# Trials of microbiological control in common marmosets

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SINCE 1952

# Microbiological status of common marmosets in laboratory

- Low risk of zoonoses and severe infectious diseases
- Generally, non specific pathogen free (SPF)/non barriered facility
  - Quarantine and check of high-risk zoonotic pathogens in marmosets (*Salmonella*, *Shigella*, …) and humans (measles, tuberculosis,… ) are conducted.
  - Some facilities use outside enclosure for environmental enrichment (Bakker et al., 2015)
  - A SPF colony has been developed at Barshop Institute for Longevity and Aging  Studies (Ross et al., 2017).
  - SPF definition is controversial.
- **Some health problems caused by opportunistic pathogens**

# Pathogens detected in common marmosets in the Central Institute for Experimental Animals (CIEA)

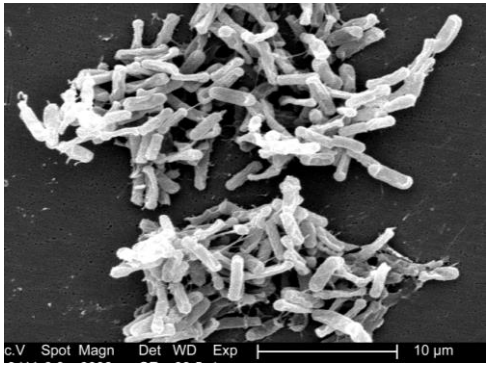
| Pathogen | | Disease |
|---|---|---|
| Protozoa | ***Pentatrichomonas hominis*** | **No, Diarrhea?** (Inoue et al., 2015) |
| Bacteria | **Enteropathogenic *Escherichia coli* (EPEC)** | **Diarrhea (bloody)** (Hayashimoto et al., 2016) |
| | ***Clostridium difficile*** | **Diarrhea, pseudomembranous colitis** |
| | ***Clostridium perfringens*** | **Sepsis** (Yasuda et al., 2016) |
| | ***Klebsiealla pneumoniae*** | **Sepsis** |
| | ***Helicobacter* spp.** | **Unknown** |
| Viruses | **Callitrichine herpesvirus 3 (CalHV-3; lymphocryptvirus)** | **No, lymphoma?** |

Trials of microbiological control in common marmosets

# *Clostridium difficile* infection and Fecal Microbiota Transplantation in common marmosets

# *Clostridium difficile*

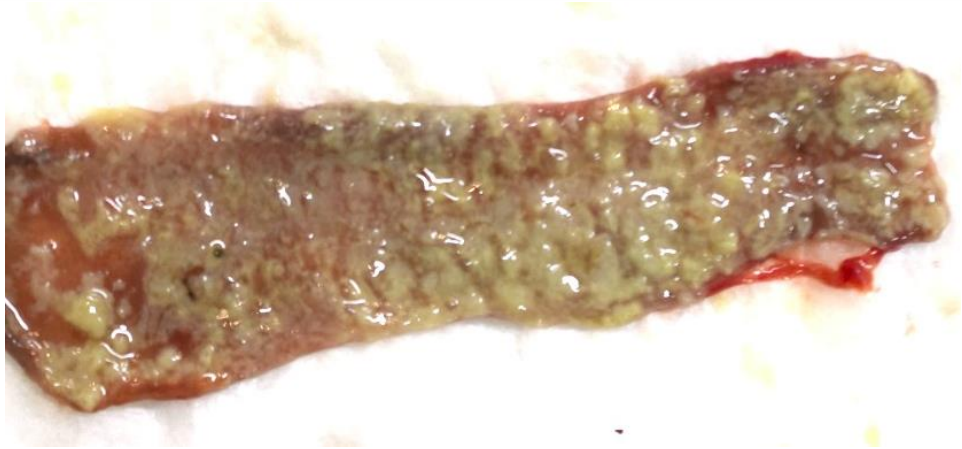
- ***Clostridium difficile*** is a spore-forming, Gram-positive anaerobic bacillus that is naturally found in the intestinal tract of humans and animals, and in the environment.
- It is a common cause of **nosocomial and antibiotic-associated diarrhea** in humans.

