Advances in research on Rotavin-M1

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Abstract:

"Rotavin-M1" is an oral live-attenuated vaccine that prevents diarrhea in children under five years old, produced from rotavirus strains G1P [1] on Vero cells. This vaccine has been studied for 16 years beginning with monitoring the local circulating strain and eventually establishing a seed lots system. The safety and immunogenicity of the vaccine were found equivalent to internationally-imported vaccines.

Keywords: ***G1P, Rotavin-M1, vaccine.***

Classification number: ***3.2***

Introduction

The discovery of the rotavirus as one of the major causes of diarrhea in children has led to the expansion of research in order to develop vaccines to prevent or reduce the incidents and mortality from this disease. Epidemiological studies of diarrhea caused by rotavirus were particularly useful as prerequisites for research into a vaccine. Specific preventative measures in Vietnam support and protect general public health, especially that of infants and young children. According to statistic data, the annual incidence of diarrhea caused by rotavirus accounts for over 50% of children under age of five years old hospitalized in pediatric hospitals in Vietnam, and about 5,300 - 6,800 deaths were reported annualy in children under five years old, accounting for 8 to 11% of the mortality rate among children of the same age. Among about 1.64 million of those born annualy, it is estimated that 820,000 clinical visits, 122,000 to 140,000 cases of hospitalizations, and 2,900-5,400 deaths were due to diarrhea caused by rotavirus. Rotavirus causes great damage to the national economy. Annual cost used for the treatment of rotavirus diarrhea in Vietnam amounted to US$ 5.3 million, of which US$ 3.1 million was direct medical expenses, US$ 685,000 was the costs spent in other sectors than health, and US$ 1.5 million was used for indirect costs. "Rotavin-M1" is an oral live-attenuated vaccine preventing diarrhea in children under five years old, produced from rotavirus strains G1P [1] KH0118 on Vero cells as identified at the Center for Research and Production of Vaccines and Biologicals - Ministry of Health.

Materials and Methods

Materials

• Vero cells from ATCC Vero cell bank of the WHO;

• Primary monkey kidney cells of Macaca mullata;

• MA104 cells provided by the US CDC;

• Rotaclon Kit;

• Reagents for determining the concentration of antigen by creating clusters of the rota necrosis and immunofluorescence method;

• RT-PCR Kit;

• Production strain type G1P8 (KH0118);

• DMEM cell culture (Dulbecco's Modified Eagle Medium) - Gibco, Cat. No 31600 pH 6.8 to 7.0, 5% fetal calf serum;

• DMEM cell washing (Dulbecco's Modified Eagle Medium) - Gibco, Cat. No 31600 pH 6.8 to 7.0, no fetal calf serum, no trypsin, no herpes;

• DMEM (Dulbecco's Modified Eagle Medium) - Gibco, Cat.No 31600 pH from 7.0 to 7.15, Herpes 0,0125 mM, trypsin 20 µg/ml;

• Medium 199, Gibco, Cat.No 31100;

• Fetal calf serum (Fetal Bovine Serum-Certified, HI Gamma Irradiated) - MP Biomedical LLC, Germany, Formula number: 2916454;

• 0.25% trypsin solution 1x, Gibco, Cat.No.15050-065;

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• Solution Hanks' Balanced Salt Solution (HBSS) 1x, Gibco, Cat. No.14175-103;

• Neomycin antibiotic solution, Sigma, N1142, Lot 104K2321;

• Plastic Bottle 225 cm^3;

• Incubators, plastic pipette using 01 types, pipe aid and other tools;

• Medium dispenser;

• Other equipment and materials sufficient for the research process.

Methods

Step 1: Monitoring of diarrhea caused by the rotavirus was conducted at the Pediatric hospitals in Vietnam and pathogenic circulating strains were identified by the RT-PCR method.

Step 2: Establishment of an attenuated virus seed strains system is performed by cloning the virus, multiple culture passages of virus lines, Vero cell adaptive virus line, and virus purification.

