The small round virus-like particles observed in our study had a structure which was not clear. However, it seems it has some surface structures.

Figure 8 shows typical caliciviruses which were highly purified in Japan. Electron micrograph showing virus morphology when viewed along the two fold axis(square cross) five fold axis(ten spiked spheres) and threefold axis (star of David).

As we can see in the left photograph, generally the star of David configuration is a characteristic surface morphology of caliciviruses. Some of our small round virus-like particles resemble caliciviruses. But the particles seen in our study cannot be identified as caliciviruses by morphological criteria alone.

To identify the small round virus-like particles, the particles should be purified further and their antigenicity compared with that of other small round viruses by immune electron microscopy. Studies on buoyant density of the particles, analysis of their nucleic acids and capsid proteins, and attempts to adapt them to cell culture systems need to be carried out.

Generally, the symptoms of small round virus infection are rather mild compared to that of rotavirus infection. But these viruses sometimes play a role in outbreaks in closed populations such as schools or orphanages.
To control viral diarrhoeal diseases, we must assess the relative importance of these adenoviruses and small round viruses.as causative agents for acute non bacterial gastroenteritis.

Preparation for EM

10% (wt/vol) stoolsuspension in PBS

Low speed centrifugation 8000g for 40 min

Filtrate the supernatants with filter paper No. 6(Toyo Roshi)

Ultracentrifugation 100,000g for 90 min at $4^{o}C$

Resuspended pellets in 3 or 4 drops distiled water

Stain with 2% PTA(pH 7.0)

Figure 1

Results of studies of Rotazyme negative faecal specimens from children with acute gastroenteritis

Type of virus Found	Number of Specimens
Adenovirus	16(5.3%)
Small round Virus	9(3%)
total posiive	25(8.3%)
Rtavirus*	2
total negative	271

Figure 2

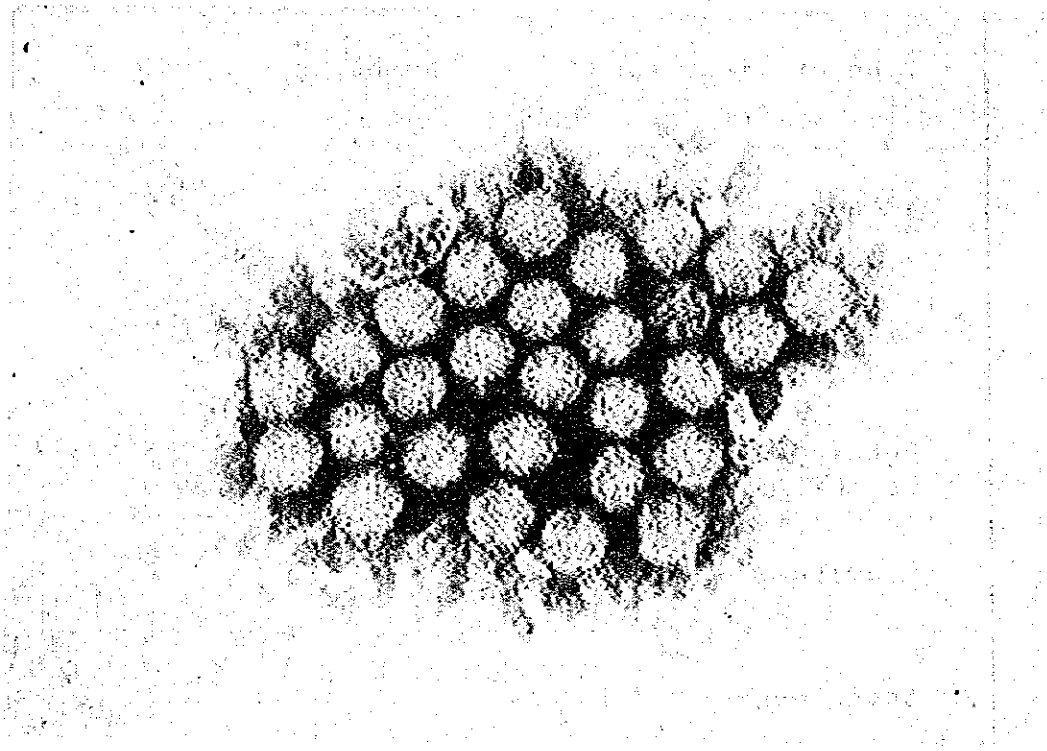


Figure 3

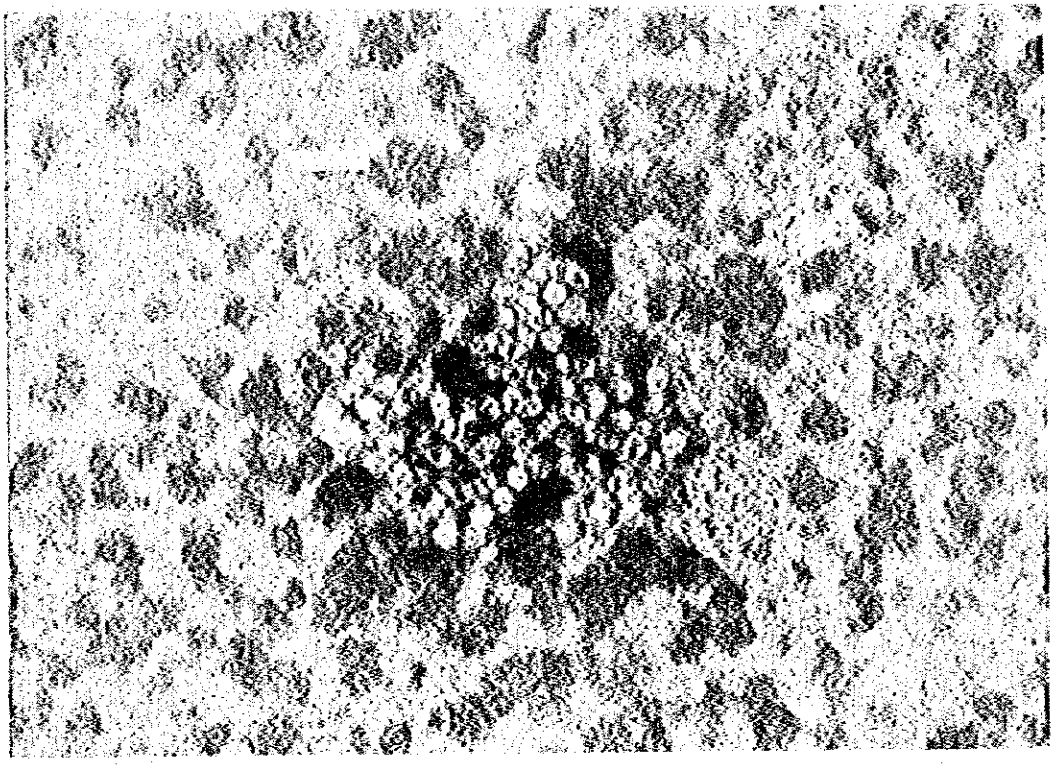
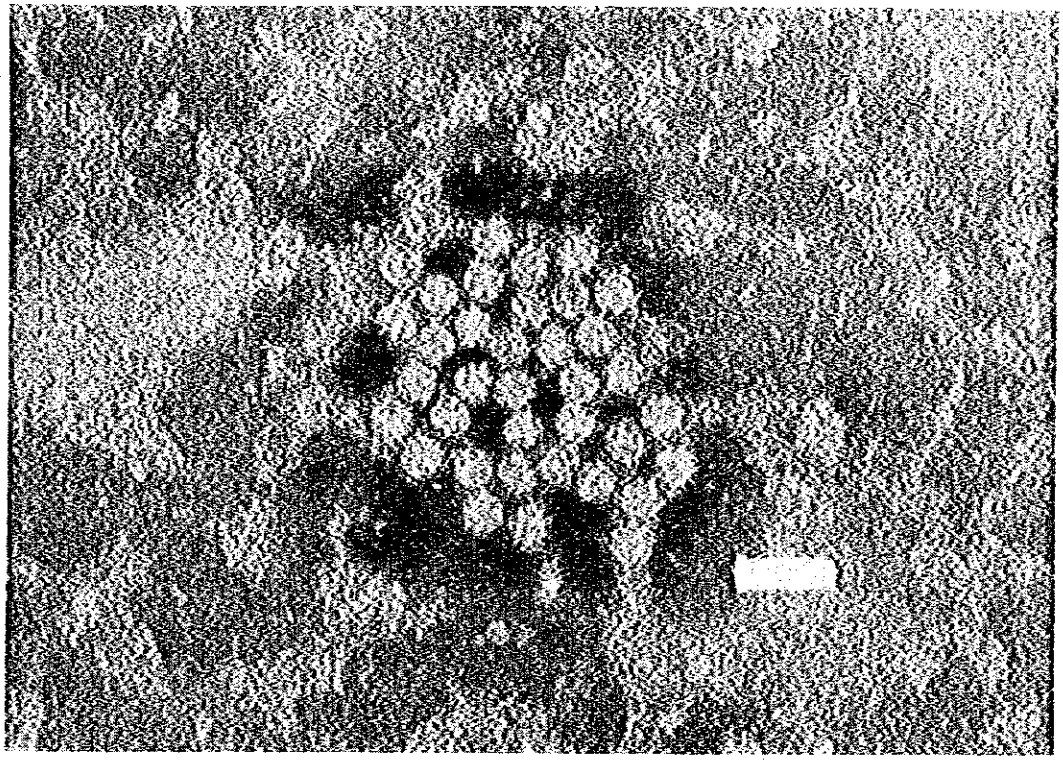
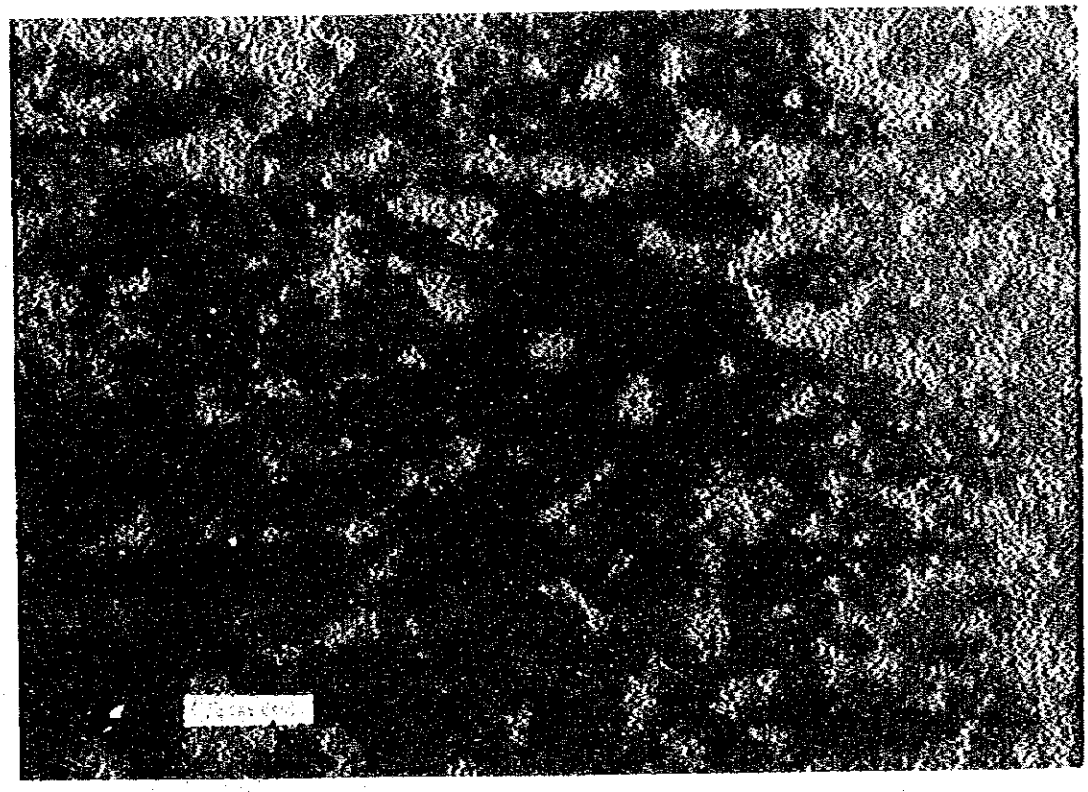


Figure 4





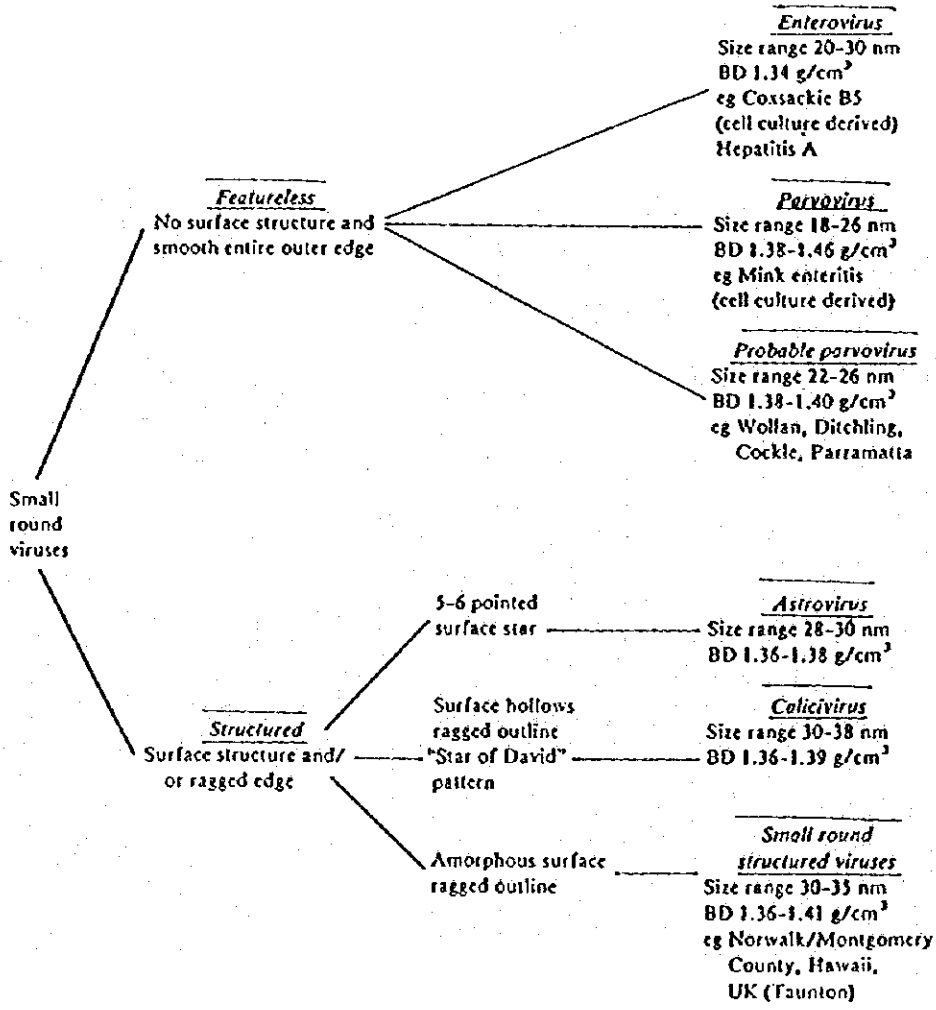

Small round viruses
Featureless
No surface structure and smooth entire outer edge
Enterovirus
Size range 20-30 nm
BD 1.34 g/cm³
eg Coxsackie B5 (cell culture derived)
Hepatitis A
Parvovirus
Size range 18-26 nm
BD 1.38-1.46 g/cm³
eg Mink enteritis (cell culture derived)
Probable parvovirus
Size range 22-26 nm
BD 1.38-1.40 g/cm³
eg Wollan, Ditchling, Cockle, Parramatta
Structured
Surface structure and/or ragged edge
5-6 pointed surface star
Astrovirus
Size range 28-30 nm
BD 1.36-1.38 g/cm³
Surface hollows ragged outline "Star of David" pattern
Calicivirus
Size range 30-38 nm
BD 1.36-1.39 g/cm³
Amorphous surface ragged outline
Small round structured viruses
Size range 30-35 nm
BD 1.36-1.41 g/cm³
eg Norwalk/Montgomery County, Hawaii, UK (Taunton)

