

46th Joint Working Conference on Viral Diseases
The Japan – United States Cooperative Medical Science Program
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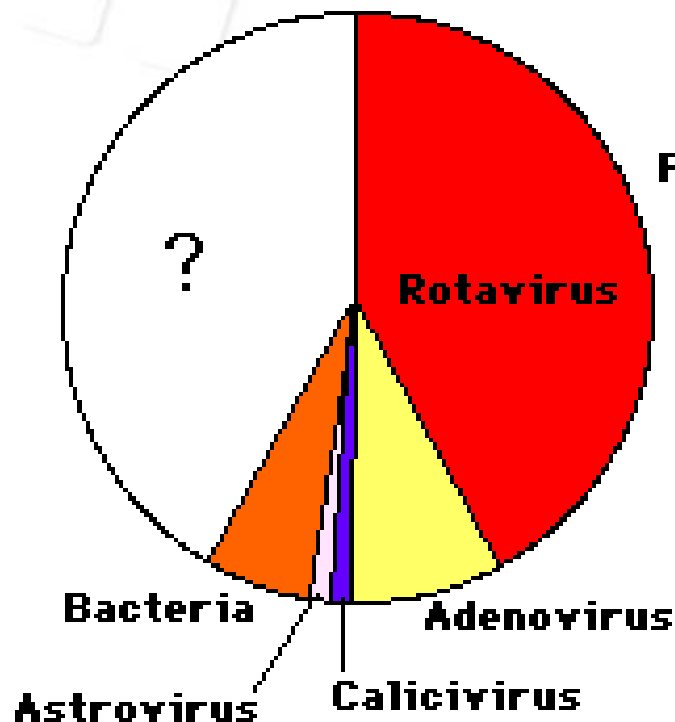
**Immunoglobulin Y (Ovalgen RV) as adjunct
to standard supportive therapy for
rotavirus-associated diarrhea among
pediatric patients**

Shofiqur Rahman et al.

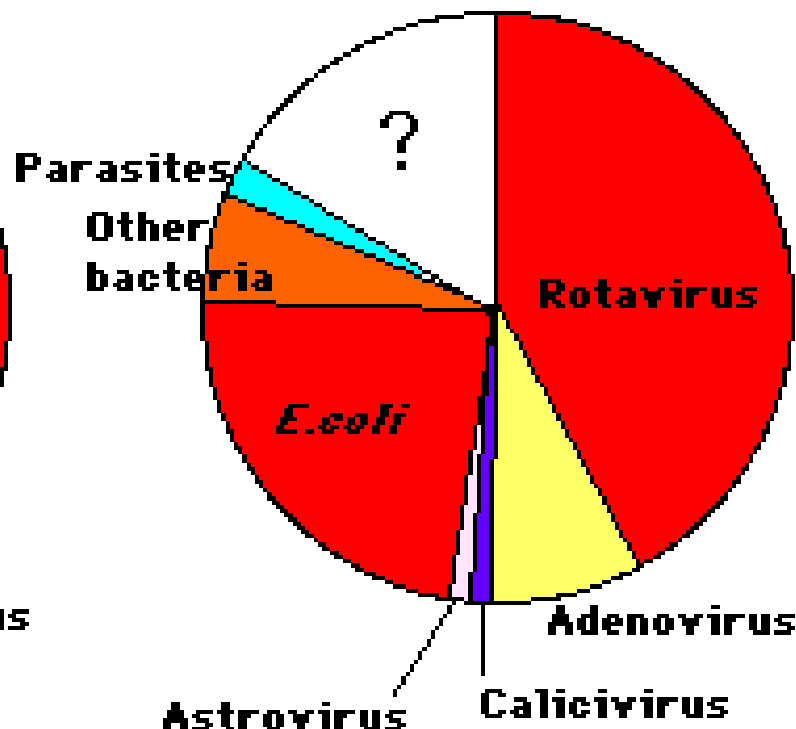
Immunology Research Institute in Gifu, Japan