Step 3: Establishement of a rotavaccine production process in a laboratory and laboratory and animal experiment evaluation.

Step 4: Evaluation of the safety and immunogenicity of the vaccine by three phase clinical trials using volunteers as under the GCP process issued by the Ministry of Health.

Step 5: Vaccine production after licensing in accordance to approved procedures.

Results and discussion

Surveillance of rotavirus caused dirrhoea in Vietnam [1-14]

Vietnam is one of the countries in Southeast Asian that participates in monitoring diarrheal diseases caused by the rotavirus. This network is organized by the World Health Organization (WHO). Since 1998, the surveillance system has been set up in three regions of the country, at the Paediatric Hospital and Saint Paul Hospital in Hanoi, the Children's Hospital in Hai Phong, Khanh Hoa General Hospital, and the Children's Hospital I and II in Ho Chi Minh City. Monitoring results have shown that among children with diarrhea caused by rotavirus, children of 6-24 months of age accounted for 90% of cases. The rotavirus strain has been found circulating in South and Central Vietnam throughout the year at higher rates than the North.. In the North, due to the four distinct seasons at the subtropical climate zone, the incidence of diarrhea due to rotavirus have been found to fluctuate according to seasonal changes, with a maximum peak lasting from November to May (winter - spring season) and decreasing considerably in other seasons. The detection rate of rotavirus diarrhea in patients was 55.64% at the Paediatric Hosptal in Hanoi, 49.84% at Saint Paul Hospital, 59.28% at the Children Hospital in Hai Phong, 58.32% at Khanh Hoa General Hospital, and 58.82% at the Children's Hospital I, and 58.65% at the Children's Hospital II in Ho Chi Minh City. The average incidence of children with rotavirus acute diarrhea was 57.39% nationwide, and rotavirus strains circulating in the country in 1998-2011 period were mainly P [1] and G1.

Establish the seed lost system for rota virus vaccine production [15, 16]

Based on the results obtained from epidemiological surveillance during the 1998-2011 period, rotavirus strains G1P [1] have been chosen through selective cloning and by multiple tissue culture passages; first in MA104 cells, then in primary monkey kidney cells (pMKC) and in Vero cells. The purified virus on Vero cells was used to establish the master seed virus (MSV) and working seed virus (WSV). The obtained seed strain systems was confirmed via gene sequence and tested for adventitious agents at the Center for Prevention and Disease Control (CDC-USA), the Vietnam Academy of Science and Technology, and the National Institute for Vaccine and Bio-medical products. The strain system has been tested successfully in monkeys of 6-12 months old in safety and in immunogenicity and certified by the National Institute for Vaccine and Bio-medical products for use in rotavirus vaccine production in Vietnam since 2007.

The virulent strains of rotavirus used to establish the system of MSV was the strain code KH0118. This rotavirus strain was isolated from stool samples of patients with acute gastroenteritis caused by the rotavirus. KH0118 strain creation process is summarized as follows:

KH0118 rotavirus strains isolated from clinical specimens KH0118 using three continual isolations on MA104 cells in 1-2 µg trypsin/ml supplemented DMEM media not contained fetal bovine serum, according to rotavirus isolation procedures developed by CDC (Atlanta, USA). This strain is preserved at the Center for Research and Production of Vaccines and Biologicals (POLYVAC), located at 135 Lo Duc, Hanoi, Vietnam, to be used as raw materials to produce master seed rotavirus strains.

Cloning method (according to CDC's protocol): Each clone was inoculated into 0.1 ml medium culture suspension, then the infected MA104 cells were put into 2 ml DMEM and incubated for 72 hours at a temperature of 37^0C, in a shake-condition of 4 cycles/min, in a 5% CO_2 incubator. The rotavirus genetically homogeneous clones with the highest titers were then selected.