Figure 7

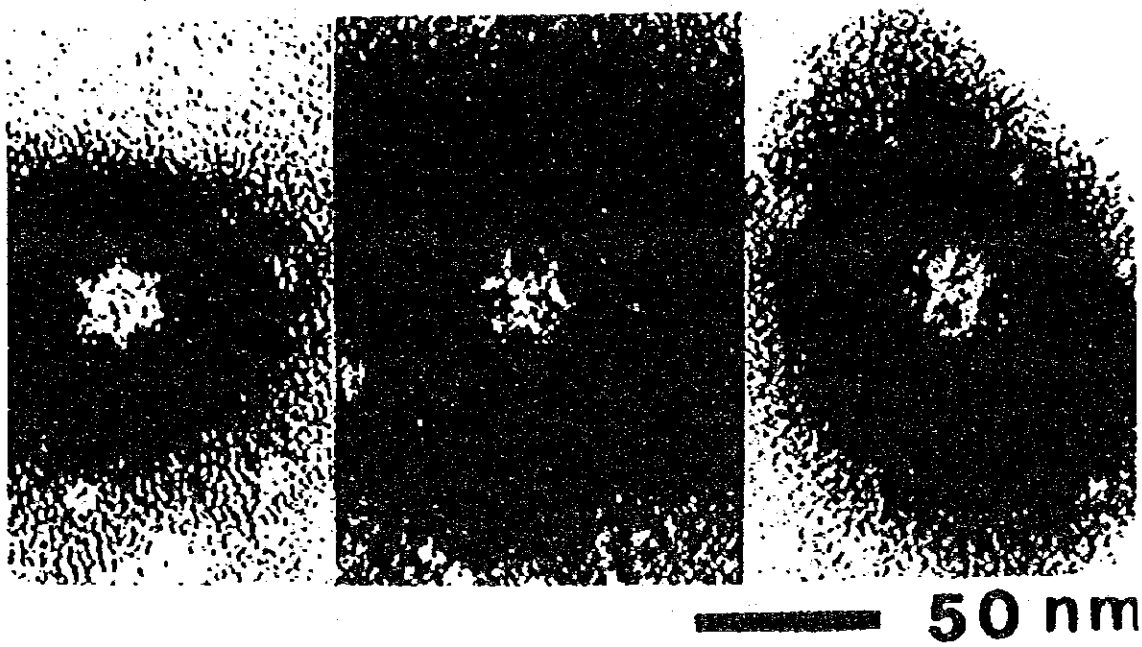


Figure 8

小児下痢症患児糞便の電顕的観察

寺嶋秀幸[1]，　宮崎千明[2]，　佐藤成大[3]，
P.O. CGAJA[4]，　C. ICHORO[4]　and G.W. NAKITARE[4]

1　札幌医科大学小児科学教室
2　九州大学医学部ウイルス学教室
3　岩手医科大学医学部細菌学教室
4　Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

ロタウイルス以外のウイルス性急性胃腸炎起因ウイルス及び候補ウイルスに関する報告はこの１０年来数多くなされている。現在迄，アデノウイルス，コロナウイルス，ノルウォーク因子，カリシウイルス，アストロウイルス，音更因子などがヒトのウイルス性下痢症の起因ウイルスとして知られているが，その他に数多くのウイルス様粒子が下痢症患者の糞便より電子顕微鏡によって検出されている。今回我々はケニアにおけるウイルス性下痢症の実態を明らかにする目的で，ロタウイルス以外のウイルス性下痢症の病因論的研究を行った。

材料と方法：モンバサを中心とするケニアのcoast地方で集められた下痢症患児の糞便より，ロタザイムによりロタウイルス感染症を否定された３００検体の糞便を対象とした。糞便は10％乳剤を低速遠心，化学濾紙，超遠心を用い約１００倍に濃縮しリンタングステン酸２ネガティブ染色をし，JEM 100S で鏡検した。

結果：１６検体よりアデノウイルスが検出された。アデノウイルスのtypingは行っていない。さらにこの内の2検体よりアデノの衛星ウイルスが検出された。３％に相当する9検体より直径３０～３５ nm 前後の小球型ウイルスが検出された。このウイルス様粒子は大部分の検体において大凝集塊として存在し，表面構造の観察は困難を極めた。我々はそのなかから粒子が孤立性に散在している検体を選び出し，注意深い観察を行ったところ，形態学的にカリシウイルスに類似した粒子を見出すことができた。しかし一部にはアストロウイルスとも思われる表面構造を呈している粒子も存在した。さらに詳細な表面構造の観察と，免疫電顕法（以下ＩＥＭと略記）によるカリシウイルス，音更因子との抗原性の異同を検討する目的でsucrose cushion を用いてさらなるウイルス様粒子の精製を試みた。しかし，ＩＥＭに用いる程の量の粒子の回収に失敗し，この試みは不成功に終った。さらに我々はケニアにおけるカリシウイルスの抗体保有率を調査する目的で，小児から成人に至る迄の各年令の血清検体５０を札幌医大小児科学教室へ送った。Radio immuno assay により抗体価が測定された結果，約７０％の

検体においてカルシウイルスに対する抗体が証明された。

考察：ケニアにおいてロタウイルス以外のウイルス性下痢症病原（又は候補ウイルス）ウイルスを検出し，同定するという初期の目的は残念ながら達成することはできなかった。しかしながらアフリカの地においても，small round virus 様粒子が存在するということを示し得たことは大きな意義があったと考える。近い将来ロタウイルスがvaccinationによりコントロールされた次の段階でのウイルス性下痢症における問題点は集団生活者におけるoutbreakのコントロールである。学校，乳児院においては，カリシウイルス，音更因子などのいわゆる小球形ウイルスが流行を起すことが知られている。これらのウイルスによる急性胃腸炎の症状は一般にはロタウイルスより軽症であるといわれているが，しかし音更因子などではロタウイルス感染症に匹適するなどの臨床症状を呈することもあることも報告されており，決して軽く見過すことのできない問題であると考える。電顕により直接患者糞便検体よりウイルス様粒子を検出し，その粒子の同定まで行うという作業は，様々なウイルス実験学的操作を含んでおり，この様なtechnologyの伝達はケニアにおけるウイルス学のlevel upに大きく貢献するであろうと考える。

3.1.1.7

PREVALENCE OF ANTIBODIES AGAINST ROTAVIRUS AND POLIOVIRUSES IN RESIDENTS OF COASTAL PROVINCES OF KENYA

S. SATO[1], C. MIYAZAKI[2], H. TERASHIMA[3], P.M. TUKEI[4], G.W. NAKITARE[4], P.O. OGAJA[4] and C. ICHORO[4]

[1] Department of Bacteriology, School of Medicine, Iwate Medical University, Morioka, Japan

[2] Department of Virology, School of Medicine, Kyushu University, Fukuoka, Japan

[3] Department of Pediatrics, Sapporo Medical College, Sapporo, Japan

[4] Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya

Serum antibody against rotavirus (simian rotavirus 11) and 3 serotypes of poliovirus in residents of coastal provinces of Kenya were examined by enzyme linked immunosorbent assay (ELISA) and neutralization test (NT), respectively. Sera were obtained directly from patients of the Coast Province General Hospital in Mombasa, Kenya, from December 1983 to Feburaly 1984. These sera were part of the blood collection for clinical examination in the hospital laboratory.

Prevalence of rotavirus-antibody: Percentage of anti-rotavirus antibody positive sera in different age groups is shown in Table 1. Sixty % of infants under 1 year of age (4 - 12 months) had antibody to rotavirus. The rate of sera with anti-rotavirus antibody was found to increase to 100% at 1 to 2 years of age. Then, the antibody-positive ratio fell off gradually after 3 years of age. In these areas, most of the children seemed to be infected with rotavirus until 1 to 2 years of age. The decreased serum antibody level to rotavirus, as observed in older age groups (over 4 years of age), seemed to suggest an important role of local immunity in the intestine for protection against rotavirus infection.

Prevalence of polio-antibodies: Percentage of sera with anti-poliovirus antibody in each age groups is also shown in Table 1. Antibody-positive ratio increased age by age and almost 100% of the persons over 8 years of age acquired immunity to all 3 serotyes. However, in seroepidemiology, quite a few children under 8 years of age are not immunized against polio-virus in the coastal areas of Kenya. Therefore, a proper vaccination system against pollovirus should be considered in these areas.

Table 1

Anti-rotavirus antibody detected by ELISA and neutralizing antibody to poliovirus Type 1,2,and 3 in different age groups

Age Group	% with anti-rotavirus antibody	% with anti-polio 1 antibody	% with anti-polio 2 antibody	% with anti-polio 3 antibody
4-12 months	60 (3/5)	40 (2/5)	80 (4/5)	40 (2/5)
1 year	100 (10/10)	70 (7/10)	80 (8/10)	40 (4/10)
2 years	100 (7/7)	71 (5/7)	71 (5/7)	71 (5/7)
3 ,,	80 (4/5)	40 (2/5)	80 (4/5)	80 (4/5)
4-7 ,,	50 (5/10)	90 (9/10)	100 (10/10)	30 (3/10)
8-17 ,,	29 (2/7)	100 (10/10)	100 (10/10)	100 (10/10)
18-25 ,,	20 (1/5)	100 (10/10)	100 (10/10)	90 (4/5)

ケニア国沿岸地方住民におけるロタウイルスおよびポリオウイルス1，2，3型に対する血清抗体調査

佐藤成大[1]，　宮崎千明[2]，　寺嶋秀幸[3]，　P.M. TUKEI[4]，
G.N. NAKITARE[4]，　P.O. OGAJA[4]　and C. ICHORO[4]

1　岩手医科大学医学部細菌学教室
2　九州大学医学部ウイルス学教室
3　札幌医科大学小児科学教室
4　Virus Research Center, Kenya Medical Research Institute
　Nairobi, Kenya

モンバサを中心とした，ケニア国沿岸地方の住民について，ロタウイルスおよびポリオウイルス1，2，3型に対する血清抗体の保有状況を調査した。ロタウイルスに対する抗体はＳＡ11を抗原としたＥＬＩＳＡ法により測定し，ポリオウイルスに対する抗体は，Sabin株(1，2，3型)を用い中和法により測定した。血清は１９８３年１２月から，１９８４年２月までの３ケ月間に，モンバサのCoast Province General Hospitalの採血室および小児科病棟において，直接患者から採取した。これらの血清は臨床検査用に採血したものの一部である。

抗ロタウイルス抗体の保有率は４～１２ケ月の乳幼児で６０％（３／５），１～２才の小児で１００％（１７／１７）に達し，この地方にロタウイルスが広く浸淫していることがわかった。ポリオウイルスに対しては，８才をすぎるとほぼ１００％の者がどの型に対しても抗体を獲得していたが，〔１型：１００％（２０／２０），２型：１００％（２０／２０），３型：９３％（１４／１５）〕，７才以下では１型に対する抗体の保有率は６８％（２５／３７），２型は８４％（３１／３７），３型は４８％（１８／３７）と，抗体陰性のものがかなり存在することが判明した。従って今後，より適切な予防接種の実施が望まれる。

3.1.1.8 Summary and Recommendation

Yasuo CHIBA

Department of Pediatrics, Sapporo Medical College, Sapporo, Japan

Childhood mortality in Kenya is high and infantile mortality rate reaches 10% on an average. Although there are a number of medical problems ralated to childhood health, such as general hygiene and malnutrition, communicable diseases are still a major cause of their death.

Viruses infecting respiratory tract such as measles virus and respiratory syncytial virus (RSV) are well known to induce more severe illnesses in young and malnourished children. The initial research activity of the Kenya/Japan communicable diseases research and control project (CDRCP) has been involved in establishing a rapid diagnosis for the identification of viruses which cause febrile upper or lower respiratory tract illnesses. The results suggested ubiquitous distribution of RSV, adeno and parainfluenza viruses among the child population.

Diarrhoeal illnesses are also an important and an urgent problem for childhood health in this country and the CDRCP concentrated research activity on the epidemiological survey of enteric virus infection from 1981 to 1984. Although a number of viruses including picornavirus were identified, human rota virus (HRV) was assigned as the most important pathogen for this illness.

As determined by enzyme-linked immunosorbend assay (ELISA)

to detect HRV in stool specimens, the infection was observed monthly with the maximum incidence of 34%(Nyeri) and 50%(Mombasa) among children aged less than 3 years old. The HRV infection was found less often in rainy (cold) seasons during this period, although it was detected in every month in which the study was conducted. It is possible that the alteration in the incidence of HRV infection in rainy seasons may simply reflect the increase of diarrhoeal illness due to bacteria in the population. However, more detailed studies will be necessary to determine factors related to these alterations in the incidence of HRV infection.

Poly acrylamide gel (PAG) electrophoresis of rota viral genomic RNA is useful for the identification of HRV subgroups and also for distinguishing one strain from another. Specimens obtained from the coastal district and Nairobi were employed for this analysis and it was found that these HRV strains consisted of both subgroup 1 and 2, with the strains of the later subgroup being detected more frequently. Long term observations for the oc ence of new HRV strains and their sequential changes may be useful for selection of an HRV vaccine which may be available in the near future.

An epidemiological survey was extended to a rulal area, the Kilifi district, which has a relatively low population but a high infantile mortality. HRV infections were detected even in this small community, with a manner somewhat different from that of the large city. Comparative analysis using PAG electrophoresis

of viral RNA have demonstrated that strains detected in epidemics of HRV in Kilifi were those which appeared initially in Mombasa, nearly a month before. Thus, it was suggested that there is a close association between an epidemic of HRV infection in a small village and those in a neighboring large city.

Viral diarrhoeal illness may be caused by non HRV agents. However, no investigation so far has been made for these agents in this country. The CDRCP installed an electlon microscopy and studies for the detection of non HRV diarrhoeal illness were initiated in 1983. Among HRV-negative stool obtained from the coastal area, calici-like particles were detected in 3% of the specimens and adeno virus particles were also detected in 8%. This may be the first observation of the small-round diarrhoeal viruses in East Africa. The relative importance of these agents among childhood viral diarrhoeal illness remains to be elucidated.