Rotavirus – leading cause of diarrhea among children in all countries

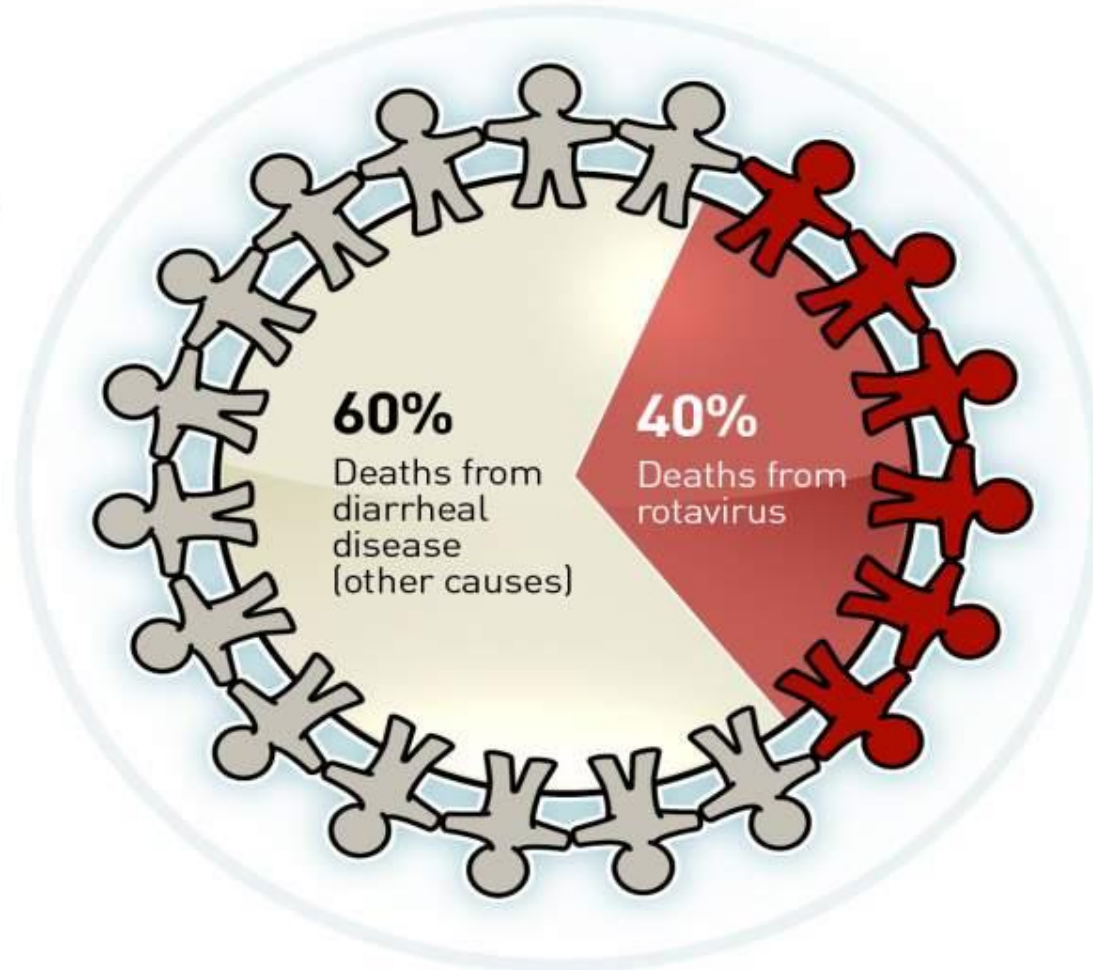
Developed Countries



Developing Countries



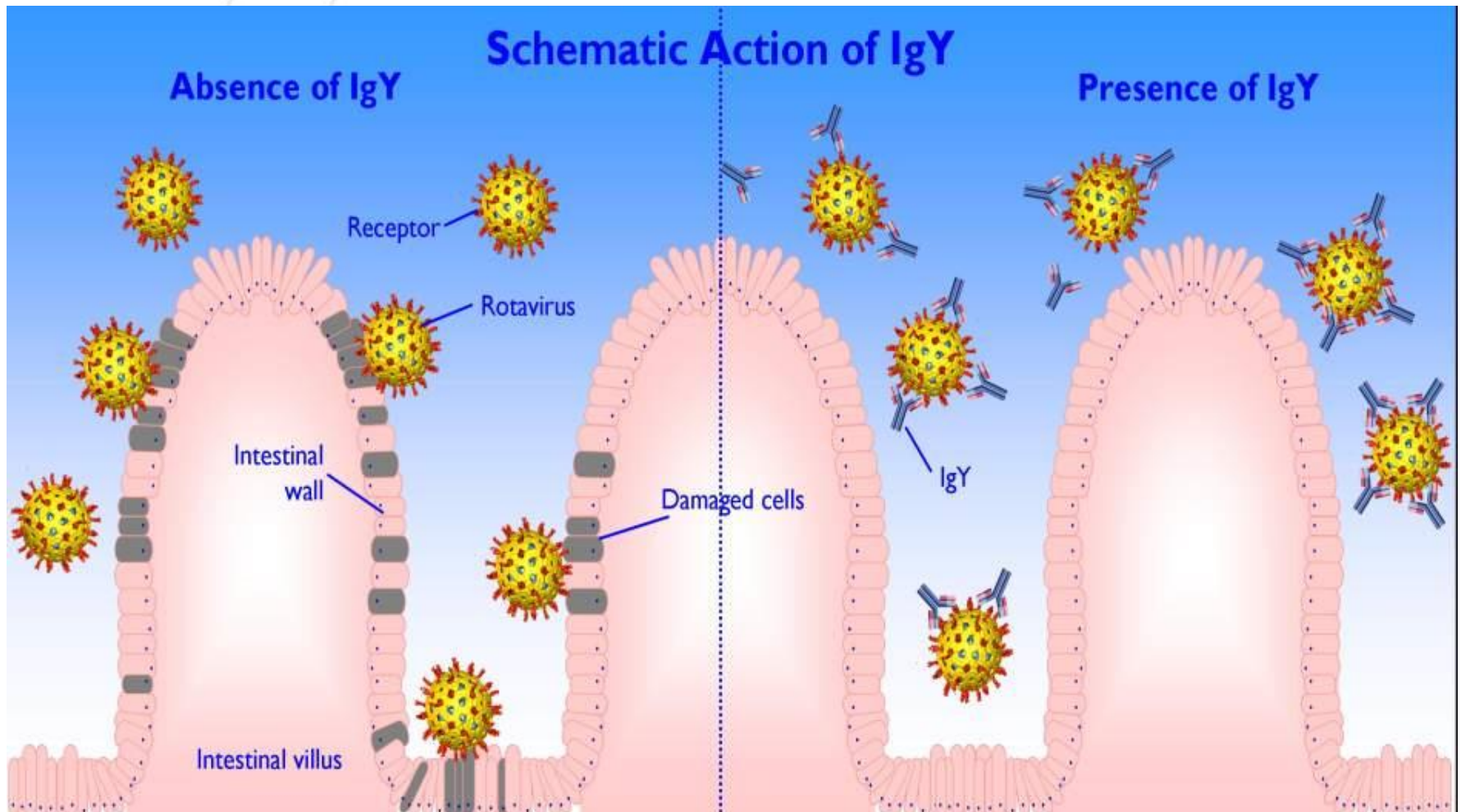
Death rate due to rotavirus infection



Rotavirus – a global problem

- Each year rotaviruses cause approximately 111 million episodes of gastroenteritis in children, which result in:
 - 25 million visits to clinics,
 - 2 million hospitalizations, and
 - 352,000 to 592,000 deaths.
- On a worldwide basis, nearly every child
 - experiences rotavirus gastroenteritis by age 5,
 - 1 in 5 visits a clinic,
 - 1 in 65 is hospitalized, and
 - 1 in 293 dies.
 - Children in the poorest countries account for 82% of rotavirus deaths