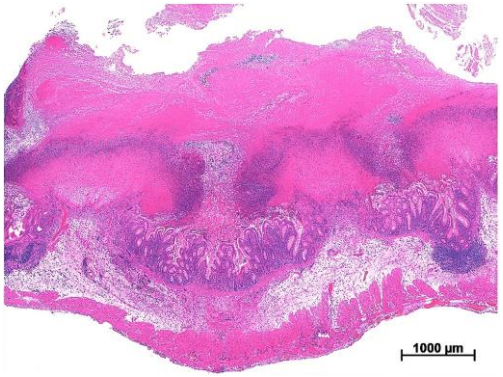


http://phil.cdc.gov

- It proliferates by **imbalance of intestinal bacterial flora (dysbiosis)**, produces toxins, and causes symptoms ranging from mild diarrhea to severe pseudomembranous colitis.
- In New World monkeys, *C. difficile* associated colitis was found in cotton-top tamarins (Rolland et al., 1997).
- Recently, *C. difficile* has been found in some marmoset colonies in Japan (Yamazaki et al., 2017).

# A pseudomembranous colitis case of common marmoset



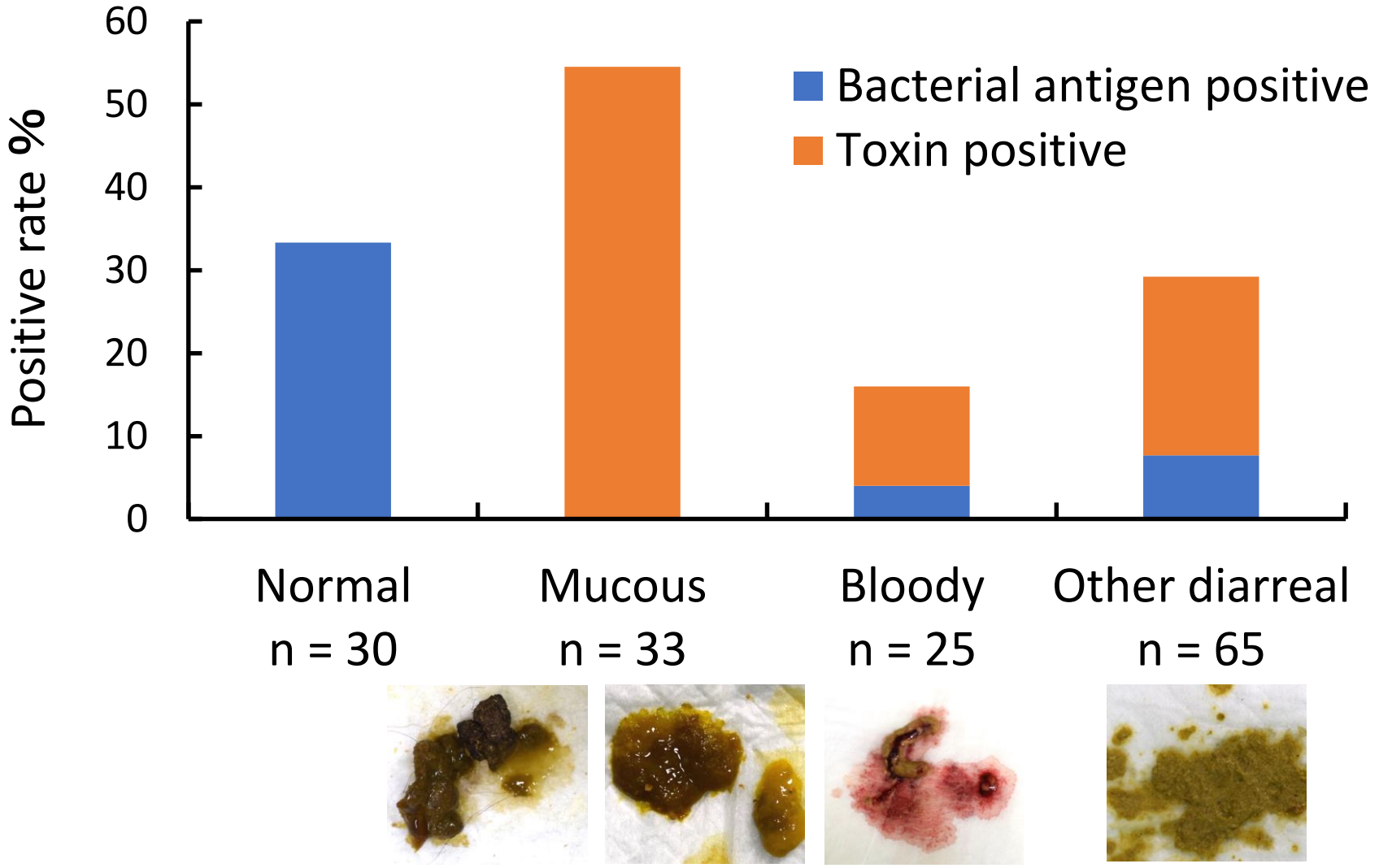



'Pseudomembrane' was formed in mucosa of the enlarged colon.