From all these results, it is apparent that HRV is the most important agent as a cause of viral diarrhoeal illness in infants and young children regardless of geographical differences. However, these studies are rather preliminary for the disclosure of all aspects of the HRV infection in Kenya.

It is likely that the importance of HRV in childhood diarrhoeal illness will be gradually recognized in Kenya. Although final control of HRV infection could be achieved by active immunization of children with an appropriate vaccine which may be available in

the near future. The present situation in this country requires prompt action in order to cope with the infection. Extension of the research activity on HRV infection is nesessary in both recruitment of manpower and areas of investigation. A long term regular surveilance of HRV infection, and more clinical or hospital based studies are mandatory for the wider recognition of this illness in pediatric practice. Thus, the research hereafter preferably should have two aspects; one as a laboratory service and another for establishing basic data for the future trial of HRV vaccine.

Specific research subjects should include;

—— A long term and extensive epidemiological survey of HRV infection in terms of morbidity and mortality. Influence of socioeconomical status, mode of living, and geographical differences should also be evaluated.

—— Transmission of HRV between communities. An outbreak of HRV infection in a rural area may be associated with an epidemic in a neighboring large city.

—— Detailed information on types and subgroups of HRV prevailing in communities and their sequential changes. PAG analysis of HRV genomic RNA and monoclonal antibody to serotype specific antigens should be employed for these purposes.

—— Clinical evaluations for host factors related to aggravation

of HRV infection, such as the influence of nutrition, anemia and other underlying illnesses. Evaluation on the methods of feeding infants may also be important.

—— Studies of non rota diarrhoeal viruses, such as calici and adenovirus are necessary for an accurate estimation of viral infections among various diarrhoeal illnesses.

For these purposes, it is necessary to establish a research center which has branch laboratories in several districts or in each province, if possible. The major activity of the center laboratory would be to make a detailed analysis of specimens and centralize the information obtained from branch laboratories, in order to give appropriate advice to a relevant ministry which is responsible directly for medical practice. The center laboratory should have enough equipment and manpower to make progress in research activity. Education of research technologists, specialized in viral diarrhea, who will be directed to branch laboratories is also an important role of the research center. The branch laboratories should have close communication with the center laboratory in every aspect of activity. However, great effort should be exercised on responding to the local needs for the diagnosis of HRV infection and on obtaining information regarding epidemics of diarrhoeal illness in local areas. Such laboratory services should greately enhance the wider recognition of these illnesses.

まとめと助言

千葉靖男
札幌医科大学小児科学教室

ケニアにおける小児死亡率は高く，乳児の場合，平均して１０％に達する。これらの根底には，一般的な衛生状態，あるいは低栄養など，多くの医学的問題があるが，伝染性疾患は依然として，その最も大きな原因である。

麻，respiratory syncytial (RS)ウイルスなど，呼吸器疾患ウイルスが幼若乳児，あるいは低栄養乳児において，より重篤な感染を引起こすことは良く知られている。したがって，当プロジェクトは始め，このような呼吸器ウイルス性疾患の迅速診断の確立に努めた。また，これらの結果から，ＲＳ，パラインフルエンザ，およびアデノウイルスが小児の間に広く浸透していることが明らかとなった。

一方，下痢性疾患もまた，ケニアにおける小児の健康上，重要，且緊急を要する問題である。我々は１９８１年から１９８４年迄の間，この点についての研究を集中的におこなった。下痢疾患児の便についてのウイルス学的検索では，ピコルナウイルスを始めとして多くのウイルスが検出されたが，ヒトロタウイルス（ＨＲＶ）が最も重要な病原体であることが明らかとなった。

即ち，３才未満の下痢症小児につき酵素抗体法（ＥＬＩＳＡ）により便中のＨＲＶの検索をおこなったところ，ほぼ毎月ＨＲＶ感染が認められ，ニエリでは最高３４％，モンバサでは５０％の頻度に達した。なお，ＨＲＶ感染は，この期間，雨期にいく分減少する傾向がみられたが，この原因は現在のところ明らかではない。細菌性下痢症の相対的増加もその理由の一つとして考えられるが，今後の検討が必要である。

ＨＲＶの遺伝子ＲＮＡのポリアクリルアミドゲル（ＰＡＧ）電気泳動はＨＲＶのsubgroupの同定，および，あるＨＲＶ株の他の株からの識別に極めて有用である。海岸地域，およびナイロビで得られた株につきＰＡＧ分析をおこなったところ，両地区においてＨＲＶ subgroup１および subgroup２の存在が確認された。また，これらの地域では subgroup２のＨＲＶが相対的に多いことも明らかとなった。これらの点につき，今後，長期にわたり観察し，その変化を明確にすることは，将来投与されるかも知れないＨＲＶワクチンの選定にとって貴重なデーターとなり得るものと考えられる。

ＨＲＶについての疫学的検索を，さらに，人口が比較的少なく，且，乳児死亡率の高い地域（キリフイ村）にまで広げてみた。この地域においてもＨＲＶ感染が検出されたが，その流行様式は多少，大都市のそれとは異なっているように思われた。また，ＰＡＧの分析で見ると，この村におけるＨＲＶの流行は，その１ケ月前，モンバサ地区で新に出現したＨＲＶ株により

発生したことが明らかになった。つまり，小集落におけるＨＲＶ流行は隣接する大都市のＨＲＶ感染の流行と密接な関係にあることが明らかとなった。

ウイルス性下痢症はＨＲＶ以外のウイルスによってもおきるが，ケニアにおいて，この点についての研究は全くなされていなかった。当プロジェクトでは電子顕微鏡の設置後，非ＨＲＶ下痢症の研究を開始した。その結果，海岸地域で得られたＨＲＶ陰性検体のうち，３％にカリシ様ウイルス粒子が，また，８％にアデノウイルスが検出された。これは小球形下痢ウイルスについての東アフリカで始めての知見である。ウイルス下痢症のうちで，これらの占る割合，および重要性については今後，さらに検討される必要がある。

以上の結果から，地域的な違いにかかわらず，ＨＲＶが乳児および幼児下痢症の原因として重要な位置を占ることは明らかであるが，未だ，当国におけるこの下痢症の全ての問題点の解明には到っていない。

小児ＨＲＶ感染の重要性は今後，さらに，広く認識されるであろうと思われる。ＨＲＶ感染の最終的コントロールは，将来，適当なワクチンの使用により達せられる望みもあるが，ケニアの現状を見た場合，早急に何んらかの対処策を講じる必要がある。ＨＲＶについての研究の拡大，研究員の増員，あるいは対象地域の拡張などが，当面の課題となる。また，長期にわたるサーベイランス事業，あるいは，より臨床に密接した研究も必要と考えられる。これらは小児科臨床における本症の重要性認識のためにも不可欠である。したがって，今後のＨＲＶ感染の研究は，このような直接的なサービス業務としての面と，将来におけるワクチン投与のための基礎データー確立の相方を目的とすることが望まれる。

具体的には以下の研究テーマが含まれるべきであろう。

――長期，且広範な疫学的サーベイランスにより，本症の罹患率，および死亡率を明らかにする。社会経済的要因，生活様式，および地域差の影響についての研究。

――ＨＲＶの地域間の伝播について。例えば，小集落における本症流行が隣接する大都市におけるそれと密接な関係にある可能性が存在する。

――地域において流行しているＨＲＶの subgroup，および血清型とその経時的変化をPAG電気泳動，あるいは単クローン抗体を用いた血清反応による解析する。

――ＨＲＶ感染重篤化に関係する因子の解明，特に，栄養状態，貧血，あるいは他の基礎疾患について。また，乳児の栄養法と本症の臨床経過との関係についても，今後，検討される必要がある。

――カリシ，あるいはアデノウイルス等，非ＨＲＶ因子のウイルス下痢症において占る割合についての研究。

このような研究目的遂行のため，下痢症研究センターの設置と，それに所属する地方機関の設置を提唱する。センターの主要な活動は送付された検体についてのより高度なウイルス学的検索であり，また，情報の収集，および，直接医療に関係する省庁への助言にある。したがって，このような活動を推進する上で，研究センターは充分な資材と，研究者を確保する必要がある。また，地方機関に配属される下痢症研究者の育成も大事な課題である。一方，地方機関はセンターと連携を保つことは当然であるが，実験室診断を通じて，直接，地域医療に貢献するとともに，各地域のＨＲＶ感染の流行を正確に把握するよう努める。このような活動を通じて，本症の重要性をさらに広く認識させることが可能であると思われる。

3.1.2.1

VIBRIO CHOLERAE O1 ISOLATED IN KENYA

M. IWANAGA[1*], K. MORI[1**] and J N. KAVITI[2]

[1] Department of Bacteriology, Institute for Tropical Medicine, Nagasaki University, Nagasaki , Japan

[2] National Public Health Laboratory Service, Nairobi, Kenya

Biological and serological analyses of 272 isolates of *Vibrio cholerae* O1 from six epidemics and from a few sporadic cases in Kenya were carried out. All of the isolates were identified as *V. cholerae* biotype El Tor, and 210 out of 272 isolates were hemolytic as examined by Feeley's method.

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* Present Adress: Department of Bacteriology, Ryukyu University, School of Medicine, Okinawa, Japan

** Present Adress: 2nd Department of Internal Medicine, Nagasaki University, School of Medicine, Nagasaki, Japan

ケニア国分離 Vibrio cholerae 01

岩永正明[1*]，森　賢治[1**]，J.N. Kaviti[2]

1　長崎大学熱帯医学研究所病原細菌部門

*現：琉球大学医学部細菌学教室

**現：長崎大学医学部第２内科学教室

2　National Public Health Laboratory Service, Nairobi, Kenya

１９６０年代初期から始まった第７回コレラパンデミーは未だに終息しない。その原因菌であるエルトールコレラ菌の性状の一部，特に溶血性や溶原性に変化が認められてきている。これまでアジア地域で分離されたものの性状はよく報告されているが，アフリカ分離株についての情報は少い。本報では１９８０年，１９８１年にケニア国で６流行例と散発例から分離した２７２株の性状を述べる。

５流行例では数株の稲葉型と１株の彦島型を含むものの小川型主流の流行であり，１流行は稲葉型によるものであった。全株ファージN抵抗性で，２株がポリミキシンB感受性を示し，１２株がVP反応陰性であったが，すべてエルトール型と判定した。

Feeley法による溶血性は，１４２株で完全溶血，４９株が溶血，１９株が弱溶血を示し，62株が非溶血であった。弱溶血を除外しても分離株の７０％が溶血を示しているのは東南アジアでの分離株の多くが非溶血であるのと異なっている。教室に保存されていた１９７５年ケニア国分離の６４株を試験したところ，弱溶血を除く溶血株は１１％で，今回の分離株のうち１９８０年のもので５４％，１９８１年８６％と増加傾向が認められた。

カッパファージ溶原性および同感受性による型別では５流行よりの株の９０％以上が溶原性セレベス型で，稲葉型による１流行では６６株中６５株がcured typeであった。全体としてUbol型が８株みられた。これも近年の東南アジア分離株の多くがcured typeであるのと異なっていた。

アモキシシリン，クロラムフェニコール，リファンピシン，ストレプトマイシン，テトラサイクリンに対しては全株感受性であったが，テトラサイクリンが多用されているので，その耐性菌の出現に注意する必要があると思われた。

Journal of Clinical Microbiology

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3.1.2.2

THE ROUTES OF CHOLERA SPREADING IN KENYA

M. IWANAGA[1,*], K. MORI[1,**] and J.N. KAVITI[2]

[1] Department of Bacteriology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[2] National Public Health Laboratory Services, Nairobi, Kanya

The routes of cholera spreading in Kenya was guessed by the type classification of *Vibrio cholerae* isolated in 1980 and 1981. The strain classification was made by serological type and phage-prophage type. Three main routes of spreading, from Turkana to the South along the Rift Valley, from Western Kenya around lake Victoria to highland cities and from the South Coast to highland cities, were suspected.

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* Present Adress: Department of Bacteriology, Ryukyu University, School of Medicine, Okinawa, Japan

** Present Adress: 2nd Department of Internal Medicine, Nagasaki University, School of Medicine, Nagasaki, Japan

ケニア国内におけるコレラの伝播経路

岩永正明[1*]，　森　賢治[1**]，　J.N. KAVITI[2]

1　長崎大学熱帯医学研究所病原細菌学部門
（*現：琉球大学医学部細菌学教室）
（**現：長崎大学医学部第２内科学教室）
2　National Public Health Laboratory Services, Nairobi, Kenya

１９８０年および１９８１年にかけてケニア各地で分離したコレラ菌の型別によって，ケニア国内におけるコレラの伝播経路を推定した。菌の型別は抗血清およびカッパファージによって行なった。その経過３つの主要経路が推定された，即ちツルカナ地区からリフトバレーに沿って南下する経路，ビクトリア湖周辺部から中央高原都市へ，また南部海岸地区から中央高原都市へ至る経路である。

熱帯医学　２３巻４号，２１７－２２２頁，１９８１年

3.1.2.3

BACTERIOLOGICAL STUDY ON THE DIARRHOEAL DISEASES IN KWALE DISTRICT, COAST PROVINCE, KENYA

A. UTSUNOMIYA[1], K. MORI[1,*], T. HAYASHI[1,*], M. IWANAGA[1,**], T. NAITO[1], Z.W. GATHERU[2], F. SANG[2], N. KOSKE[2] and J.N. KAVITI[3]

[1] Department of Bacteriology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[2] Kenya Medical Research Institute, Nairobi, Kenya

[3] National Public Health Laboratory Service, Nairobi, Kenya

The aim of this study is to clarify the etiology and ecology of diarrhoeal diseases in Kwale District, Coast Province, Kenya. Nine hundred and ninety-nine diarrhoeal specimens were collected during the period between October 1981 and July 1982, and they were bacteriologically examined. The isolation rates of enteropathogens were as follows: 22.8% for *Shigella*, 8.0% for enteropathogenic *Escherichia coli* (EPEC), and 3.5% for *Salmonella*. *Vibrio cholerae* and *Vibrio parahaemolyticus* were not isolated during this period. The isolation rates of the pathogens got high during the rainy season, such as 42.1% in April, 57.5% in May, and 53.0% in June. *Shigella* was the most predominant pathogen, and EPEC was isolated with relativery high frequency especially in October, March and June. In the bloody diarrhoeal stools, *Shigella* was isolated in 76.0% (124/163) while EPEC and *Salmonella* were 4.9% and 2.4% respectively. But the other 18.4% of the bloody diarrhoea was negative for these pathogens. Among a total of 228 *Shigella* isolates, the subgroup *Sh. flexneri* was most predominant (168/228= 73.6%), and the second predominant subgroup was *Sh. sonnei* (12.2%). Regarding the serovar of 168 strains of *Sh. flexneri*, 71 strains belonged to type 1-a (42.3%), and 39 strains revealed type 1-b (23.6%). In the age group older than 5 years, the isolation rate of the enteropathogens was more than 30%, but in the group below 5 years, it was 20.8%. From these results, it can be stressed that the study on the infections with *Campylobacter jejuni*, enterotoxigenic *E. coli*, and rota virus should be carried out as said by WHO. In the drug sensitivity test using tetracycline, streptomycin, chloramphenicol, ampicillin and nalidixic acid, the resistant *Shigella* strains against tetracycline were outstanding. About 50% of the *Shigella* strains appeared clinically resistant (MIC> 25 mcg/ml). Highly resistant strains (MIC>100 mcg/ml) were seen in 12.2%. The rate of resistant strains against the multiple drugs was low. In Kwale District, most of the diarrhoeal patients are treated with tetracycline, so it is possible that Tetracycline-resistant strain will increase and persist. Enteropathogenic *E. coli* was detected from the drinking tap water. It suggests that the tap water is one of the focus of infection.