Mechanism of Egg Antibody Against Rotavirus





Ovalgen RV: In-vitro studies

Rotavirus strains used in this study

Strain	Serotype
Human origin:	
EW48	Natural reassortant
EW28	Natural reassortant
Wa	G1 P[8]
KU	G1 P[8]
M37	G1 P[6]
S2	G2 P[4]
1076	G2 P[6]
YO	G3 P[8]
HK	G4 P[8]
Horse origin: HO-5	G3 P[12]
Cow origin: Shimane	G6 P[5]
Pig origin: S-80	G1 P[7]

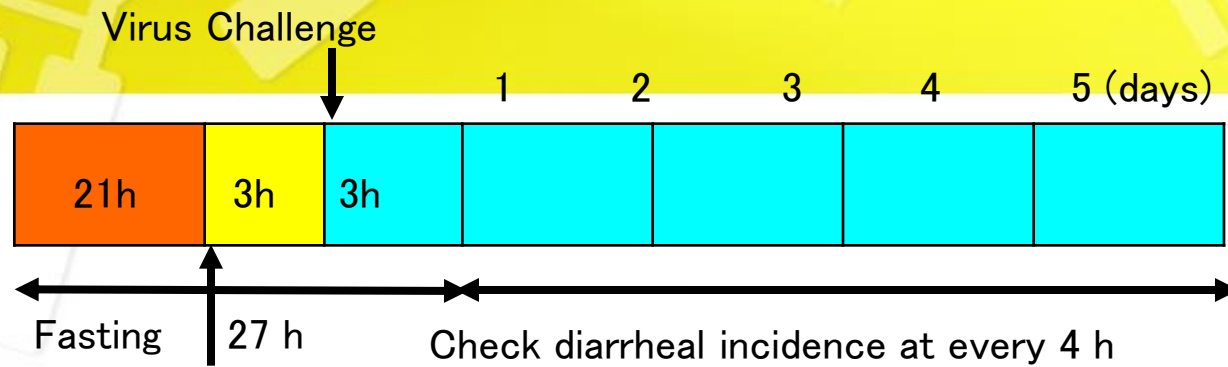
Cross neutralization activity of Ovalgen RV with human and animal rotavirus strains

IgY Samples	Neutralization titer/0.1 ml IgY* against different human rota strains											
	408	248	Wa	KU	M37	S2	1076	YO	HK	HO-5	Shimane	S-80
Anti-EW48	5120	2560	10240	5120	10240	2560	>40960	40960	10240	5120	<20	1280
Anti-EW28	2560	>40960	5120	5120	5120	5120	>40960	20480	40960	1280	<20	640
Rotamix (Ovalgen RV)	10240	>40960	20480	20480	20480	10240	>40960	>40960	>40960	5120	<20	2560
Control	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20

*IgY titer is expressed as dilution factor of 1 gm hyperimmunized IgY powder that reduced the fluorescent focus (FF) count by >50% in the FF inhibition assay.

Ovalgen RV: In-vivo mouse model studies

Mouse challenge protocol



Study Outline IgY administration

Aims of Test:	Occurrence and recovery of Diarrhea
Length of Study:	Five days
Virus:	Human Rota Virus (EW28/48)
Mouse Strain:	ICR Suckling Mice (5 days)
Test Groups:	Six groups (n =10 x 6= 60)
Control Group:	One group (n =10 x 1= 10)
Dosage:	2.5 mg pure IgY per mouse
Challenge Route:	Administered orally
Test Parameter:	1) Degree (%) of diarrheal onset 2) Reduction of diarrheal incidence

Suckling mice groups



ICR pregnant mouse



4 days baby mouse (Suckling mouse)

Mice groups:

Group	Inoculation	No. of Mice
1	PBS	10
2	EW28 IgY	10
3	EW48 IgY	10
4	PBS + HRV 248	10
5	PBS + HRV 408	10
6	EW28 IgY + EW28	10
7	EW48 IgY+ EW48	10

Effect of Rotamix IgY against EW28-induced diarrhea in suckling mice

Treatment group (n=10/group)	Day 1 No.(%) diarrhea	Day 2 No.(%) diarrhea	Day 3 No.(%) diarrhea
Placebo IgY	0	5(50)	7(70)
Rotamix IgY 5 mg/ml	0	1(10)*	1(10)*
Rotamix IgY 2.5 mg/ml	1(10)	1(10)*	1(10)*
Rotamix IgY 1.25 mg/ml	2(20)	5(50)	4(40)
Rotamix IgY 0.625 mg/ml	3(30)	8(80)	7(70)