Attenuated rotavirus master seed strain by multiple passages: The MA104 cells in 10 g trypsin/ml supplemented DMEM were infected with 0.25 ml of obtained genetically homogeneous rotavirus and were cultured in a condition of 5% CO_2, and shook with rotation speed of 4 cycles/min for 72

hours at 37^{0}C. After the incubation, rotavirus lines with the highest titers obtained from six repeated subcultures in the same culture conditions were selected for use in the next step.

In order to adapt rotavirus strain on primary monkey kidney cells, 12 passages were performed on primary monkey kidney cells in 10 mg trypsin/ml supplemented DMEM. Samples were incubated for 72 hours at 37^{0}C, in 5% CO_2 and shake condition of 4 rounds/min. The rotavirus culture clones with the highest titers were selected.

To adapt the rotavirus strain on Vero cells, 0.25 ml of the attenuated virus suspension was adapted in primary monkey kidney cells and passaged 17 times on the Vero cells in 20 micrograms of trypsin/ml supplemented DMEM by using the same culture condition for each culture passage (72 hours of incubation at 37^{0}C in a 5% CO_2 incubator, and shaken at 4 cycles/min).

Isolation of adapted to Vero cells rotavirus master seed strain: The isolated Vero cells that adapted to the rotavirus master seed strains were obtained using a double cloning procedure.

First cloning: The Vero cell adapted virus suspensions were diluted from 10^{-1} to 10^{-7}. The concentrations from 10^{-2} till 10^{-7} were used to infect the Vero cells, and the later were incubated in a shaking condition of 3-5 rounds/minute at 37^{0}C. After the virus was harvested, virus concentration was determined by EIA and the virus samples with highest optical index (OD) of concentration of 10^{-6} were chosen for second cloning.

Second cloning: Using the same procedure as for the first cloning. The harvested virus suspension with the highest OD were selected and used as purified attenuated rotavirus strains type G1P [1].

Establish attenuated rotavirus seed strains: For obtain attenuated rotavirus seed strains, the purified attenuated rotavirus strain G1P [1] has been subcultured five times consecutively on Vero cells, then stored at -70^{0}C at the POLYVAC. This seed virus strain is used to produce working seed rotavirus strain for production of vaccine.

Development of vaccine production process and establishment of standard requirements [5]

"Rotavin-M1" is an oral live-attenuated vaccine used for the prevention of diarrhea in children under five years old, caused by rotavirus strains G1P [1] on Vero cells, identified at the POLYVAC.

"Rotavin-M1" production process:

(a) Culure the monolayer of Vero cells in plastic bottles in a 5% fetal calf serum supplemented DMEM and incubate at 37^{0}C for three days to obtain enough cell layers for vaccine production; after washing two times with DMEM without trypsin and Herpes, pH of 6.8 to 7.0, incubate the harvested cells in new DMEM at 37^{0}C for three hours;

(b) Activate the WSV strains G1P [1] KH0118 using trypsin with concentrations of 15 µg/ml at 37^{0}C for 30 minutes (in 37^{0}C thermal bath). Before this step, $CaCl_2$ (300 g/l) a concentration of two µl/ml is added to the cell solution at room temperature for at least 30 minutes;

(c) Virus absorption: Empty the cell culture bottles prepared in step 'a', add 3 ml of virus suspension activated in step 'b' into the culture bottle to obtain concentrations of infected cells reached to 0.006 FFU/ml. Let absorb forone hour at 37^{0}C, then slightly shake the culture vessels for 15 minutes each;

(d) Add the fresh medium to the infected cell cutures. The culture vessels, after absorption step, are met with 70 ml of 20 µg trypsin/ml supplemented DMEM, pH 7.0 to 7.15; then incubate the vessels at 37^{0}C in a static state;

(e) Harvest the vaccine Virus suspension: Virus suspension is harvested at day three, after adding the fresh medium from step 'd'. Before the havest, shake the culture bottle. Put the culture bottles at -20^{0}C and then thaw them. Transfer the virus solution from culture bottles into plastic bottle at 2.5 liters. The pool cell solution is preserved at -60^{0}C, and then thaw out once again;