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*Present Adress: 2nd Department of Internal Medicine, Nagasaki University, School of Medicine, Nagasaki, Japan

**Present Adress: Department of Bacteriology, Ryukyu University, School of Medicine, Okinawa, Japan

ケニア国南部海岸地区における細菌性下痢症

宇都宮明剛[1]，　森　賢治[1*]，　林　敏明[1*]，　岩永正明[1**]，　内藤達郎[1]，
Z.W. GATHERU[2]，　F. SANG[2]，　N. KOSKE[2] and J.N. KAVITI[3]

1　長崎大学熱帯医学研究所病原細菌学部門
（*現：長崎大学医学部第２内科学教室）
（**現：琉球大学医学部細菌学教室）
2　Kenya Medical Research Institute, Nairobi, Kenya
3　National Public Health Laboratory Service, Nairobi, Kenya

ケニア国南部海岸地区（Kwale District, Coast Province）で細菌性下痢症の原因病原体の検出を１９８１年１０月から１９８２年７月までの１０カ月間にわたって行った。

６カ所の医療施設に下痢を主訴として訪れる外来患者の便を検体とし，９９９検体について検査を行い，赤痢菌２２.８％，病原大腸菌8.0％，サルモネラ3.５％の結果を得たが，コレラ菌，腸炎ビブリオはこの期間には検出されなかった。最大雨期の４，５，６月に検出率が高く，それぞれ，４２.１％，５７.５％，５３.０％であり，赤痢菌が最優勢の病原菌であった。

１６３の血便検体からは，赤痢菌７６.０％，病原大腸菌4.９％，サルモネラ2.４％が検出されたが，３９検体（１８.４％）は陰性の結果であった。２２８株の赤痢菌の血清型別では，フレキシネル２ａが７１株（４２.３％），フレキシネル１ｂが３９株（２３.６％）と優勢で，ソンネ菌は２８株（１２.２％）であった。年齢別の検出頻度では，５歳以上のグループでは３０％以上であったが，５歳以下のグループでは２０.８％にすぎなかった。

テトラサイクリン，ストレプトマイシン，クロラムフェニコール，アンピシリン，ナリディキシン酸に対する薬剤感受性試験を行った結果，赤痢菌の５０％は，２５γ／ml 以上の耐性を示し，特にテトラサイクリンに対して，１００γ／ml の高度耐性を示すものが１６.１％にみられた。しかし，多剤耐性を示すものは少なかった。５種の薬剤中，ナリディキシン酸が最も強い抗菌力を示した。

住民の利用する飲料水の細菌学的検査の結果，病原大腸菌が検出され，水系感染の危険性があることが示唆された。

熱帯医学　２４巻４号，２３５－２５２頁，１９８２年

3.1.2.4

Serovar and Drug Sensitivity of *Salmonella* Isolated in Kenya

A. UTSUNOMIYA

Department of Bacteriology, Institute for Tropical Medicine,
Nagasaki University, Nagasaki, Japan

Thirty-three strains of *Salmonella* isolated in Kwale District, Coast Province, Kenya, were tested for their serovars and drug sensitivities to tetracycline, streptomycin, chloramphenicol, nalidixic acid and aminobenzyl-penicillin. Number of strains belonging to O-groups B, C 1, C 2, D 1, E 1, E 4, G 1, and O were 16, 1, 1, 3, 2, 1, 7 and 2 respectively. The serovars detected were 16 as shown in Table 1, and 6 strains of *Sal. kiambu*, 5 of *Sal. goodwood* and 4 of *Sal. heidelberg* were predominant. As shown in Table 2, the strains resistant to one and two drugs were only 3 and 1.

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ケニアで分離されたサルモネラ属菌の血清型と薬剤感受性

宇都宮明剛　　長崎大学熱帯医学研究所病原細菌学部門

１９８１年１１月から１９８２年７月までの間，ケニア国南部海岸地区で下痢症の原因細菌の調査を行い，９９９の下痢便検体から赤痢菌２２８株，病原大腸菌８０株と共に，サルモネラ属菌３５株を分離した（宇都宮ら，１９８２）。本報ではサルモネラ属菌の３３株について，その血清型と分類株個々の薬剤感受性値を記述した。血清型別の結果，８種類のＯ群と１６種類の血清型がみられ，Sal. kiambu が最も多く６株（１８.１％），次いでSal. goodwood ５株（１５.１％），Sal. heidelberg 4株（１２.１％），Sal. arechavaleta 3株（9.0％）で，Sal. typhimurium は1株であった。またチフス菌，パラチフス菌は検出されなかった。薬剤感受性試験の結果では，１００mcg/ml以上の耐性を示したものはアンピシリンに対する１例のみで，多剤耐性株も見出されなかった。１９６６年－１９７６年の日本国内分離株，および１９７９年内に大阪空港検疫所で調査され，旅行者下痢患者から分離された薬剤耐性サルモネラ属菌の耐性頻度と比較して，ケニア住民から分離されたサルモネラ属菌は，極めて高い感受性を示したといえる。

熱帯医学　２５巻　２号，６５－７１頁，１９８３年

3.1.2.5

EPIDEMIOLOGY OF CHOLERA IN KENYA IN 1983

M. EHARA[1], S. WATANABE[1], Y. ICHINOSE[1], S. SHIMOTORI[2], T.K. ARAP SIONGOK[3], A.M. KIBUE[4] and F.C. SANG[4]

[1] Department of Bacteriology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[2] School of Health Sciences, Kyushu University, Fukuoka, Japan

[3] Division of Communicable Diseases and Control, Afya House, Nairobi, Kenya

[4] Kenya Medical Research Institute, Nairobi, Kenya

More than 17,000 rectal swab specimens were examined for Vibrio cholerae O 1 at Cholera Laboratory in Homa Bay District Hospital. Out of these specimens, 1301 cases were cholera-positive excluding repeated specimens. The number of admitted cases and healthy carriers were 788 and 513.

Infection rate of V. cholerae was lowest in the age-group, 13-19 of male. It was highest in female of child-bearing age-group(15-39). Monthly variation of cholera positive cases showed two peaks in March and July and closely co-related with the dry season. Monthly variation of cholera by age-group also showed two peaks in March and July. The former peak was created by age-group of 20-39 and the latter was by 13-19. The most affected locations were Karachuonyo(438) and Kanyada(360). Then Gembe, Kasipul, Kabuoch and Kamagambo followed them. The mortality of the admitted cases was 9.0 %. One hundred and sixty one cases were thought to be family contacts, however most of the others were sporadic cases. In 1982 tetracycline resistant strains were isolated in Kenya, though the number was small.
Most of the strains isolated in 1983 were tetracycline resistant.

ケニアにおけるコレラの疫学的観察

江原雅彦[1]，　渡辺繁徳[1]，　一瀬休生[1]，　霜鳥翔一[2]，
T.K. ARAP SIONGOK[3]，A.M. KIBUE[4] and F.C. SANG[4]

1　長崎大学熱帯医学研究所病原細菌学部門
2　九州大学医療短期大学部
3　Division of Communicable Diserses and Control, Afya House, Nairobi, Kenya
4　Kenya Medical Research Institute, Nairobi,Kenya

Kipindupindu はスワヒリ語でコレラを意味し，１９７１年以後にできた新造語で，それ以前にはなかった。第７回 cholera pandmy がケニアに波及したのがこの年で，それ以来，何故かビクトリア湖周辺にコレラは定着している。日本－ケニア間の伝染病研究対策プロジェクトの一部を紹介する。Homa Bay であつかった約１７,０００検体のうち，１,３０１人がコレラ菌陽性で，７８８人が入院し，５１３人は健康保菌者であった。罹患率は１３－１９才の男性に低く，１５－３９才の女性に高かった。季節的変動は明らかで，乾期に多く，雨期に減少した。KarachuonyoとKanyada の２ Location にコレラは集中している。入院患者の死亡率は９％と高く，種々の問題を含んでいる。１６１症例が family contacts と考えられ，初発は成人女性に多く，二次感染は子供に多くみられた。一方分離株のほとんどがテトラサイクリン耐性のエルトール小川型菌であった。

3.1.2.6

THE CHARACTERIZATION OF VIBRIO CHOLERAE ISOLATED IN KENYA IN 1983

Y. ICHINOSE[1], M. EHARA[1], S. WATANABE[1], S. SHIMOTORI[2], J.N. KAVITI[3], A.M. KIBUE[4], F.C. SANG[4] and J. NGUGI[4]

[1] Department of Bacteriology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[2] School of Health Science, Kyushu University, Fukuoka, Japan

[3] National Public Health Laboratory Service, Nairobi, Kenya

[4] Kenya Medical Research Institute, Nairobi, Kenya

Since the emergence of resistant strains of Vibrio cholerae to tetracycline in Tanzania was reported by Mhalu et al, there has concerns on the emergence of similar resistant cholera strains in Kenya. Actually, since 1982, some resistant strains of Vibrio cholerae to tetracycline have been isolated in Kenya. We collected as many specimens as possible of these strains to characterize biochemically and bacteriologically.

Two hundred and forty four strains of V.cholerae were collected from the cholera patients in Homa Bay District Hospital, Migori Health Center, Ombo Hospital, etc in Nyanza Province. All the strains of V.cholerae isolated in Kenya in 1983 are El Tor, Celebes original type except one untypable strain. Environmental cholera survey was done after cholera outbreak was subsided. Fourteen strains of NAG vibrio (31.8%) and 11 strains of Aeromonas (25%) were isolated from water samples in Nyanza Province. No V.cholerae O-1 was isolated.

One hundred and eighty three out of 244 strains of Vibrio cholerae were resistant to tetracycline, streptomycin, and ampicillin. However, strains resistant to gentamycin were not isolated.

ケニア国における１９８３年分離コレラ菌の性状について

一瀬休生[1]，　江原雅彦[1]，　渡辺繁徳[1]，　霜鳥翔一[2]
J.N. KAVITI[4], A.M. KIBUE[4], F.C. SANG[4] and J. NGUGI[4]

1　長崎大学熱帯医学研究所病原細菌学部門
2　九州大学医療短期大学部
3　National Public Health Laboratory Service, Nairobi, Kenya
4　Kenya Medical Research Institute, Nairobi, Kenya

タンザニアにおいてテトラサイクリン耐性コレラ菌の出現がMuhaluらに報告されて以来，隣国のケニアにおいても薬剤耐性コレラ菌の出現に関心が払われてきた。このため我々はできるだけ多くの菌株の分離につとめ，生化学的，細菌学的に性状検査を行なった。

調査地域はツルカナ地方を除くモンバサ周辺の南部海岸地区，ナイロビ市，ビクトリア湖周辺地域を中心に行なわれ，その地域の基幹病院，診療所の入院患者からコレラ菌分離につとめた。その結果，コレラ浸淫地域であるニアンザ地方のHoma Bay District Hospital, Migori Health Center Omo Hospitalから２４４株のコレラ菌を収集することができた。この１９８３年ケニア分離株は，１株のuntypable strainを除くと全ての株が生物型エルトール型でセレベス原型であることが判明した。

薬剤感受性試験では２４４株のコレラ菌のうち１８３株がテトラサイクリン，ストレプトマイシン，アンピシリンの３剤に対し耐性を示す多剤耐性菌であることが判明した。しかし，ゲンタマイシンに対する耐性菌は検出できなかった。又，環境調査としてコレラ流行がおさまった後ニアンザ地方の河川，井戸からコレラ菌の分離を試みた。コレラ菌は検出できなかったにもかかわらず，その結果４３検体中３１.８％からＮＡＧビブリオ２５％からエロモナスが検出され，コレラ浸淫地域では，ＮＡＧビブリオが多数検出されるという事実を支持する結果であった。

3.1.2.7

A STUDY ON ENTEROTOXIGENIC ESCHERICHIA COLI INFECTION IN KENYA

S. WATANABE[1], M. EHARA[1], Y. ICHINOSE[1], S. SHIMOTORI[2], J.N. KAVITI[3], A.M KIBUE[4], F.C. SANG[4], J. NGUGI[4]

[1] Department of Bacteriology, Institute for Tropical Medicine, Nagasaki Univeristy, Nagasaki, Japan

[2] School of Health Science, Kyushu University, Fukuoka, Japan

[3] National Public Health Laboratory Service, Nairobi, Kenya

[4] Kenya Medical Research Institute, Nairobi, Kenya

The epidemiology of enterotoxigenic Escherichia coli (ETEC) was studied in children with diarrhoea and healthy people in Kenya. A total of seven hundred and eighty-two stool specimens from pediatric diarrhoeal cases ranging in age from 0-14 years and four hundred and seventy-four from healthy people were processed to identify ETEC. 47 ETEC giving an infection rate of 6.0% were detected from pediatric cases and the majority of isolates, 24 were heat stable enterotoxin (ST) producers. 21 heat labile enterotoxin (LT) producers came in a close second and only two produced both toxins. It was notable that 10 of those positive cases had mixed infections with other enteropathogens. Although ETEC could inflict illness in all age groups of children, the peak incidence of isolation was in the 25-30 month age group. In the study of healthy persons, a total of twenty-one ETEC isolates giving an infection rate of 4.4% were identified. Out of them 13 were ST producers, 7 LT producers and the rest was ST and LT producer. This work has clearly demonstrated that ETEC might play a significant roll in childhood diarrhoea and that much attention should be paid to healthy carriers.

ケニア国における毒素原性大腸菌の感染状況

渡辺繁徳[1]，　江原雅彦[1]，　一瀬休生[1]，　霜鳥翔一[2]，
J.N. KAVITI[3]，　A.M. KIBUE[4]，　F.C. SANG[4] and J. NGUGI[4]

1　長崎大学熱帯医学研究所病原細菌学部門
2　九州大学医療短期大学部
3　National Public Health Laboratory Service, Nairobi, Kenya
4　Kenya Medical Research Institute, Nairobi, Kenya

ケニア国の小児下痢患者と健康人における毒素原性大腸菌（ＥＴＥＣ）の感染状況を調査した。０歳から１４歳までの小児下痢患者から７８２，健康人から４７４の便検体が採取された。小児患者では４７例（6.7％）がＥＴＥＣ陽性でそのうち耐熱性毒素（ＳＴ）産生株が２４例で一番多く，次いで易熱性毒素（ＬＴ）産生株が２１例，両毒素産生株は２例であった。４７のＥＴＥＣ陽性例のうち１０例が他の病原菌との混合感染であった事は注目に値する。ＥＴＥＣの陽性率を年令別に見ると２歳－２歳半が最も高かった。健康人では２１例（4.4％）がＥＴＥＣ陽性でそのうちＳＴ産性株が１３例，ＬＴ産生株が７例，残り１株が両毒素産生株であった。ケニア国においてもＥＴＥＣは小児下痢症に限らず健康人に対しても重要な役割を果たしていることがわかった。

3.1.2.8

Survey on Campylobacter jejuni in Kenya

S. Shimotori[1], M. Ehara[2], S. Watanabe[2] Y. Ichinose[2], P. G. Waiyaki[3], A. M. Kibue[3], F. C. Sang[3], and J. Ngugi[3]

1) School of Health Science Kyushu University, Fukuoka, Japan.