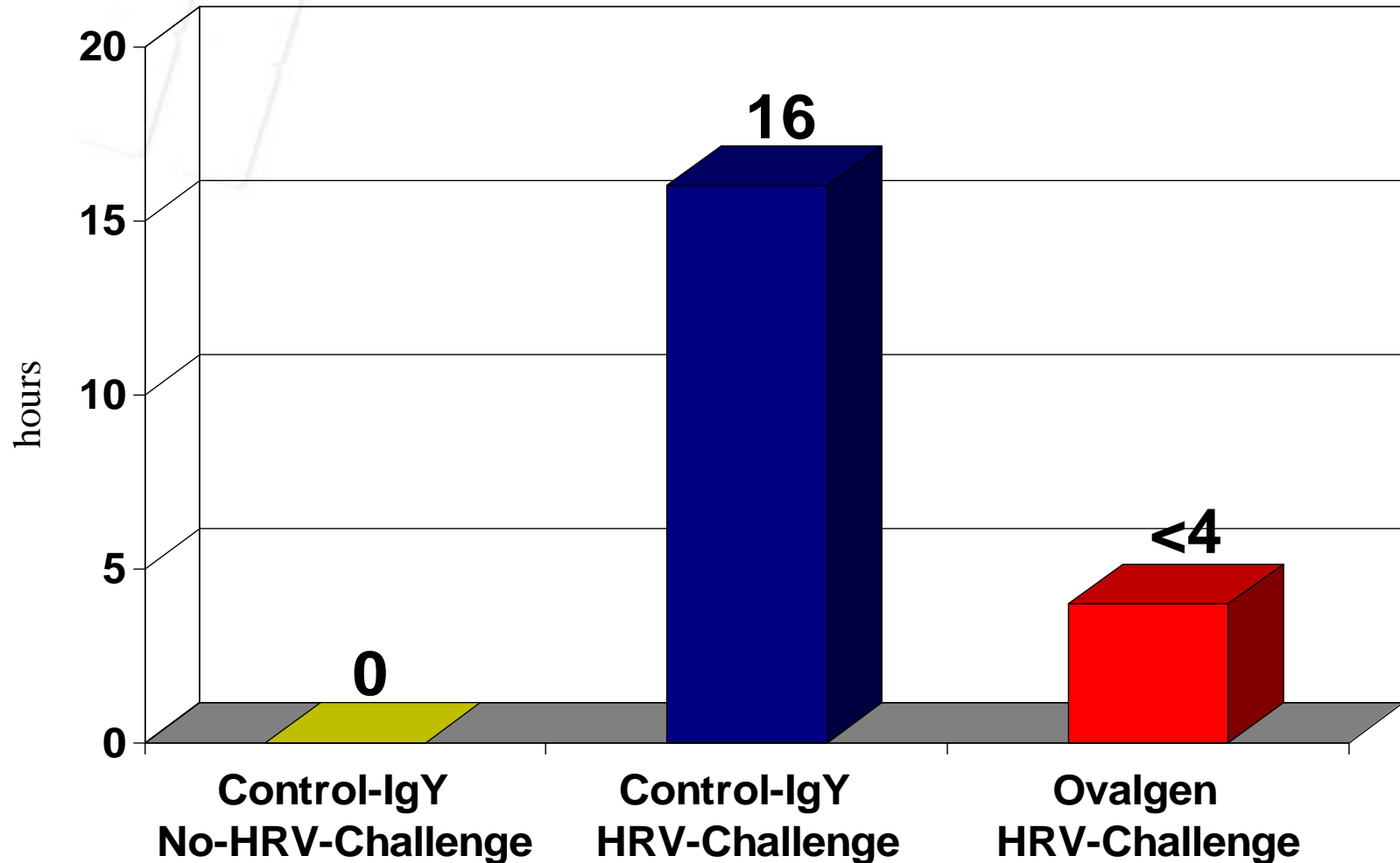
* Significant difference between control (placebo) and Rotamix IgY groups ($P \leq 0.05$, chi-square test)