(f) After being thawed, virus suspension is pooled in a big tank. A crude sampling of 50-100 ml is taken for inspection. Finally vaccine virus suspension is filtered using housing filters with diameters of 0.65 µm. Semi-finished vaccine solutions are preserved at <-60^{0}C;

(g) Prepare vaccine dilution to have G1P [1] virus concentration in each dose (2 ml) greater or equal to $10^{6.3}$ PFU/ml with 35% sucrose;

(h) Fill the Rotavin-M1 in glass vials of sighle dose, 2 ml per dose, storage at ≤-20°C.

Rotavin-M1 component:

Each 2 ml dose of vaccine contains:

Live human Rotavirus, attenuated strain of G1P [1]: ≥ $10^{6.3}$ PFU

Sucrose: 35%

Kanamycin: ≤10 µg/ml

DMEM (Gibco).

Rotavin-M1 clinical trial [17-20]

Rotavin-M1 is produced by the POLYVAC within the frame of state projects coded as KC10.03/06-10. Three loads of vaccines are shown to have good results for safety and immunogenic when tested in a laboratory and used on experimental animals (preclinical), especially on young rhesus monkeys, Macaca mulatta. At the same time, the vaccine was checked by the National Institute of Vaccines and Biologicals

in 3/2009 and approved by the Ethics Council of the Ministry of Health for clinical trials with three phases according to the Health Ministry's GCP. The vaccine was used by 30 adults, and 1,000 infants each of 6-12 weeks old in Thanh Son, Phu Tho province and Thai Binh City. Results confirmed the safety of the vaccine in adults, and children aged 6-12 weeks, which is the equivalent of this seen in Belgium's Rotarix vaccine. Rotavin-M1 is also showed to have a high immunune respose, equivalent of that of Belgium Rotarix vaccine.

Safety:

- Out of 30 adult volunteers at Thanh Son, Phu Tho

During the trial, no unwanted reactions were seen, no cases were lost, and no side effects appeared noticeable after 28 days after each vaccine dose was administered. After the first vaccine dose, two volunteers with viruses from the stool samples from day ten were isolated, but not of type G1. After the second vaccine dose administration, no stool samples were taken from ten days of continuous isolatation with the virus. The blood and biochemical test data, including red blood cells, leukocytes, platelets, SGOT, SGOP, and urea from after the first dose one and second dose administration revealed no difference compared to that of before taking the vaccine [17].

- Out of 200 healthy infants aged 6-12 weeks old in Thanh Son, Phu Tho [18]

The biochemical and blood cell indicators (red blood cells, leukocytes, platelets, SGOT, SGOP, and urea) after each vaccine dose administration were at normal level. Within 30 minutes after vaccine intake, no cases of symptoms appeared in place. In the first seven days after taking dose 1, 2, and 3, fever and vomiting occured negligibly in some cases. From day 8-30 after second and third dose administration, the rate of cases with fevers were found as the same in different groups. 2.5% of the children got diarrhea, but none of these cases were isolated with the vaccine virus. The same results were observed with the group of children taking Rotavin 10^6 FFU. Some cases of diarrhea have isolated strains of the vaccine virus in the feces of those taking Rotavin ($10^{6.3}$FFU). About excreted the vaccine virus in their stool, within seven days after the first dose, the percentage of children isolated with vaccine virus in stool varied from 47.5 to 55%. This rate was highest on day six and day seven. After the second dose, the proportion of children excreting virus was reduced compared with those who did after dose one (<20%). After the third dose, this rate had increased compared with the data observed after the second dose (approximately 45%), but lower than the rate after first dose and there was no difference between the proportion of children excreted virus among the group administered vaccine dose of 10^6 or $10^{6.3}$ FFU/ml. In 60-90 days after the first dose, none of case hospitalized due to unwanted reactions.