2) Department of Bacteriology, Institute for Tropical Medicine, Nagasaki, Japan.

3) Kenya Medical REsearch Institute, Nairobi, Kenya.

We reported the findings of Campylobacer jejuni surveys which were carried out during the latter part of 1983 at the pediatric clinic of the Coast General Hospital and Mvita Clinic in Mombasa. The bacteriological examination was made on the children with acute diarrhoea less than 3 years old who came to both facilities. C., jejuni was isolated from approximately 12.6 % of diarrhoic stools. In addition, data on infection rate of the other enteropathogens, mixed infection cases, age and sex distribution in the cases of Campylobacter enteritis were presented.

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ケニアにおけるCampylobacter jejuniの調査

霜鳥翔一[1)]，江原雅彦[2)]，渡辺繁徳[2)]，一瀬休生[2)]，
P.G. Waiyaki[3)]，A.M. Kibue[3)]，F.C. Sang[3)]，J. Ngugi[3)]

1) 九州大学医療技術短期大学部
2) 長崎大学熱帯医学研究所
3) Kenya Medical Research Institute, Nairobi, Kenya

１９８３年の後半，われわれはMombasa 地区にあるCoast General Hospital およびMvita Clinic の両施設において，３回にわたってCampylobacter jejuni の感染調査を実施してその成績を報告した。

細菌検査は急性下痢症状を示す３才以下の小児を対象に行ない，12.6％の患者からC. jejuniが検出された。さらにわれわれは，これらの小児下痢患者７７９人について，C. jejuni以外の下痢起因菌の感染並びに混合感染例，Campylobacter腸炎の年令別および性別分布についてものべた。

3.1.2.9

Summary and Recommendation

Shoichi Shimotori

School of Health Science Kyushu University, Fukuoka, Japan

1) THE PROGRESS OF THE PROJECT IN THE BACTERIOLOGICAL SECTION

Our project was performed with two major aims: i) epidemiological survey of cholera and the project was launched in 1919. enteropathogens survey of diarrhoeal diseases other than cholera. The first 2 years were spent on the preparation for the practical work: preparation of materials and equipment, selection of Kenyan counterparts, their basic bacteriological instruction, and selection of regions and facilities for surveying. We started substantial survey in October 1980. During the first 6 months,.till the end of March 1981, examination for enteropathogens was performed on the outpatients of diarrhoea at the Coast General Hospital (Mombasa District) and at the Provincial General Hospital (Nyeri District). A similar survey was carried out at the Kenyatta National Hospital (Nairobi) and in the Mombasa District in June and July, 1981. During the 10 months, from October 1981 through July 1982, our bacteriological survey team made a general epidemiological survey on bacterial diarrhoea mainly at the Kwale District (Coast Province) and its surrounding areas; bacteriological examination was made on 999 stool samples from the outpatients of diarrhoea at 6 medical facilities in those areas and on the drinking water used by the inhabitants. The results are: some enteropathogens exept V.cholerae and V.parahaemolyticus were detected in about 30% of the diarrhoeal patients. This survey provided us with the first approach to the elucidation of the actual state of the manifestation and prevalence of bacterial diarrhoea in that region.

In Kenya, there has been a prevalence of cholera since its invasion in 1970; since then no year has passed without occurrence of cholera. The regions where cholera has been especially prevalent are roughly divided into the following three: i) North-West District bordering Sudan and Uganda, ii) Western Province around Lake Victoria and iii) South Coast bordering Tanzania.

In 1981, we examined 280 strains of V.cholerae which were sent to National Public Health Laboratory Service (Nairobi) from the epidemic areas as well

as sporadic cases. The route by which cholera spreads in Kenya was followed by the type classification of V.cholerae isolated in 1980 and 1981. The strain classification was made by serological type and phage-prophage type. Consequently, three main routes of spreading from Turkana to the South along the Rift Valley, from Western Kenya around Lake Victoria to high land cities and from the South Coast to high land cities were indicated.

Our investigations during the 2 years, from April 1982 until the end of the project, can be roughly classified into the following 6 divisions: i) examinations of traditional enteropathogens (Shigella, Salmonela, enteropathogenic E.coli, V.parahaemolyticus and V.cholerae) for the children with acute diarrhoea at the Coast General Hospital and the Mvita Clinic in the Mombasa District of Coast Province, ii) survey of EPEC and ETEC from normal healthy subjects (villagers and secondary school students in the Kwale District of Coast Province and prisoners in the Kisumu District of Nyanza Province), iii) epidemiological analysis of the prevalence of cholera in the South Nyanza District, iv) observations on the characteristics of V.cholerae isolated in Kenya in 1983; especially on its drug sensitivity, v) bacteriological survey of contamination for the water of Lake Victoria and the drinking water used by the inhabitants around the lake, and vi) survey of Campylobacter for the children with acute diarrhoea at the Coast General Hospital and the Mvita Clinic in the Mombasa District of Coast Province. The results of those investigations are described in the next chapter.

2) AN EPIDEMIOLOGICAL SURVEY OF CHOLERA

(a) Epidemiological analysis of the prevalence of cholera in the South Nyanza District in 1983

The aspects of the prevalence of cholera in this region were analyzed from different angles; for this analysis we employed, as data, the admission records of the patients of cholera who had been hospitalized at the Homa Bay Hospital or at the Migori Health Center in 1983 and the records of bacteriological examinations for those patients' households (contacts) which were

made simultaneously with their hospitalization. The results can be summarized as follows: i) The age and sex distribution of V.cholerae carriers for the inpatients at both facilities and their contacts is: high V.cholerae-positive rates were observed for the 13-to-19 age group and the 20-to-39 age group for both sexes; for both age groups females present significantly higher rates than males. ii) The admission period: one week or less in 60% of the patients at both facilities; there are also some cases of long-hospitalization: 9-to-14 days in 12.6% and 15 days or more for 2.8%; those figures would probably suggest that the diagnosis or treatment of cholera is not always carried out properly. iii) The number cases of cholera and V.cholerae carriers among their households (contacts) for each month presents 2 peaks: 42.5% for March and 23.1% for July in proportion to the whole; both of the months are the turning points from the dry season into the rainy season in Kenya. Those data clearly indicate a close relationship between the prevalence pattern for cholera and seasons in Kenya. The elucidation of the intervening factors will be needed through closer epidemiological surveys of cholera in future. iv) The death rate for the inpatients of cholera is some 9%, which is extremely high. This percentage, as well as the high rate of long-hospitalization, which is mentioned inii), suggest that the treatment of cholera and/or measures taken before hospitalization are not always proper.

(b) Characterization of Vibrio cholerae isolated in Kenya in 1983

Since the emergence of resistant strains of V.cholerae to tetracycline in Tanzania was reported by Mhalu et al[1], a concern on the emergence of resistant cholera strains has been expressed even in Kenya. Actually, since 1982, some resistant strains of V.cholerae to tetracycline have been isolated in Kenya. Therefore, we collected as many specimens as possible mainly from the cholera patients admitted in Homa Bay district Hospital, Migori Health Center, Ombo Hospital and others in Nyanza province, and drug resistanceand biochemical properties in these isolates were examined in detail. In addition, we also carried out environmental cholera survey during the subsidence of cholera endemic in this area.

Five important conclusions arise from this study. First of all, the only biotype of cholera strains so far isolated in Kenya is El Tor, although most of the strains isolated recently in Bangladesh[2] are changing in biotype from El Tor to Classic. Secondly, all the cholera strains isolated in Kenya were Celebes, original type. The third conclusion concerns the changes in hemolytic rate of the isolates since the original studies carried out in Africa in 1970. Biotype El Tor was originally differentiated from biotype Classic by the ability to hemolyse sheep RBC. However, quite a large number of biotype El Tor which are non-hemolytic have been reported. It seems, however, that the change of hemolytic pattern is very significant. Gallut reported that almost all strains isolated in Africa in 1970 and 1971 were non-hemolytic. The rate of hemolysis has increased dramatically since then. In Kenya, 11% of isolates were hemolytic in 1975, whereas 83% of isolates were hemolytic in 1980/81. In our study, 73% of isolates were hemolytic. In contrast, in the Philippines, the hemolytic rate seems to be decreasing year by year, and non-hemolytic strains have now become dominant. Therefore, the hemolytic behavior of vibrio should be followed yearly. Concerning the results of examination of the water samples which is supposed to be used for the domestic consumption including drinking water, no V.cholerae could be isolated, whereas 14 strains of NAG vibrio (31.8%) and 11 strains of Aeromonas (25%) were isolated. This fairly high isolation rate of NAG vibrio may suggest that NAG vibrio infection occures simultaneously in the cholera endemic area. As we expected, NAG vibrio were also isolated in the water samples from Lake Victoria, however, NAG vibrio and Aeromonas were isolated from the flowing river samples much more than from the stagnant river samples. The examination of the water samples should be done repeatedly when cholera is epidemic in the area. The last point to be mentioned is the emergence of resistant strains of V.cholerae against tetracycline. Tetracycline which has been considered to be very effective against cholera infection, showed a very poor anti-vibrio activity. These strains are also resistant to other drugs. Epidemics resulting from cholera strains resistant to tetracycline have already been reported by Towner

et al[3], in 1979. Thirty four strains of V. cholerae isolated in Homa Bay in 1982 were examined for susceptibility to tetracycline. The MIC of 14 strains was more than 50 microgramme/ml. The development of antibiotics resistance against V.cholerae 01 appears to be due to the extensive therapeutic and prophylactic use of drugs. Some important problems remain to be solved: firstly, an effective and cheap drug against cholera should be chosen, with due consideration being given to the most appropriate drug for pediatric and pregnant cases. Secondly, particular attention should be paid to the care of hospital patients, and to the improvement of laboratory diagnostic techniques in rural areas where cholera is endemic. Thirdly, an effective use of ORS (oral rehydration solution) in rural areas should be promoted and the discontinuation of extensive therapeutic and prophylactic use of drugs should be considered. Finally, it is vitally important to continue to do the drug susceptible tests to monitor the resistance of V.cholerae to the various drugs currently being used in Kenya to combat the disease.

3) AN ETIOLOGICAL SURVEY OF DIARRHOEAL DISEASES OTHER THAN CHOLERA

(a) Campylobacter jejuni in children with diarrhoea in Mombasa district.

IN Kenya, very little information on the incidence and prevalence of C.jejuni among patients with diarrhoea is available. Gikonyo et al[4] in 1981 and Wamola et al[5] in 1983 worked at the Kenyatta National Hospital, and examined many stool specimens from children with diarrhoea. Those studies established an important association between gastroenteritis and infection with C.jejuni in this country. Our works was undertaken to determine the he frequency of C.jejuni among children with acute diarrhoea in Mombasa. In addition, we also analyzed some epidemiological characteristics of C.jejuni infection in these children. Although our work was focused on Campylobacter gastroenteritis, the species were simultaneously examined for the detection of Salmonella, Shigella, Vibrio cholerae, Enterotoxigenic E.coli, Enteropathogenic E.coli, NAG vibrio, Vibrio fluvialis and Aeromonas hydrophila.

A total of 779 stool specimens were examined bacteriologically. Out of these specimens 98 strains of C.jejuni were isolated. During the first survey in July 1983, out of a total of 314 specimens examined , 54 C.jejuni were isolated giving an isolation rate of 11%. During the second survey carried out in September, 256 specimens were examined and 16 C.jejuni were isolated. This constituted an isolation rate of 5.4%. In the third survey carried out in November, out of 209 specimens examined, 28 C.jejuni were isolated. This gave an isolation rate of 12.2%. The overall isolation rate for all the three surveys was 12.6%.

The results on the prevalence of enteropathogens other than C.jejuni were as follows: there were a total of 91 EPEC, 26 ETEC-ST 23 ETEC-LT, 17 Shigella species, 13 Salmonella species and 3 V.cholerae non O-1. Taking the three surveys together, C.jejuni was isolated more frequently than the other enteropathogens. V.fluvialis and A.hydrophila also were isolated from the children with acute diarrhoea at the ratio of 0.6 and 1.1%, respectively. Mixed infection cases were found among 98 C.jejuni positive cases. In 12 cases, EPEC were isolated along with C. jejuni. There were also three cases of double infections with Shigella. There were two cases of mixed in-

fection with Salmonella species and EPEC. The age distribution of children with Campylobacter enteritis indicates that children of up to 2 years of age were more frequenctly infected than older ones. The peak incidence was seen in the 19-24 month age group. Therefore, we confirmed the finding that the peak incidence of Campylobacter enteritis is in children aged 24 month or less.

V. fluvialis and A. hydrophila were also isolated really from the children with diarrhoea. Examination for these two bacteria, however, should not be excluded in the survey of diarrhoeal diseases in Kenya.

(b) Survey on Enterotoxigenic E. coli(ETEC) infection in Kenya.

The survey was carried out in Coast Province and South Nyanza District. Seven hundred and eighty-two stool specimens were collected during the three survey carried out in Mombasa. Forty-seven ETEC were isolated from children with diarrhoea. This showed an infection rate of 6.0%. Two isolates of 47 ETEC produced both LT and ST, 21 isolates produced LT only, and 24 isolates produced ST only. This indicated that there were more ST producers than LT producers. On the other hand, in ETEC examination of a total of 474 stool specimens obtained from healthy persons living South Nyanza and Kwale, 21 ETEC were isolated from them. The infection rate was 4.4 %.

The distribution of ETEC isolation by age from pediatric diarrhoea cases showed that the peak incidence was in the 25-30 month age group, giving an isolation rate of 11.8 %, while the lowest isolation rate was in the 0-6 month age group, 4.2 %. The sex distribution of ETEC infection showed a slightly higher rate of isolation in males than in females. However, the difference was not significant statistically.

In Kenya, it appears that from the limited data available the rate of isolation of ETEC is relatively low in comparision with that of other enteropathogens such as Salmonella, Shigella and Campylobacter species and that reported elsewhere[6)7)]. Therefore, it was suggested that ETEC infection is not likely to be the major cause of diarrhoea in children at least

in Kenya. Further study on ETEC infection, however, should be performed through one year to make clearer the rate of ETEC infection in a total of diarrhoeal cases and seasonal variation of prevalence of ETEC in these pilot areas.