Effect of Rotamix IgY against EW48-induced diarrhea in suckling mice

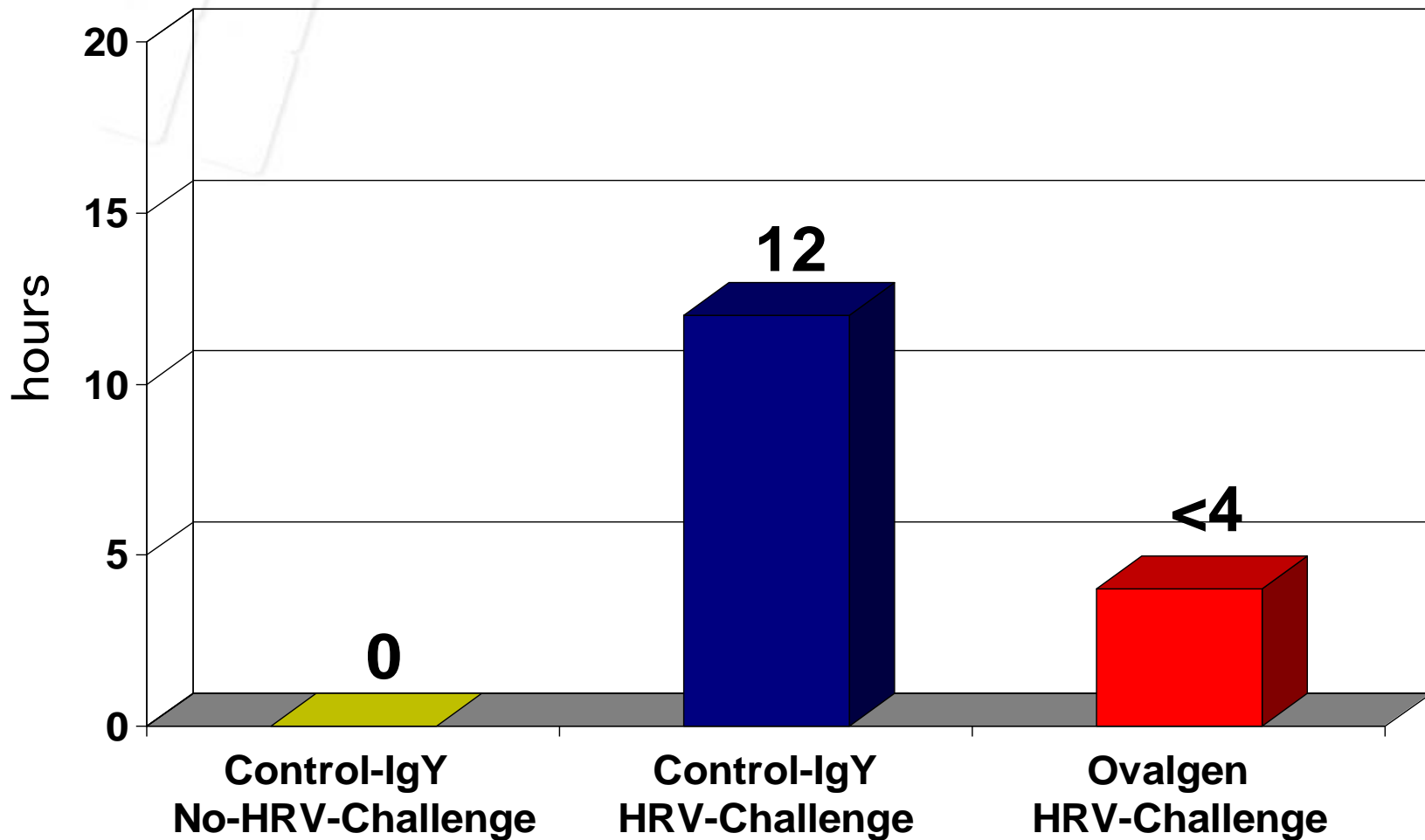
Treatment group (n=10/group)	Day 1 No.(%) diarrhea	Day 2 No.(%) diarrhea	Day 3 No.(%) diarrhea
Placebo IgY	1(10)	7(70)	8(80)
Rotamix IgY 5 mg/ml	0	1(10)*	1(10)*
Rotamix IgY 2.5mg/ml	1(10)	2(20)*	3(30)*
Rotamix IgY 1.25 mg/ml	3(30)	5(50)	6(60)
Rotamix IgY 0.625 mg/ml	2(20)	6(60)	9(90)

*Significant difference between control (placebo) and Rotamix IgY groups ($P \leq 0.05$, chi-square test)

Effect of Ovalgen RV on diarrhea duration of mice challenged with EW28 strain



Effect of Ovalgen RV on diarrhea duration of mice challenged with EW48 strain



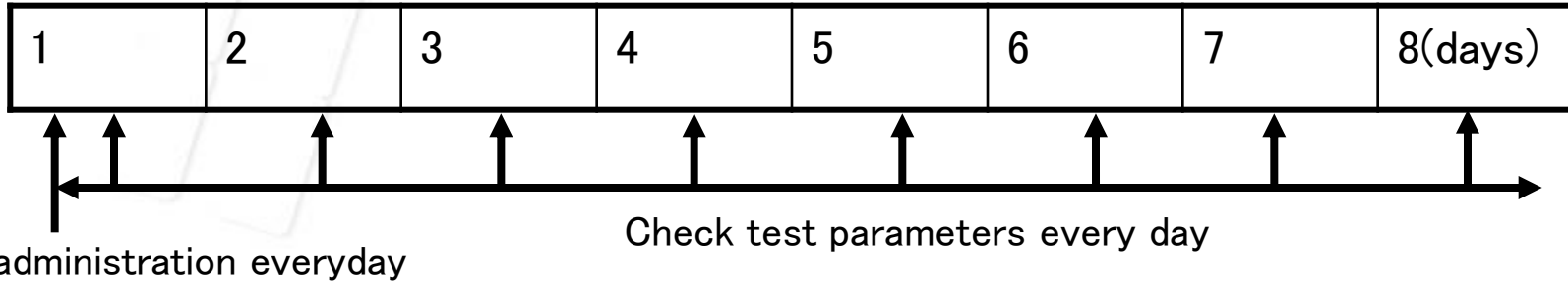
Summary of in-vivo studies

- IgY had no side effect on suckling mice.
- IgY reduced diarrhea rate in challenged suckling mice.
- Diarrhea duration was shorter in test groups compared to control group.