- Out of 400 children in Thanh Son, Phu Tho and 400 children in Thai Binh province [18]

None of the children appeared to have undesirable reactions after 30 minutes of vaccination. No vomiting, abdominal pains, or allergies were seen in the children during the first seven days after the first dose of the vaccination in both study sites, excepted 1.3% of cases with fever (2% in placebo group), 2.67% with diarrhea (3.02% in placebo group), 0.17% with anorexia or refused the feeding (0.5% in placebo group); 1.5% of children crying (3% in placebo group), and 1% with cough (2.51% in placebo group). seven days after the second vaccination dose, none of the children were vomiting, had loss of appetite or not eating, but the children experienced fever 2.12% (2.72% in the placebo group), 1.24% of children with had pepper flow (placebo 0.54%), 0.18% of the children had abdominal pains (placebo 0%), 1.42% of the children experienced crying (placebo 1.09%), 0.88% of the children had a cough (placebo 0.54%), and 0.18% of the children experienced allergic reactions (none in placebo group).

From 8 to 30 days after taking first vaccine dose, none of children at both study sites had allergic reactions, but 10.67% of them had fevers (7.04% in placebo group), 0.17% had vomiting, 2% had diarrhea (2.01% in placebo group), 0.17% had abdominal pain (0.5% in placebo group), 0.83% had anorexia or refused feeding; 10.83% cried (7.04% in placebo group) and 2.5% experienced a cough (2% in placebo group). Within 8-30 days after the second dose, no abdominal pain cases were observed, but 11.33% of children had fevers (8.15% in placebo group), 0.35% had vomiting, 0.71% had diarrhea (2.17% in placebo group), 1.59% had anorexia andrefusal of feeding, 9.56% cried (7.61% in placebo group), 3.09% had a cough (1.09% in placebo group) and 0.18% had allergies. In summary, 15.22% of any unwanted reactions were observed in children during the 30 days of vaccine administration, while this rate in group taken the placebo was 14.67%, no statistically significant difference between these two ratios was found.

From day 0 to 30 days, at two trial sites, after taking one dose of vaccine, no allergic cases were found, 11.50% of children had fevers (8.04% in the placebo group), 0.17% had vomiting, 4.17% had diarrhea (4.52% in placebo group), 0.17% had abdominal pain (0.5% in placebo group), 0.83% had anorexia or refusal of feeding (0.5% in placebo); 11.33% had crying (8.04% in placebo) and 3% had a cough (3.02% in placebo group). In 30 days after taking

the second dose of vaccine, 12.74% had fevers (9.78% in placebo group), 0.35% had vomiting (0.54% in placebo group), 1.95% had diarrhea (2.72% in placebo group), 0.18% had abdominal pain (none in placebo group), 1.59% had anorexia or refused to feed (0.54% in placebo group); 10.44% were crying (8.15% in placebo group), 3.72% had a cough (1.63% in placebo group) and 0.35% had a cough.

So in 30 days after taking the first and second doses of the vaccine, at both study sites, the proportion of children with symptoms such as vomiting, fever, diarrhea, abdominal pain, coughing, or crying, were low and no statistical significance difference between the groups of children receiving vaccines and placebo.

Among the 95 cases with diarrhea, it appeared during the follow-up time (30 days after each dose), four children were isolated with rotavirus in their feces, including three typed as the vaccine virus G1P [1] strain. All these diarrhea cases were observed within the virus excretion period after vaccination, other symptoms of gastroenteritis related were not found.