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まとめと助言

霜鳥翔一

九州大学医療技術短期大学部

1. 細菌部門におけるプロジェクト活動の沿革

細菌部門はこのプロジェクトの遂行にあたって，二つの主要な目的を設定した。その一つはコレラの疫学調査であり，他はコレラ以外の下痢症の病因調査である。このプロジェクトが開始された１９７９年からの約２年間は業務遂行のための準備に費された。この間，研究資材の整備，ケニア側カウンターパートの人選と彼らに対する細菌学の基礎的教育および調査対象地域と施設の設定がなされた。最初の実質的な調査活動は１９８０年１０月から始められ，１９８１年３月までの６ケ月間にMombasa 地区（Coast General Hospital）およびNyeri 地区（Provincial General Hospital）において外来下痢患者を対象に起因菌の検査を実施した。さらに同年６，７月にはNairobi（Kenyatta National Hospital）およびMombasa 地区において，同様の調査を実施した。その後１９８１年１０月から翌年７月までの１０ケ月間に，われわれ細菌調査チームはCoast 州のKwale 地区を中心に，細菌性下痢症の総合的疫学調査を行ない，同地区内６ケ所の医療施設を訪れた下痢患者の便，９９９検体および住民の利用すを飲料水の細菌学的検査を行なった。その結果，下痢患者の約３０％から，コレラ菌と腸炎ビブリオを除くいずれかの病原性腸内細菌が検出された。この調査によって，この地域における細菌性下痢症の発生と流行の実態が初めて明らかにされた。

ケニアにおけるコレラ流行は，１９７０年に同国へコレラ侵襲があって以来，今日まで毎年患者発生がみられている。その主たる流行地域は１）スーダン・ウガンダ国境沿いの北西地区，２）ビクトリア湖周辺のWestern 州，３）タンザニア国境沿いの南海岸の３地域に大別することが出来る。われわれは１９８１年にSouth Coast 州のKwale 地区において コレラの調査を行ない，４～７月の４ケ月間に約3,０００のコレラ患者が細菌学的に確認された。

１９８２年４月以降プロジェクト終了までの約２年間におけるわれわれの活動は，おおむね次の６項目に区分される。すなわち，ⅰ）Coast 州Mombasa 地区のCoast General Hospital およびMvita Clinicにおける急性下痢症児からの赤痢菌，サルモネラ，病原性大腸菌（ＥＰＥＣ），腸炎ビブリオおよびコレラ菌の検査，ⅱ）Coast 州Kwale 地区における健康者（villagers およびSecondary School students）およびNyanza 州Kisumu 地区における健康者（因人）を対象としたＥＰＥＣと毒素原性大腸菌（ＥＴＥＣ）の調査，ⅲ）South Nyanza 地区におけるコレラ流行の疫学的解析，ⅳ）１９８３年にケニ

，で分離されたコレラ菌の性状，特の分離菌の薬剤感受性，V）コレラの非流行期中におけるビクトリア湖水および周辺住民の飲料水の細菌学的汚染調査，VI）Coast 州 Mombasa 地区の Coast General Hospital および Mvita Clinic における急性下痢小児を対象としたキャンピロバクターの調査，が実施された。これらの結果については次項で述る。

2. コレラの疫学調査

(a) １９８３年の South Nyanza 地区におけるコレラ流行の疫学的解析

１９８３年の１年間に Homa Bay Hospital と Migori ヘルスセンターに入院したコレラ患者の入院記録および同時に実施した患者家族（接触者）の細菌検査記録をもとに，この地域におけるコレラ流行の様態を様々な角度から分析した。それらの結果はおおむね次のとおり要約出来る。i）これらの両施設に入院したコレラ患者および接触者におけるコレラ菌陽性者の年令および性別分布は，いずれの場合も１３～１９および２９～３０才の両年令グループにおいてとくに高い陽性率を示し，かつこの両年令グループとも女性が男性よりもコレラ菌の陽性率は有意に高かった。ii）コレラ患者の両施設における入院期間は一週間以内が全体の６０％を占めている。しかし，長期入院例も少なくなく，9～14日間の入院患者が１２.６％，１５日以上が2.8％であった。これはコレラの診断あるいは治療が必ずしも適切ではないことを示唆するものと云える。iii）コレラ患者の発生数，および患者家族（接触者）のコレラ菌陽性者数の月別推移をみると，ケニアにおける乾期から雨期への移行期に一致して，３月と７月とに二つのピークがあり，それぞれ年間のコレラ陽性総数の４２.５％と２３.１％である。このようにケニアにおけるコレラの流行パターンと季節との間には密接な関係があるものと思われるので，それらの間に介在する因子の解明が，今後の詳細なコレラの疫学調査によってなされる事が必要である。iv）入院コレラ患者の死亡率は約９％を示し，他の流行国に比べてきわめて高率である。これは前述 ii）の長期入院例が多い事実と合せて，コレラの治療あるいは発病から入院までの間の処置が必しも適切でないことを再び示唆する。

(b) １９８３年にケニアで分離されたコレラ菌の性状について

１９７９年に Mhalu らはタンザニア流行株がテトラサイクリンに対して耐性を獲得していることを報告して以来，テトラサイクリンが専らコレラの治療および予防薬として用いられているケニアにおいて，コレラ菌の薬剤耐性の問題に特に警戒がなされていた。しかし，１９８２年のケニアにおけるコレラ流行分離株中に初めてテトラサイクリン耐性菌が検出されたことから，われわれは流行地域からその後のコレラ菌分離株を多数集めて，各種薬剤に対する感受性試験および生化学的性状を詳細に調べた。また，流行地におけるコレラの発生と流行の原因を探る目的で，流行の静止期（１１月～１２月）にビクトリア湖水および飲料水の細菌検査を実施した。

これらの検査結果からは次の結論が得られた。すなわち，ⅰ）ケニアにおいて分離されたコレラ菌の生物型はすべてエルトール型である。ⅱ）プロファージ型別の結果，分離株はすべてセレベス型の原型に属し，従って，第7回コレラパンデミーの起因菌に一致した。ⅲ）溶血性株の分離率をみると，１９７５年の分離株では１１％であったが，１９８０～８１年においては８３％の分離株が溶血性を示した。さらに今回のわれわれの調査では，最近のケニア流行株の７３％が溶血性を示した。アフリカ流行株の溶血性に関しては，Gallut の報告があり，彼らが調べた１９７０年および１９７１年流行のコレラ菌すべてが非溶血性であったとのべている。このようにコレラ菌の溶血株の分離率は年ごとに変動していることは興味深く，今後も毎年の追跡調査が必要である。ⅳ）コレラ流行の静止期（11～１２月）に行なった住民の飲料水およびビクトリア湖水の細菌検査の結果は，コレラ菌はすべて陰性であった。しかし，分類学上コレラ菌ときわめて近縁のＮＡＧビブリオ（非凝集性ビブリオ）が３１.８％のサンプルから検出され，また，Aeromonas 属も２５％のサンプルが陽性であった。コレラの発生と流行は，その地域における水のコレラ菌汚染と深い関連性がある。従って，流行地における飲料水あるいは河川湖水の定期的な細菌検査の実施は，コレラ発生および蔓延を監視する上で不可欠である。

ⅴ）ケニアにおいて最近分離されたすべてのコレラ菌がテトラサイクリンに耐性を獲得していることが今回の調査で確認された。このような薬剤耐性コレラ菌の出現は長期にわたる薬剤の濫用に起因するものと考えられる。従って，この為の緊急対策として，効果的で廉価な薬剤を選択して，その適切な使用法を指導すること。また分離コレラ菌について薬剤感受性試験を常時実施して耐性菌の出現と薬剤効果をモニターすることが要求される。ⅵ）コレラ患者の治療には抗菌剤の使用にのみ依存すべきではなく，バングラディシュなどの他の流行地において早くから応用されている経口輸液の導入を急ぐべきである。

3. コレラ以外の下痢症の病因調査

(a) Mombasa 地区における小児のキャンピロバクター腸炎

ケニアにおいてCampylobacter jejuni に起因する下痢症の流行に関する情報はきわめて少なく，わずかに１９８１年 Gikonyo らと１９８３年の Wamola らの報告を見るのみである。彼らは Kenyatta National Hospital において小児の急性下痢患者を対象に C. jejuni の検査を行ない，この菌による感染がきわめて高率に発生していることを指摘している。われわれは１９８３年の後半に3回にわたって，Mombasa 地区を中心に，急性下痢症状を示す小児を対象に C. jejuni の検査を行なった。また，この検査の為に採取した検体は，同時にサルモネラ，赤痢菌，コレラ菌，ＥＴＥＣ，ＥＰＥＣ，ＮＡＧビブリオなどの他の下痢起因菌についても調べた。3回の調査で合計７７９検体がCoast General Hospital および Mvita Clinicの２施設で採取された。検査の結果，

その内の９８検体からC. jejuniが検出され，検出率は１２.６％であった。これは他のアメリカ諸国あるいは発展途上国における調査結果にほぼ一致する。C. jejuni 以外の検査結果はＥＰＥＣ陽性が９１例で最も多く，次いでＥＴＥＣ－ＳＴ ２６例，ＥＴＥＣ－ＬＴ １７例，赤痢菌１７例，サルモネラ１３例およびＮＡＧビブリオ３例の順であった。この他にV. fluvialis およびA. hydrophila も少数例ではあるが検出された。C. jejuni が陽性となった９８人の小児下痢患者の内，他の下痢起因菌との混合感染が認められた例が相当数確認された。その内訳は，ＥＰＥＣとの混合感染が１２例，赤痢菌との混合感染が３例，サルモネラおよびＥＰＥＣとの３病原菌の混合感染が２例であった。次に，小児におけるC. jejuni 感染の年令別分布をみると，生後１２～２０ケ月の年令グループに最も陽性例の多いことが示された。従って，この結果はこれに関しての研究者の一般的見解によく一致した。

今回実施したV. fluvialis およびA. hydrophila の検査では，これらの菌による小児の下痢症例はきわめて稀であった。しかしながら，東南アジアおよび他の地域においてこの種の細菌による下痢症の報告は必ずしも少なくないので，ケニアにおける下痢症の調査には，これらの下痢起因菌は決して除外すべきではなく，今後さらに多くの小児下痢患者を対象とした調査が行なわれる必要がある。

(b) ケニアにおける毒素原性大腸菌（ＥＴＥＣ）の調査

この調査は１９８３年に，主として Coast 州および South Nyanza 地域で実施された。Coast 州においては前述のキャンピロバクター腸炎の調査対象と同じ小児下痢患者について，同時にＥＴＥＣの検査を行った。その結果は６０％にＥＴＥＣが検出された。この内，ＬＴとＳＴの両腸管毒を産出する大腸菌が２株，ＬＴのみの産出菌が２１株，ＳＴのみの産出菌が２４株であった。一方，South Nyanza および Kwale 地区に住む健康者４７４人についてもＥＴＥＣの検査を実施した。この場合のＥＴＥＣ検出率は4.4％で前述の小児下痢患者の検出率との間に有意の差は認められなかった。小児下痢患者におけるＥＴＥＣ感染率の年令別分布をみると，２５～３０ケ月の年令グループが最も高率であることが示された。新生児においてＥＴＥＣ感染が比較的低率であったことは，前述のキャンピロバクター感染の場合と同様である。

今回のわれわれが行った調査の限りでは，ケニアにおけるＥＴＥＣ感染率は，キャンピロバクターおよび赤痢菌の感染に比べて低い結果が示された。従って，ＥＴＥＣが，少なくともこの国における主要な下痢症の起因菌ではないことが示唆された。しかしながら今後は，年間を通してのより詳細な調査を実施することにより，この国におけるＥＴＥＣの感染率と季節的変動をより正確に把握しておく必要があろう。

3.1.3.1

THE PREVALENCE OF INTESTINAL PROTOZOA IN NAIVASHA, KITUI, MACHAKOS, TAVETA AND NANDI HILLS AREAS IN KENYA

M. ISEKI[1], K. HAYASHI[2*], S.M. GATIKA[3] and T.K. ARAP SIONGOK[3]

[1] Department of Medical Zoology, Osaka City University Medical School, Osaka, Japan

[2] Department of Virology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

[3] Division of Communicable Diseases and Control, Ministry of Health, Nairobi, Kenya

During the period from May to November in 1980, a total of 2,114 stool specimens were collected from individuals living in Naivasha, Kitui, Machakos, Taveta and Nandi Hills areas in Kenya, and were examinedmined for intestinal protozoa by formol-ether concentration method followed by iodine-staining.

Out of 2,114 specimens 673 (31.8%) were positive for Entamoeba histolytica, 1,105 (52.3%) for Entamoeba coli, 102 (4.8%) for Endolimax nana, 184 (8.7%) for Iodamoeba butschlii; 176 (8.3%) for Giardia lamblia, and 220 (10.4%) for Chilomastix mesnili. The total positive rate, which means the percentage of positive persons for any kinds of intestinal protozoa, was 75.1%.

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* Present Adress: Institute of Hublic Health, Ohita Prefecture, Ohita, Japan

ケニア諸地域住民の腸管寄生原虫感染状況

井関基弘[1]・林　薫[2]・Simon M. Gatika[3]
T. K. Arap Siongok[3]

1　大阪市立大学医学部医動物学教室
2　長崎大学熱帯医学研究所ウイルス部（現：大分県公害衛生センター）
3　Division of Disease Control and Research, Nairobi, Kenya

１９８０年５月から１１月の間にケニアのNAIVASHA, KITUI, MACHAKOS, TAVETA および NANDI HILLSの住民2,114人から採取した糞便についてホルマリン・エーテル法により腸管寄生原虫のシストの検査を行ない感染状況を調査した。

その結果，赤痢アメーバは31.8%，大腸アメーバは52.3%と極めて高率を示し，その他は小形アメーバ4.8%，ヨードアメーバ8.7%，ランブル鞭毛虫8.3%，メニール鞭毛虫10.4%であった。腸トリモニナスおよびEntamoeba hartmanniも少数例検出されたが，大腸バランチジウムやイソスポーラなどは検出されなかった。総陽性率（陽性総数／検査総数）は75.1%にもおよび，飲料水，食物など生活環境が糞便によって高度に汚染されていることが示唆された。陽性率に男女間の有意差は認められなかった。年令別にみると，４才以下の乳幼児でもすでにかなり高率に感染がみられるが，ランブル鞭毛虫を除き，特に３０才代から４０才代で最高値を示した。ランブル鞭毛虫は若年令層ほど高い陽性率を示し，４才以下が最高であった。

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3.1.3.2

PARASITOLOGICAL FINDINGS IN DIARRHOEIC STOOLS AT THE COAST PROVINCIAL GENERAL HOSPITAL, MOMBASA

Y. Aoki[1], E. Wambayi[2], M. Iwanaga[1*], Y. Makino[1*], M.N. Maina[3]

1, Institute for Tropical Medicine, Nagasaki University, Nagasaki

2, Kenya Medical Research Institute, Nairobi , Kenya

3, Coast Provincial General Hospital, Mombasa , Kenya

The bacterial, viral and parasitic agents that cause diarrhoea were studied in June and July, 1981 at the Coast Provincial General Hospital, Mombasa, under the Kenya-Japan technical co-operation programme on communicable diseases in Kenya. The present paper deals with the aetiology of diarrhoea from the parasitological point of view.

Stool specimens were collected from 378 patients. Of these, 87 were diarrhoeic, 73 were semi-formed and 218 were formed stools. For the detection of trophozoites the freshly passed stools of the diarrhoeic and semi-formed were examined in direct saline fecal smear. The remaining of the diarrhoeic and semi-formed specimens and the formed stools were preserved in 4 % formol-saline, and then concentrated using Formaline-Ether concentration, and examined for the cysts of protozoa, ova and larvae of helminths.