Human clinical study

Clinical trial protocol

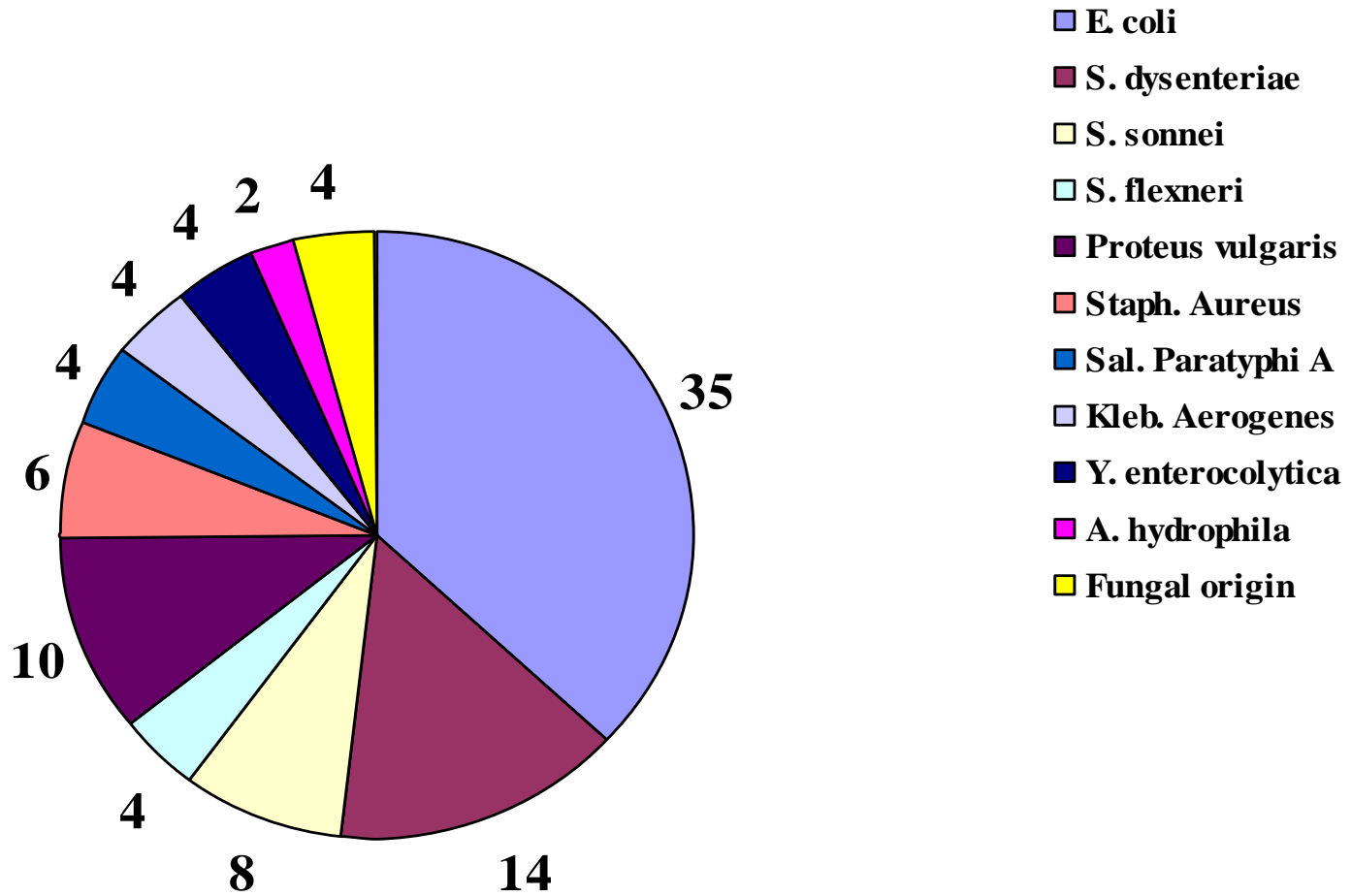


- Trial time: Jan ~ Mar, 2011
- Trial place : Dept Med Res (Central Myanmar, NayPyiTaw)
- Subjects : Total 52 (2 ~ 14 m) (Test, n = 26 ; Placebo, n = 26)
- Screening: By Immunochromato Rota stick
- Dosage: Test group: Ovalgen RV (YP): 0.5 g/every 6 hours;
2g/day
- Placebo group: Control IgY
- Test Parameters:
- 1) Stool frequency
 - 2) ORF intake
 - 3) Fecal rotavirus clearance
 - 4) Diarrhea duration

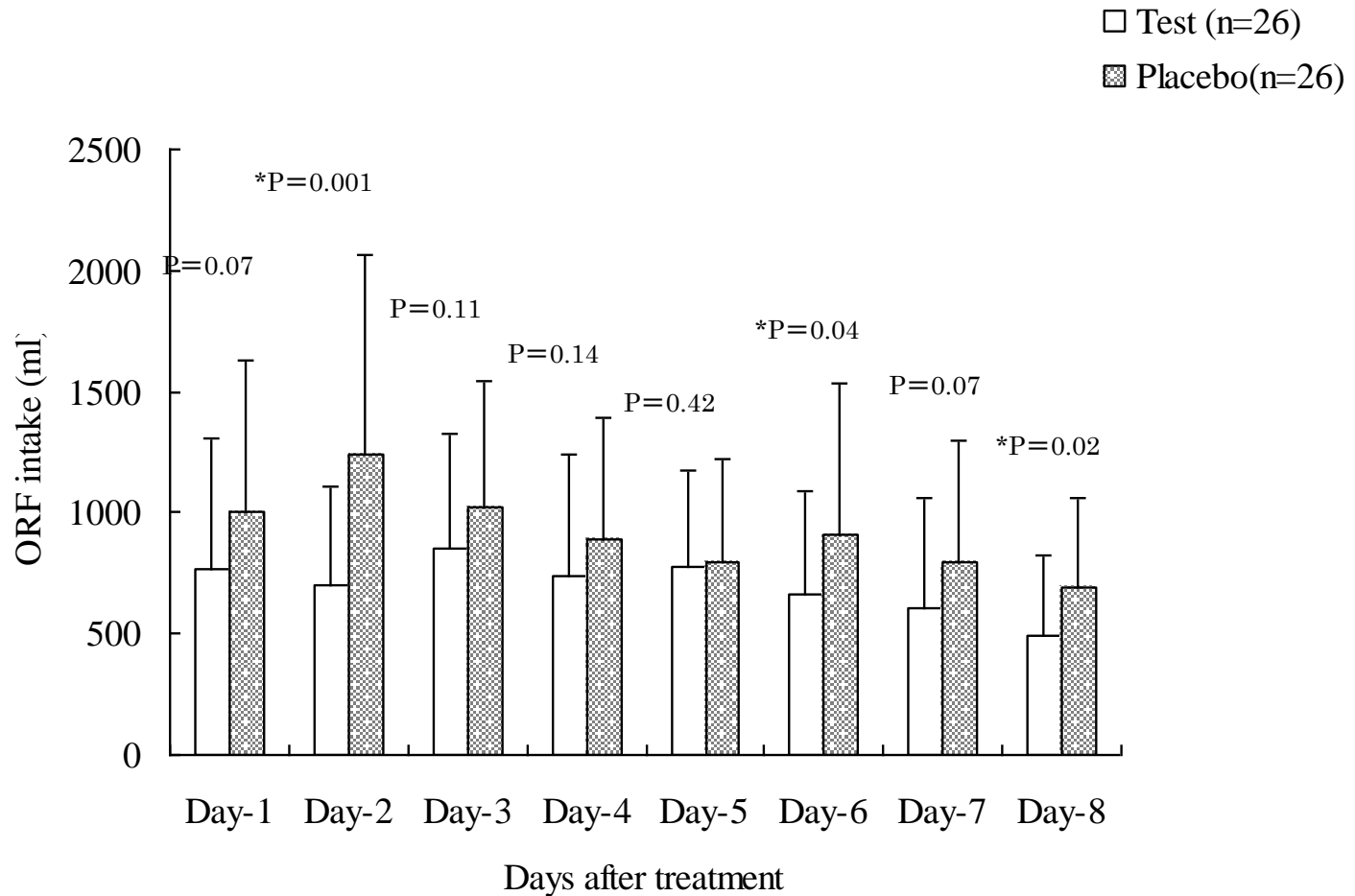
Baseline characteristics of the patients upon admission to hospital (before Ovalgen administration)