Immunogenicity:

IgA antibody:

Results of the phase II study on 200 infants in comparison to those who reveived Belgium's Rotarix vaccine showed that the IgA antibody titer after vaccination is as following:

Rotavin-M1 $10^{6.3}$ FFU, two doses at two months interval: 72.7%

Rotavin-M1 $10^{6.3}$ FFU, three doses at one month interval: 65.6%

Rotavin -M1 10^{6} FFU, two doses at one month interval: 60.5%

Rotavin-M1 10^{6} FFU, three doses at one month interval 55.9%

Belgium Rotarix two doses at one month interval: 59%

Results of phase III of the study on 800 children in Phu Tho and Thai Binh provinces compared with results from the placebo group showed that the mean percentage of children that were positive with the rotavirus-specific serum IgA in the vaccine group of 80.7% (ranging from 77.3% in Phu Tho to 84.3% in Thai Binh children). Meanwhile, in the placebo group, this rate was 13.3% (from 11.1 to 15.2% respectively in Phu Tho and in Thai Binh), corresponding to the natural virus infection of children. After vaccination with two doses, antibody titres (geometric mean) reached 85.9 (95% CI: 74.5 to 99.1), 54.5-60.1% of these had a titer ≥80.

IgA antibodies immune response level produced by the vaccine batch of high-titer (from 160 to 640) accounted for 42.35% while that produced by the batch of good titer levels accounted for only 8.55% and 14.31% produced by the vaccine batch of moderate titer reached. The batch with very high titer levels stimulated IgA immuno response in 17.69%.

IgA antibody dynamic after one year vaccinated with $10^{6.3}$ FFU of Rotavin or Rotarix showed almost stable with 54.84% in group received Belgium's Rotarix and 66,67% in those vaccinated with Rotavin-M1.

IgG antibody:

Although 100% of the children have the RV-specific IgG antibody before vaccination (transfered from mother), the proportion of children having the IgG antibody response after vaccination reached 72.8 to 85.3%. The geometric mean of the IgG titer has increased 1.4 times compared to that seen before vaccination while it decreased from 7.6 to 8.7 times in children of placebo group in both study sites.

Dose and schedule of vaccine administration:

Children of 6-12 weeks of age get the first dose with following vaccination schedule:

Rotavin-M1 $10^{6.3}$ FFU, two doses at two months interval: 80.7%

Rotavin-M1 $10^{6.3}$ FFU, three doses at one month interval: 65.6%

Rotavin -M1 10^{6} FFU, two doses at one month interval: 60.5%

Rotavin-M1 10^{6} FFU, three doses at one month interval: 55.9%

Belgium Rotarix, two doses at one month interval: 59%.

Licensed Rotavin-M1 vaccine, production and use of vaccine after licensed

The vaccine was licensed to the manufacture and used widely with high effective for disease prevention and economic efficiency. Currently, rotavirus vaccine is not included yet as part of the National Expanded Immunization program, but is being used as a health care socialized service. Before Rotavin-M1 is licensed, Rotarix and RotaTeq were the rotavaccines used in the Vietnamese market. Two of these vaccines are imported vaccines that have very high costs, so can be used mainly the big cities for people with good economic conditions. Rotavin-M1 vaccine with similar quality and with the price of 1/3 imported vaccine'price, will be highly response to the needs of Vietnamese community.

Since 2012 after Rotavin-M1 was licensed, more than 500,000 doses of the vaccine and 18 lots of the bulk vaccine amounted 200 liters equivalent to 400,000 doses has been produced. The production line and vaccine testing schedule have been established with over 60 scientists and technical staff. Several new technologies have been applied and routinely deployed in the POLYVAC as plaque cloning technique, technical limits diluted concentrations,

PCR, Sequencing etc.

Rotavin-M1 vaccine was officially approved as the national product by Decision 2441/QD-TTg of the Prime Minister. With this program Rotavin-M1 vaccine would be produced for mass serving in national vaccination programs and exporting to world markets.

Since being approved in May 2012, the Rotavin-M1 vaccine has been used in almost all provinces and cities nationwide. More than 500,000 doses of vaccine have been used, effectively preventing infection for about 250,000 children. POLYVAC has gained 40 billion in sales, benefiting about 90 billion for society in comparison to using of imported vaccines.