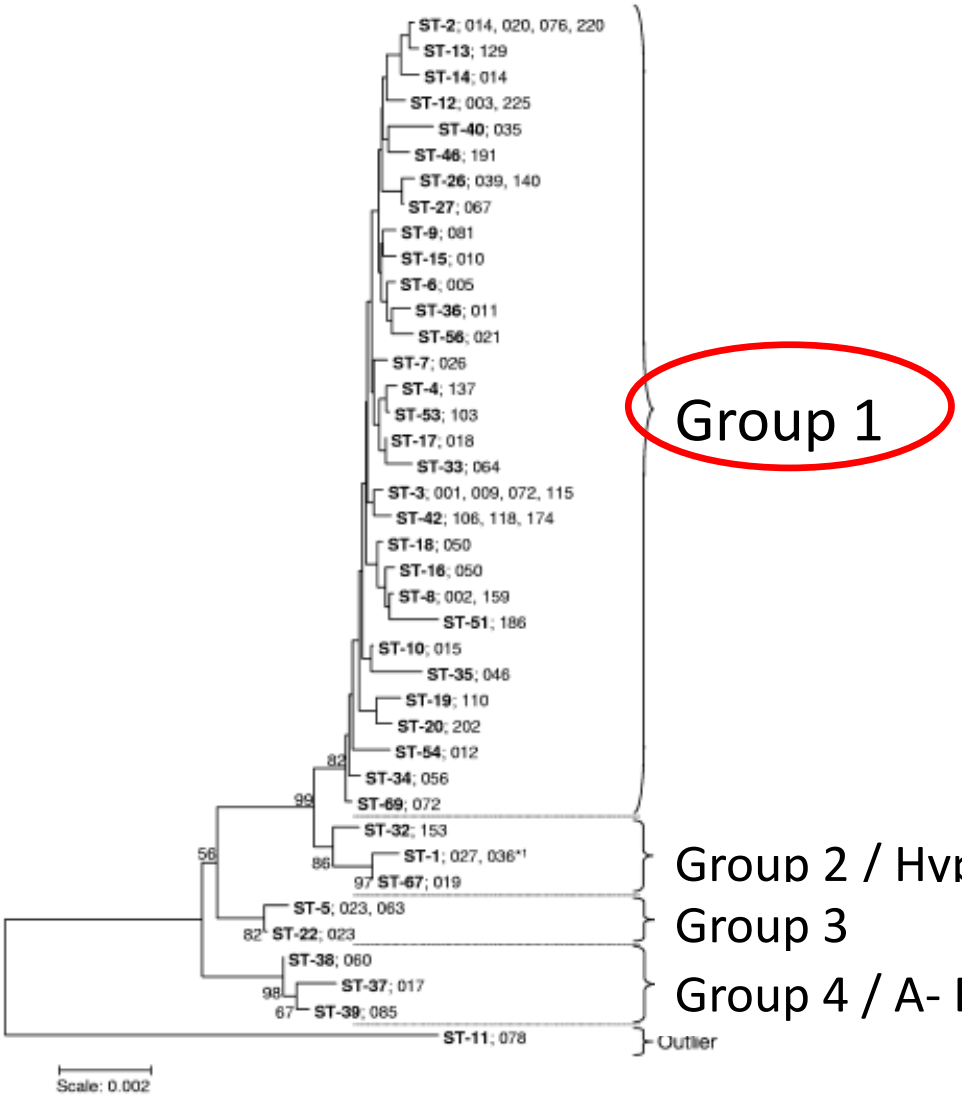
- Watery diarrhea, anorexia, acute weight loss during antibiotic (nalidixic acid) treatment against EPEC.
- ***C. difficile*-toxin positive** in rectal feces using a immunochromatographic kit


- The C. DIFF QUIK CHEK COMPLETE® (Alere) test is the only rapid cassette assay that simultaneously detects both glutamate dehydrogenase (GDH) antigen and toxins A & B of *C. difficile* in fecal specimens.