Characteristics	Test (n=26)	Placebo (n=26)
Male:Female	13:13	17:09
Resident (Urban:Rural)	16:10	12:14
Age (months)	13.8 ± 10.6†	13.5 ± 6.3
Weight (lb)	17.5 ± 4.5	18.8 ± 3.1
Breast-feeding frequency (number/day)	7.1 ± 7.0	7.8 ± 6.7
Temperature (° F)	100.5 ± 1.5	100.4 ± 1.8
Fever rate (%)	22/26 (85%)	21/26 (81%)
Fluid intake (ml/day):		
ORS and others supplement	767.3 ± 538.6	1005.4 ± 628.0
Intravenous fluid (IVF)	345.0 ± 347	592.3 ± 491.3
Diarrhea duration (h)	69.6 ± 33.6	74.4 ± 38.4
Stool frequency (number/day)	9.2 ± 5.6	8.5 ± 7.3

Co-infection in the subjects as revealed by stool microbiological tests

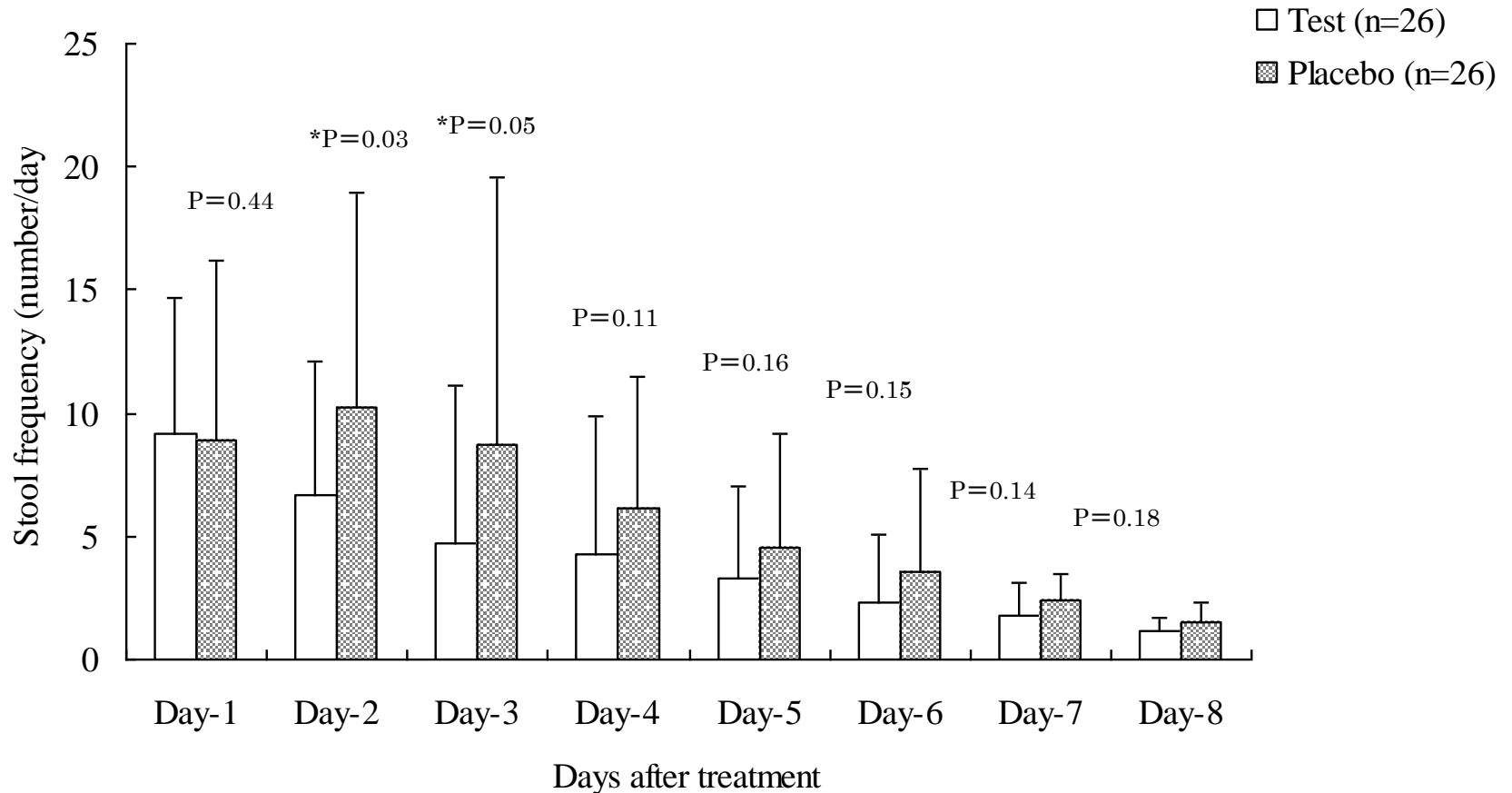


Result: mean daily oral fluid intake