Conclusions

The vaccine "Rotavin-M1" is an oral live-attenuated vaccine to prevent diarrhea disease for children under five years old, and has been produced from rotavirus strain G1P [1] on Vero cells at the POLYVAC through the Vietnamese Ministry of Health. The Rotavin-M1 vaccine has been established and developed through five stages: i) Monitoring the circulating of rotavirus strain; ii) Establishing the seed virus strain system; iii) Establishing the production process, testing and vaccine standard criteria according to WHO; iv) Implementing the clinical trials; and v) Licensing and posting of the vaccine licensure surveillance. All of these stages were performed according to the process requirement of WHO. Rotavin-M1 is safe and effective for children under five years old to prevent from the disease.

REFERENCES

[1] Nguyen Dang Hien, Le Thi Luan (2011), "Monitoring circulating strains of rotavirus in Vietnam in 2010", *Journal of Preventive Medicine*, **Vol.XXI, No.5 (123)**, pp.71-77.

[2] Le Thi Luan and Nguyen Nu Anh Thu (2003), "Genotype and phenotype of rotavirus caused accute gastroenteritis in children under 5 years old in Khanh Hoa Academy of Pediatrics, 2002", *Provision Medical Journal*, **Vol.XIII, No.4(61)**, pp.49-51.

[3] Le Thi Luan and Nguyen Nu Anh Thu (2004), "The biological characteristics of rotavirus caused diarrhea in children under 5 years old at the Children's Hospital Haiphong (7/2001-6/2002)", *Provision Medical Journal*, **Vol.XIV, No.1(64)**, p.27.

[4] Le Thi Luan and Nguyen Thuy Huong (2003), "Identify and type of rotavirus in diarrhea pediatric patients at Children's Hospital Swedish first 9 months of 2003", *Practical Journal of Medicine*, **4(451)**, pp.64-66.

[5] Le Thi Luan, Nguyen Dang Hien (2011), *Rotavin-M1 vaccine to protect diarrhea. Safety and immunnity response*, Medical Publishing House.

[6] Nguyen Dang Hien, Le Thi Luan, et al. (2012), "Circulating strains of rotavirus in 2010 in Vietnam", *Journal of Preventive Medicine*, **Vol.XXII, No.4(131)**, pp.17-21.

[7] Nguyen Dang Hien, Nguyen Thi Mai Huong, Bui Duc Nguyen, Dang Duc Anh, Le Thi Luan (2010), "Monitoring pathogenic strains of rotavirus acute gastroenteritis in children under 5 years of 2008", *Journal of Preventive Medicine*, **Vol. XX, No.5(113)**, pp.23-27.

[8] Nguyen Dang Hien, Nguyen Thi Mai Huong, Pham Thi Phuong Thao, Le Thi Luan (2011), "Monitoring results due to rotavirus in 2009 in Vietnam", *Journal of Preventive Medicine*, **Vol.XXI, No.1(119)**, pp.10-15.

[9] Nguyen Dang Hien, Pham Thi Phuong Thao, Le Thi Luan (2009), "Rota monitoring circulating strains cause diarrhea in Vietnam from 1998 to 2009", *Journal of Preventive Medicine*, **Vol. XXI, No.1(119)**, pp.5-9.

[10] Joseph Bresee, Nguyen Van Man, Le Thi Luan, et al. (2004), "First Report from the Asian Rotavirus Surveillance network", *Emerging Infectiuos Diseases*, **10(6)**, pp.988-995.

[11] Nguyen Van Man, Le Thi Luan, Dang Duc Trach, Nguyen Thi Hien Thanh, Phan Van Thu, Nguyen Thanh Long, Dang Duc Anh, Jon R. Gentsch, Roger Glass (2005), "Epidemiological Profile and Burden of Rotavirus Diarrhea in Vietnam: 5 Years of Sentinel Hospital Surveillance, 1998-2003", *JID*, **192,** Supplement 1, S127-132.