Table 1 shows the result of parasitic findings of the three types of stool examined. Out of 378 stools, 205 (54.2%) were found to be positive. In diarrhoeic specimens 44 (50.5%) were found to have one or more species of intestinal parasites, in the semi-formed 45 (61.6%), and in the formed 116 (53.2%). Faecal samples contained 4 species of amebae, 3 species of flagellates, 4 species of nematodae, and one species each from trematodae and cestodae. The common entero-pathogenic parasites are Trichuris trichiura (27.5%), hook worm(4.0%), Entamoeba histolytica (6.3%), Ascaris lumbricoides (5.3%), and Giardia lamblia (2.9%). These parasites appeared with approximately equal frequency in diarrhoeic, semi-formed and in formed stools.

Of 87 diarrhoeic stools, 74 were examined for entero-pathogenic bacteria. Entero-pathogenic bacteria were isolated from 24 cases. Of the remaining 50 specimens, 27 passed one or more species of intestinal parasites. The parasites found in diarrhoea of non-bacterial origin are listed in Table 2. The most common enteropathogenic parasite was T. trichura and followed by hook worm and E. histolytica. G. lamblia was found in diarrhoeic stools of children.

Recently the emphasis was put on the role of intestinal parasites in causation of acute diarrhoea. Diarrhoea, however, is not a characteristic symptoms of intestinal parasitic infection, and percentage of asymptomatic infection is usually high. These facts probably reflect our finding that the entero-pathogenic parasites appeared with almost equal frequency in diarrhoea and control cases. So it remains in doubt to identify the parasites found in diarrhoeic samples, even in the samples free from entero-pathogenic bacteria, as the causative agent of acute diarrhoea. Several factors, for instance, coexistence of malnutrition or bacterial enteric infection, may be involved in the aetiology of parasite-related diarrhoea.

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* Present Address: Ryukyu University, School of Medicine, Okinawa, Japan

Table 1. Parasitological Examination of Stool at Coast Prov. Gen. Hospital, Mombasa, Kenya

	Diarrheic+	Semi-formed+	Formed++	Total
No. examined	87	73	218	378
No. positive for trophozoit, cyst ova, larva	44 (50.5)	45 (61.6)	116 (53.2)	205 (54.2)
Amoebae				
Entamoeba histolytica	6 (6.9)	4 (5.5)	14 (6.4)	24 (6.3)
E. coli	6	11	48	65 (17.2)
Endolimax nana	1	2	18	21 (5.6)
Iodamoeba butschlii	2	0	9	11 (2.9)
Flagellates				
Giardia lamblia	2 (2.3)	6 (8.2)	3 (1.4)	11 (2.9)
Trichomonas hominis	7 (8.0)	4 (5.5)	ND	11 (6.9)
Chimomastix mesnili	4	3	12	19 (5.0)
Nematodae				
Ascaris lumbricoides	3 (3.4)	9 (12.3)	8 (3.7)	20 (5.3)
Hook worm	12 (13.8)	10 (13.7)	31 (14.2)	53 (14.0)
Trichuris trichiura	31 (35.6)	25 (34.2)	48 (22.0)	104 (27.5)
Rhabditis larva	1	2	2	5 (1.3)
Trematodae				
Schistosoma mansoni	0	0	3	3 (0.8)
Cestodae				
Taenia spp.	0	0	1	1 (0.3)
Mix infection	16 (18.6)	20 (27.4)	52 (23.9)	88 (23.3)

+ : Direct smear and concentration method were combined.

++ : Concentration method alone.

( ) : per cent

Table 2 Parasitological Finding of Diarrhoea of Non-Bacterial Origin

	Child	Adult	Total
No. positive for parasites	16	11	27
Entamoeba histolytica	4	2	6
E. coli	2	3	5
Endolimax nana	0	1	1
Iodamoeba butschlii	0	2	2
Giardia lamblia	2	0	2
Trichomonas hominis	4	3	7
Chilomastix mesnili	2	2	4
Ascaris lumbricoides	3	0	3
Hook worm	4	3	7
Trichuris trichiura	9	7	16
Rhabditis larva	0	1	1
Mix infection	8	7	15

ケニア国モンバサ，Coast Provincial General Hospitalにおける下痢便の寄生虫学的検査成績

青木克己[1]，　E. Wambayi[2]，　岩永正明[1*]，　牧野芳大[1*]，
M. N. Maina[3]

1　長崎大学熱帯医学研究所
（*現：　琉球大学医学部）
2　Kenya Medical Research Institute, Nairobi, Kenya
3　Coast Provincial General Hospital, Mombasa, Kenya

伝染病対策プロジェクトで，1981年6月より7月にかけ，Coast Provincial General Hospital において，下痢便の細菌学的，ウイルス学的，寄生虫学的検査が行なわれた。ここでは，主として寄生虫学的観点よりその成績を検討し，寄生虫性下痢について考える。

寄生虫学的検査を行った378検体（下痢便87．軟便73．固形便218）中，205例(54%)に寄生虫感染がみられた。原虫7種，蠕虫卵5種，蠕虫幼虫1種がみとめられたが，その内主たる原虫蠕虫の感染率は，赤痢アメーバ6.3%，ランブル鞭毛虫2.9%，鞭虫27.5%，鉤虫14.0%，回虫5.3%であった。便の性状の違いによる検出される寄生虫種の違い，また感染率の違いはみられなかった。（表1）

細菌，寄生虫学的両検査が行なわれた下痢便74検体の所見を記すと，腸管病原菌が認められたもの24例（内12例は寄生虫との混合感染），腸管病原菌の感染なく寄生虫感染がみとめられたもの27例（内1例はロタウイルスの混合感染），細菌寄生虫感染がともにみられないもの23例（内2例ロタウイルス感染）であった。尚血性下痢9例中6例に赤痢菌1例に赤痢アメーバ栄養型が検出された。

腸管病原菌が検出されなかった下痢便27例にみられた主な寄生虫は，鞭虫16例，鉤虫7例，赤痢アメーバ6例，回虫3例，ランブル鞭毛虫2例であった。（表2）モンバサ地区においては，これらの寄生虫は下痢の一要因として考慮すべきである。

一般に発展途上国における下痢の主たる要因は腸管病原菌といわれているが，近年寄生虫感染も一つの重要な要因と報告されている。

しかし寄生虫学的検査所見のみからは寄生虫性下痢を論じることは困難であり，今後寄生虫性下痢を明かにするには，細菌，ウイルスを主体とする感染性下痢，およびスプルー等の非感染性下痢の研究を同時にすすめる必要がある。

3.2.1

EPIDEMIOLOGICAL STUDY OF SCHISTOSOMA HAEMATOBIUM INFECTION IN COASTAL AREA, KENYA

1. Distribution of Schistosoma haematobium infection in Kwale district

J. Ouma[1], E. Wambayi[1], F. Waidaka[1], M. Shimada[2] and Y. Aoki[2]

[1]Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya

[2]Department of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan

Introduction

Since the general information on Schistosoma haematobium in Kwale district was scanty and not well organized, the present study was carried out, as a first step of the programme, to gather sufficient data regarding the distribution of the disease and to establish an adequate meaningful S. haematobium infection research. From September to November in 1981, the primary school children of standard 4, 5 and 6 were examined to determine the distribution of S. haematobium in order to obtain a sketch map of S.haematobium and thus select a pilot area for research. research.

Description of the study area (Figs. 1 and 2)

Kwale district is an administrative area, approximately 8,257 square kilometers in size and situated in the southern coastal of Kenya. It faces the Indian ocean in the east and bounds the northern part of Tanzania on the south. Geographically, it can be separated into three areas; the coastal strip,

the hills and the hinterland. Among the four administrative divisions, Central and Southern divisions belong to the coastal strip, Kubo division is in the hilly area and Kinango division belongs to the hinterland. The hinterland is the driest of the three zones.

The population census data of Kwale district taken in 1979 is shown in Table 1.

Materials and methods

Out of the 135 primary school in Kwale district, 41(30%) were chosen at random for the studies; 11 in Kinango division, 8 in Central, 7 in Kubo and 15 in Southern divisions. The urine samples were collected from the school children around midday and kept in a refrigerator overnight without any preservatives. The samples were examined the next morning by the sedimentation method.

Results and Discussion (Table 2)

Schistosomiasis haematobium was found to be widely distributed in the whole Kwale district except for a few locations. Approximately, about a half of the children were found to be positive for S. haematobium and the infection rate was significantly higher in males than in females (chi-square value = 10.657 degree of freedom = 1).

The infection rates differed widely from school to school. The infection rate was the highest in Kinango and Southern divisions. The other two divisions showed relatively low infection rates.

The highest infection rate in Kinango division probably indicates that in this dry area the limited water sources are highly contaminated with human excreta and consequently infec-

tion of snails fully established in this area. This hypothesis is supported by the geographical distribution of E. Coli (see the report on intestinal parasites), which is considered to be a good indicator of water contamination with faces. However, the very low infection rate in 4 schools and the differences in the infection rates among divisions may merely reflect the differences in populations of vector snails in those areas.

Table 1. 1979 Population census data of Kwale District

		Kinango	Kubo	Central	Southern	Total
Population	Male	44,351	14,318	23,377	59,700	141,746
	Female	50,362	14,932	22,950	58,373	146,617
	Total	94,713	29,250	46,327	118,073	288,363
Size of land (km^2)		3,837	454	340	3,331	8,257
Population density ($/km^2$)		24	64	135	35	34

Table 2. Infection rate of S. haematobium in each school

School	No.	MALE Exa	Neg	Pos	(%)	FEMALE Exa	Neg	Pos	(%)	TOTAL Exa	Neg	Pos	(%)
Mwagodzo	B-01	21	11	10	(48)	35	15	10	(29)	56	36	20	(36)
Lukore	B-03	58	41	17	(29)	53	40	13	(25)	111	81	30	(27)
Shimba Hills	B-07	34	29	5	(15)	35	29	6	(17)	69	58	11	(16)
Majimboni	B-08	66	43	23	(35)	52	29	23	(44)	118	72	46	(39)
Mwapala	B-09	50	30	20	(40)	52	34	18	(35)	102	64	38	(37)
Baakanda	B-13	38	16	22	(58)	15	5	10	(67)	53	21	32	(60)
Kibuyuni	B-20	32	28	4	(13)	33	33	0	(0)	65	61	4	(6)
Subtotal		299	198	101	(34)	275	195	80	(29)	574	393	181	(32)
Ngonzini	G-05	25	2	23	(92)	4	0	4	(100)	29	2	27	(93)
Nzovuni	G-07	18	0	18	(100)	8	2	6	(75)	26	2	24	(92)
Mazola	G-08	33	8	25	(76)	20	3	17	(85)	53	11	42	(79)
Yapha	G-09	19	3	16	(84)	1	0	1	(100)	20	3	17	(85)
Mtaa'	G-11	23	6	17	(74)	11	2	9	(82)	34	8	26	(76)
Makamini	G-15	13	5	8	(62)	2	0	2	(100)	15	5	10	(67)
Vigorungani	G-19	49	17	32	(65)	9	1	8	(89)	58	18	40	(69)
Mtumwa	G-23	32	13	19	(59)	5	3	2	(40)	37	16	21	(57)
Mwalukombe	G-26	26	6	20	(77)	20	7	13	(65)	46	13	33	(72)
Kilimangodo	G-32	32	14	18	(56)	19	9	10	(53)	51	23	28	(55)
Chanzou	G-35	23	21	2	(9)	0	0	0		23	21	2	(9)
Subtotal		293	95	198	(68)	99	27	72	(73)	392	122	270	(69)
Ngombeni	C-01	54	42	12	(22)	60	52	8	(13)	114	94	20	(18)
Pungu	C-04	68	49	19	(28)	48	36	12	(25)	116	85	31	(27)
Mbweka	C-08	40	21	19	(48)	27	20	7	(26)	67	41	26	(39)
Vinuni	C-11	24	16	8	(33)	14	8	6	(43)	38	24	14	(37)
Mwaligulu	C-14	64	44	20	(31)	51	31	20	(39)	115	75	40	(35)
Bilashaka	C-17	34	19	15	(44)	34	18	16	(47)	68	37	31	(46)
'amgunga	C-18	37	17	20	(54)	36	25	11	(31)	73	42	31	(42)
'oni	C-21	67	51	16	(24)	38	32	6	(16)	105	83	22	(21)
Subtotal		388	259	129	(33)	308	222	86	(28)	696	481	215	(31)
Chuwuili	S-02	49	34	15	(31)	21	16	5	(24)	70	50	20	(29)
Ngadhini	S-03	31	15	16	(52)	19	9	10	(53)	50	24	26	(52)
Mwalewa	S-04	27	18	9	(33)	14	12	2	(14)	41	30	11	(27)
Washini	S-11	19	17	2	(11)	11	11	0	(0)	30	28	2	(7)
Ramisi	S-18	52	28	24	(46)	50	32	18	(36)	102	60	42	(41)
Mwachande	S-20	16	9	7	(44)	10	7	3	(30)	26	16	10	(38)
Mwaembe	S-21	49	12	37	(90)	41	4	37	(90)	90	16	74	(82)
Jomo Kenyatta	S-24	40	12	28	(70)	30	10	20	(67)	70	22	48	(69)
Mwangulu	S-35	60	23	37	(62)	19	8	11	(58)	79	31	48	(61)
Mwandeo	S-38	31	30	1	(3)	43	42	1	(2)	74	72	2	(3)
Kilulu	S-40	18	15	3	(17)	23	17	6	(26)	41	32	9	(22)
Mivumoni	S-42	63	46	17	(27)	61	44	17	(28)	124	90	34	(27)
Nguluku	S-43	33	20	13	(39)	24	14	10	(42)	57	34	23	(40)
Maumba	S-44	36	32	4	(11)	14	8	6	(43)	50	40	10	(20)
Sham	S-48	43	6	37	(86)	29	5	24	(83)	72	11	61	(85)
Subtotal		567	317	250	(44)	409	239	170	(42)	976	556	420	(43)
Total		1547	869	678	(44)	1091	683	408	(37)	2638	1552	1086	(41)