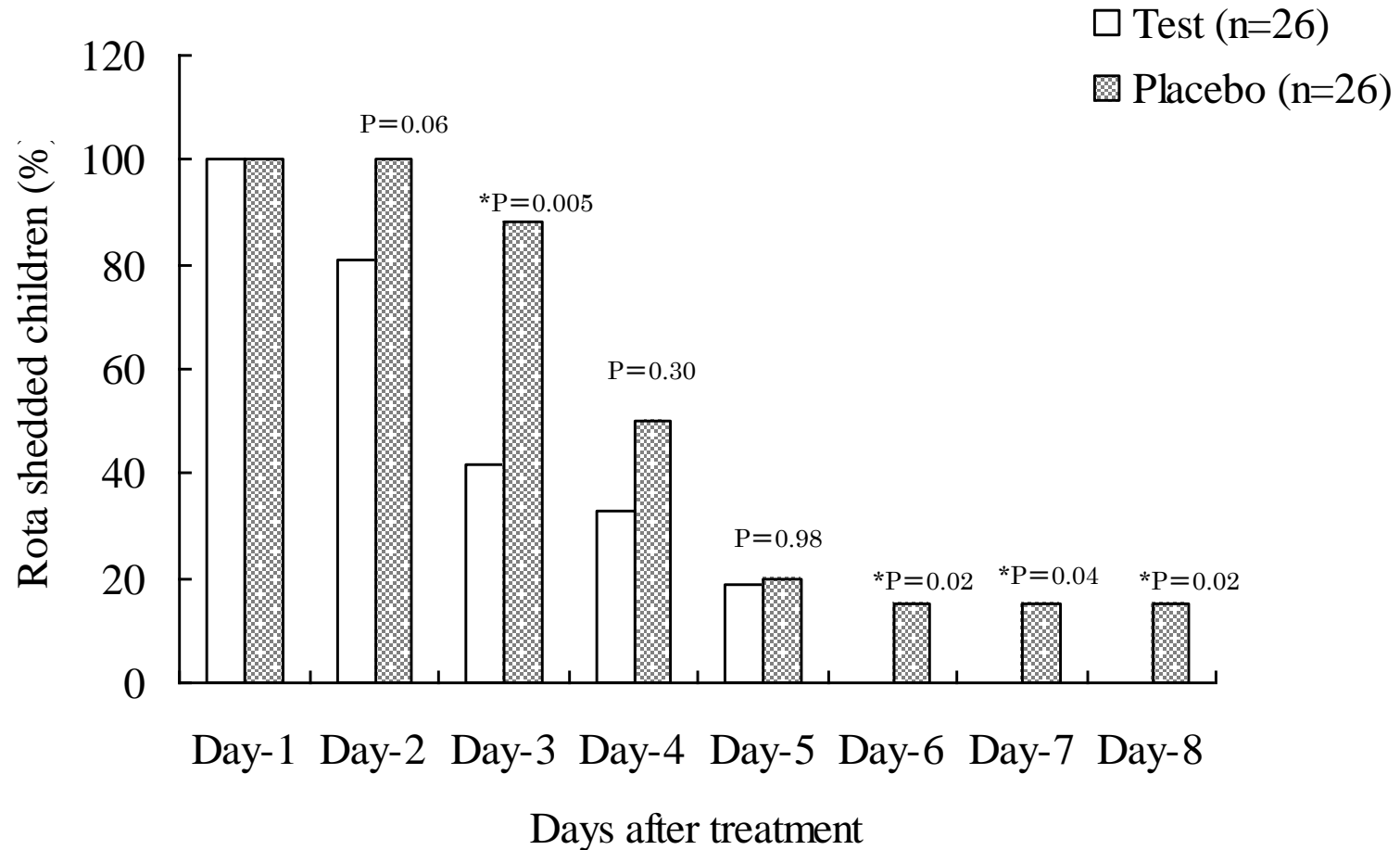
* Significant difference between the test and placebo groups (* $P \leq 0.05$, Student's t-test)

Result: stool frequency



*Significant difference between the test and placebo groups.

Result: Rotavirus shedding



*Significant difference between the test and placebo groups. (*P≤0.05, chi-square test)

Summary of human trial

- Ovalgen RV had no side effect on pediatric patients (based on questionnaire)
- ORF intake was lower in test group than in placebo group
- Diarrheal frequency decreased in test group compared to placebo group
- Rotavirus shedding frequency was significantly lower in test group than in placebo group.