[12] Nguyen Van Man, Le Thi Luan, Tran Hong Thuy, et al. (2004), *Immunocytochemical study of the rotavirus interaction with host cells in the culture*, Publising House of Vietnam National University, Hanoi, Asean-MC4, pp.6-10.

[13] Nguyen Van Man, Nguyen Van Trang, Huynh Phuong Lien, Dang Duc Trach, Le Thi Luan, et al. (2001), "The Epidemiology and Disease Burden of rotavirus in Vietnam: Sentinel Surveillance at 6 hospitals", *The Journal of Infectious Disease*, **183**, pp.1707-1712.

[14] Nguyen Van Man, Nguyen Van Trang, Le Thi Luan, Huynh Phuong Lien, Dang Duc Trach, et al. (2004), *The Epidermiology and burden of Rotavirus diarrhea in Vietnam: Five years of Sentinel Hospital Surveillance 1998-2003*, Vietnam National University Publishers, Hanoi, The proceeding of The 2nd Vietnam-Lao-Cambodia, pp.20-30.

[15] Le Thi Luan, Nguyen Thuy Huong, Nguyen Dang Hien, Nguyen Thanh Thuy, Nguyen Thi Minh Lien, Ennio Lucio Benedetti, Irene Dunia (2011), *Immunocytochemical Chacacterization of rotavirus entry into monkey kidney cells in culture. Viruses Responsible for Emerging Diseases in South - East Asia*, Jacques Monod Institute/CNRS Universite Paris Diderot - Chapter 7, pp.225-238.

[16] Luan T. Le, Trang V. Nguyen, Phuong M. Nguyen, Nguyen T. Huong, Ngo T. Huong, et al. (2009), "Development and characterization of candidate rotavirus vaccine strains derived from children with diarrhea in Vietnam", *The official Journal of the International society for Vaccine*, **27**, Supplement 5, pp.130-138.

[17] Dang Duc Anh, Nguyen Van Trang, Vu Dinh Thiem, Nguyen Hien Anh, Nguyen Dang Hien, Le Thi Luan, Nguyen Van Mao (2010), "The safety of vaccines Rotavin-M1 produced in POLYVAC on adult volunteers", *Provision Medical Journal*, **Vol.XX, No.7(115)**, pp.19-28.

[18] Dang Duc Anh, Nguyen Van Trang, Vu Dinh Thiem, Nguyen Hien Anh, Nguyen Dang Hien, Le Thi Luan, Nguyen Van Mao, Bui Duc Nguyen, Vu Thi Bich Hau, Le Thi Hong Nhung, Le Thi Kim Anh (2010), "The safety of Rotavin-M1 vaccine on children under 6-12 weeks old", *Provision Medical Journal*, **Vol.XX, No.10(118)**, pp.5-14.

[19] Vu Dinh Thiem, Nguyen Van Trang, Nguyen Thi Hien Anh, Le Huy Hoang, Nguyen Dang Hien, Le Thi Luan, Thien Anh Tong, Nguyen Van Mao, Nguyen Van Thom, Tran Van Dui, Bui Duc Nguyen, Vu Thi Bich Hau, Le Thi Hong Nhung, Le Thi Kim Anh, Ngo Thi Thanh Hoa, Nguyen Anh Tuan, Dang Duc Anh (2011), "The safety of the Rotavin-M1 in children under 6 to 12 weeks old at the Phu Tho and Thai Binh", *Journal of Preventive Medicine*, **Vol.XXI, No.2(120)**, pp.99-111.

[20] Dang Duc Anh, Nguyen Van Trang, Vu Dinh Thiem, Nguyen Thi Hien Anh, Nguyen Duc Mao, Baoming Jiang, Nguyen Dang Hien, Le Thi Luan (2012), "A dose-escalation safety and immunogenicity of a new live attenuated human rotavirus vaccine (Rotavin-M1) in Vietnamese children", *Vaccine*, A114-A121.