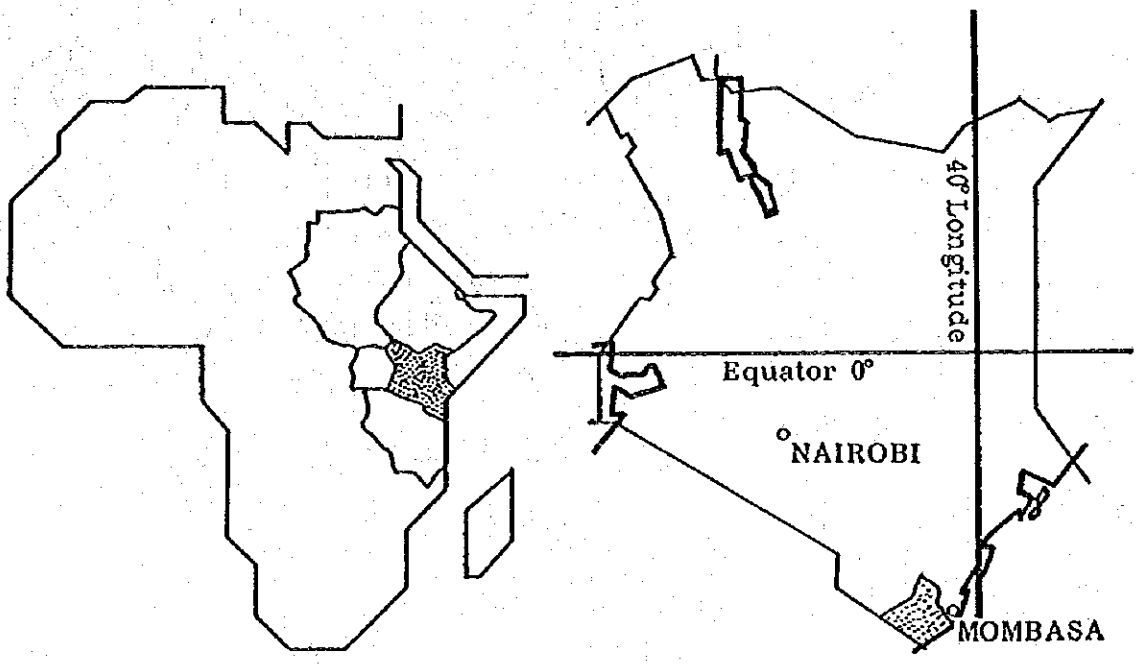


Fig. 1. Map of Kenya indicating Kwale district

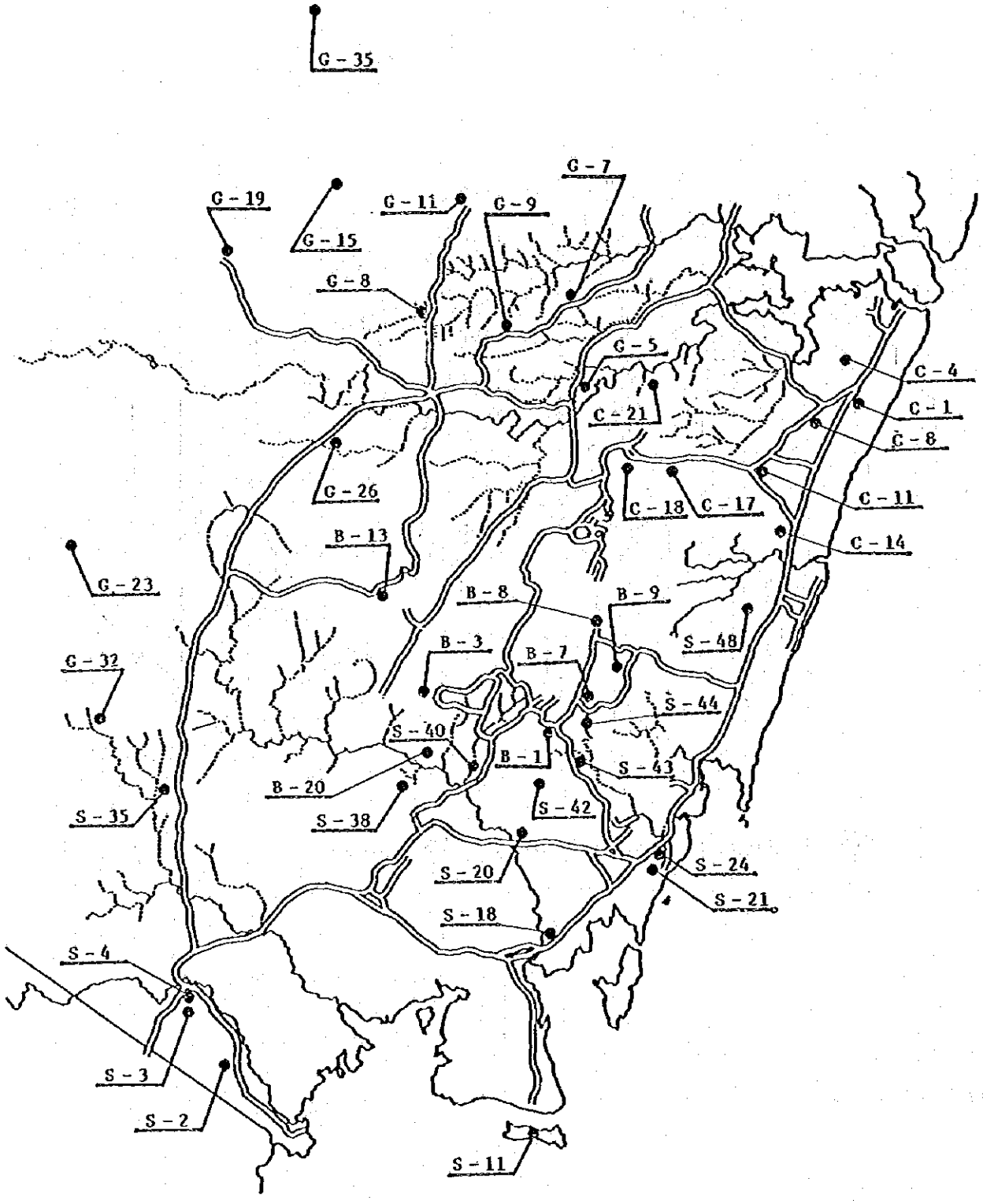


Fig. 2. Map of Kwale district.
The black dots indicate the locations of primary school, the children of which were examined for ova of S. haematobium and intestinal parasites.

ビルハルツ住血吸虫症の疫学的研究

1.クワレ地区の分布状況

J. Ouma[1], E. Wambayi[1], F. Waidaka[1]　鳩田雅暁[2], 青木克己[2]

1 Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya

2 長崎大学熱帯医学研究所寄生虫学部門

クワレ地区のビルハルツ住血吸虫症に関する調査は過去散発的なものしか見あたらず，全体を把握できる程の情報は得られていない。そこでまず全体の流行状態を知るために小学生を対象に検尿を行った。クワレ地区内には135の全学年制小学校があるが，その内の30%41校を無作為に抽出し，4.5.6年生の尿を検査した。一般にビルハルツ住血吸虫症はこの年令層で最も高い感染率を示すと考えられている。

クワレ地区はケニア共和国の南端に位置し，東はインド洋，南に国境を越えればタンザニアという所で，総面積は8,257平方キロ，ほぼ兵庫県と同じ広さを持つ。行政的に4地域(Central, Southern, Kubo, Kinango) に分けられるが，気候的には3つに区分することもできる。最も海寄りの海岸地方，やゝ海から離れた丘陵地帯，更に内陸の後背地域である。海から離れれば離れる程降雨量が少なくサバンナ的気候になると考えればよい。Central, Southernは海岸地方，Kubo は丘陵地帯，Kinango は後背地域をそれぞれ形成している。人口は1979年のセンサス結果をTable 1に示す。

検尿の結果，いくつかの例外はあるもののクワレ地区全体にビルハルツ住血吸虫は広く分布していることが明らかになった。（Table2）陽性率は全体でほぼ40%，男子が女子より有意に高い値を示した。これを行政的に分けられる4地域別にみると，後背地域のKinango で最も高く，Southernがそれに続き，Kubo, Central では比較的低い感染率であった。このことは恐らく，Kinango では充分な水に恵まれないため汚染された水をも使用せざるを得ないという状態を示していると考えられる。これらの傾向とは無関係に，調査した内の4校ではほとんど陽性者が認められず，地域的な差は単に伝搬貝の生息密度等の違いによるものかもしれない。

3.2.2

INTESTINAL PARASITIC INFECTIONS OF SCHOOL CHILDREN IN KWALE DISTRICT OF COAST PROVINCE, KENYA

M. Shimada[1], H. Nojima[2], M. Hirata[3], J. H. Ouma[4], E. Wambayi[4], S.M. Gatika[4] and Y. Aoki[1]

[1]Department of Parasitology, Institute for Tropical Medicine Nagasaki University, Nagasaki, Japan

[2]Department of parasitology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

[3]Department of Parasitology, Kurume University School of Medicine, Kurume, Japan

[4]Division of Vector Borne Diseases, Ministry of Health, and Kenya Medical Research Institute, Nairobi, Kenya

Introduction

This study was carried out to determine the distribution of intestinal protozoa and helminths in Kwale district. This provides us with some information on the sanitary condition in the area.

Materials and methods

The stool samples were obtained from the school children, whose urines were also examined for ova of S. haematobium during the period between September and November in 1981.

Each stool sample was examined for the presence of intenstinal helminths and protozoa using the formalin ether concentration method. Iodine solution was used for staining the cysts of protozoa.

Results and discussion

The results are summarized in Table 1. There was no marked difference in parasit infection rates between sexes and age groups. Some rhabditiform larvae which may indicate the infection of Storongyloides stercoralis were found in a few cases. Taenia were not found at all.

The prevalence of Entamoeba coli, Ascaris lumbricoides, hookworm and Trichuris trichiura varied from site to site(Fig. 1). Ascariasis was more common in Central and Southern divisions than in Kinango and Kubo divisions and, trichuriasis had also a similar distribution pattern. As the infection route of these two parasites is by oral ingestion, it is not surprising those two parasite infections are closely related(chi-square value=186.6 degree of freedom=1). The high population density of Central and Southern divisions may affect the sanitary condition and probably is the most important factor which provides high infection rates of ascariasis and trichuriasis in these areas rather than those in the other divisions.

Although E. coli is not generally considered a pathogen, it is a good indicator of the contamination of water by feces. The high infection rate of E. coli in Kinango division probably reflects the fact that people living in the dry area seem to have been bound to use even the contaminated water, because the safe water source has been limited in number in the area.
Hookworm has a different distribution pattern compared with those mentioned above. Although investigation should be carried out to confirm the hookworm species in this area, it is likely that Necator is more prevalent than Ancylostoma.

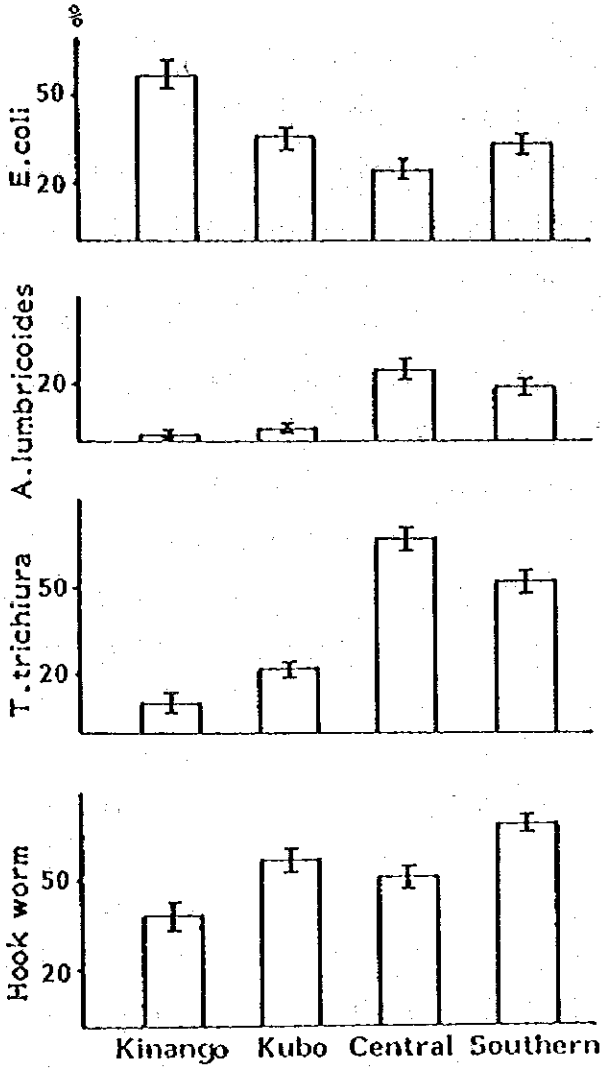
Table 1. Infection rates of parasites in each division

	Kinango	Kubo	Central	Southern	Total
E.h.	17(4.5)	26(4.9)	13(2.1)	45(5.8)	101(4.4)
E.c.	215(57.3)	190(35.5)	153(24.2)	215(32.5)	809(34.9)
E.n.	26(6.9)	8(1.5)	20(3.2)	19(2.5)	73(3.2)
I.b.	33(8.8)	29(5.4)	22(3.5)	29(3.8)	113(4.9)
G.l.	3(0.8)	4(0.7)	2(0.3)	6(0.8)	15(0.6)
C.m.	22(5.9)	18(3.4)	11(1.7)	22(2.8)	73(3.2)
A.l.	8(2.1)	23(4.3)	153(24.2)	137(17.7)	321(13.9)
H.w.	143(38.1)	307(57.4)	321(50.7)	531(68.8)	1302(56.2)
T.t.	38(10.1)	117(21.9)	416(65.7)	390(50.5)	963(41.6)
S.m.	0(0.0)	2(0.4)	1(0.2)	11(1.4)	14(0.6)
R.l.	1(0.3)	0(0.0)	1(0.2)	7(0.9)	9(0.4)
Total	375	535	633	772	2315

E.h. Entamoeba histolytica Schaudinn, 1903
E.c. Entamoeba coli (Grassi, 1879) Casagrandi and Barbagallo, 1895
E.n. Endolimax nana (Wenyon and O'Connor, 1917) Brug, 1918
I.b. Iodamoeba butschlii (von Prowazek, 1912) Dobell, 1919
G.l. Giardia lamblia Stiles, 1915
C.m. Chilomastix mesnili (Wenyon, 1910) Alexeieff, 1912

A.l. Ascaris lumbricoides Linnaeus, 1758
H.w. Hookworm
T.t. Trichuris trichiura (Linnaeus, 1771) Stiles, 1901
S.m. Schistosoma mansoni Sambon, 1907
R.l. Rhabditiform larvae

Fig 1. Infection rates of four species of parasites in each division.



クワレ地区の小学生の糞便の寄生虫学的検索

鶴田雅暁[1]，　青木克己[1]，　野島尚武[2]，　平田瑞城[3]，
J. Ouma[4]，E. Wambayi[4]，S.M. Gatika[4]

1　長崎大学熱帯医学研究所寄生虫学部門
2　鹿児島大学医学部医動物学教室
3　久留米大学医学部寄生虫学教室
4　Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya

ビルハルツ住血吸虫の分布調査と並行して同じ小学生を対象に一般的衛生状態を知るために，糞便の寄生虫学的検査を行った。検体はホルマリン・エーテル法で集卵した後，原虫嚢子の観察を容易にするためヨード染色を施した。

結果はTable 1に示している通りで，どの寄生虫についても男女間，年令層による陽性率の差は認められなかった。糞線虫と思われるラブディチス型幼虫が極めて少数認められたが，条虫類はまったく見い出せなかった。陽性例の多かった回虫，鞭虫，鉤虫，大腸アメーバの陽性率は地域によって著しい差を示した。（Fig 1）その内，回虫と鞭虫が似たような地域差を示すのはその伝搬様式が共に経口感染であることによるものと考えられる。人口密度の高いことがこの二種の伝搬にとって重要な因子とも言われているが，この結果はそれを支持しているように思える。

大腸アメーバは非病原性と考えられているが，水の汚染が強いと思われるKinangoで高い陽性率を示し，水汚染の指標として有用かもしれない。鉤虫の感染率を地域別にみると，回虫，鞭虫とはまったく異った分布パターンを示した。従ってその種は主に経口感染すると言われているズビニ鉤虫ではなく，アメリカ鉤虫の可能性が高い。