# Survey of fecal *C. difficile* toxin in a common marmoset colony using C. diff Quick Check Complete n=153 (1 month to 15 years old)



# Genetic typing of *C. difficile* from common marmosets




- Positive for both Tox A (*tcdA*) and Tox B (*tcdB*)
- Multilocus sequence typing (MLST) (Griffiths et al., 2010)

  Sequence Type: 55

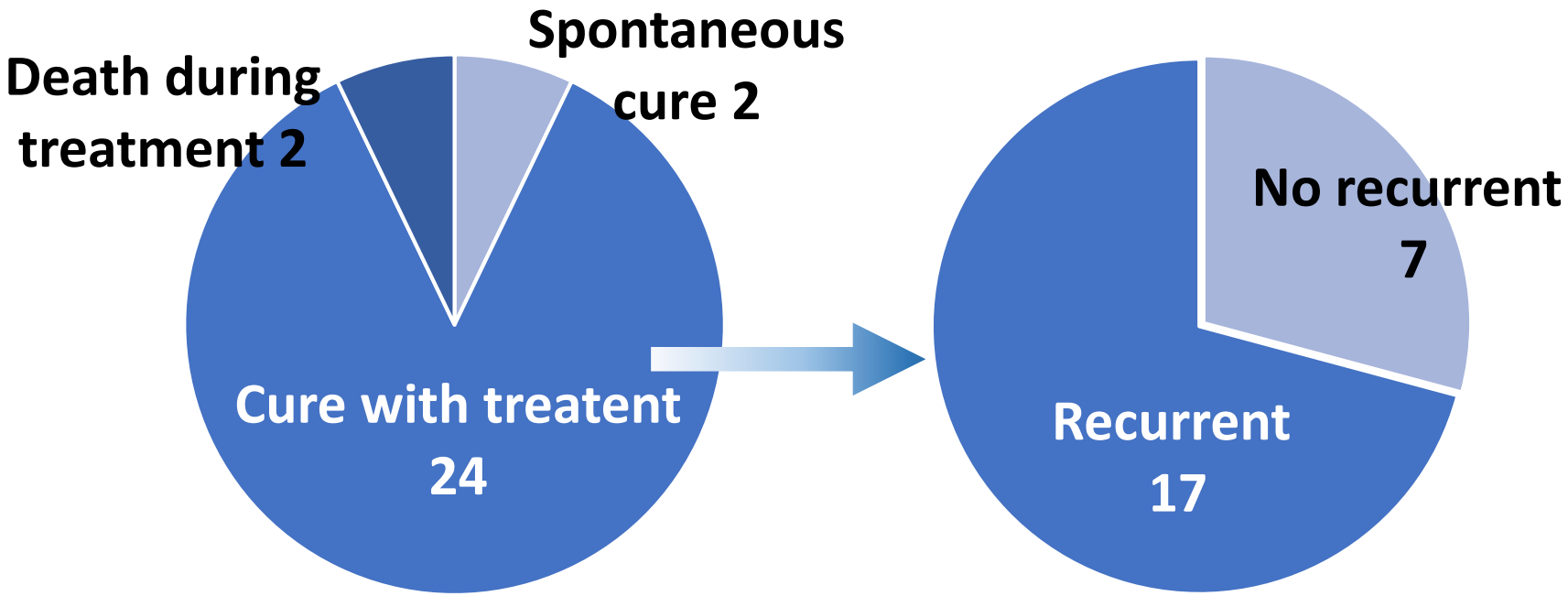
  MLST Clade: Group 1

  A major group isolated from humans and animals.

  Not hypervirulent strain

# Antibiotic therapy for *C. difficile* infection in marmosets

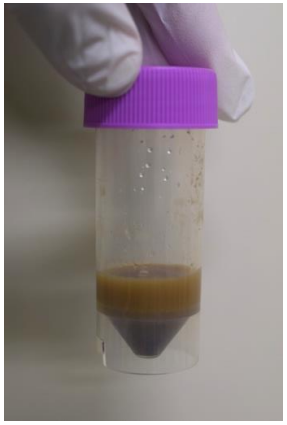
**Metronidazole** 20 mg/kg/day or **Vancomycin** 30 mg/kg/day for 5-14 days

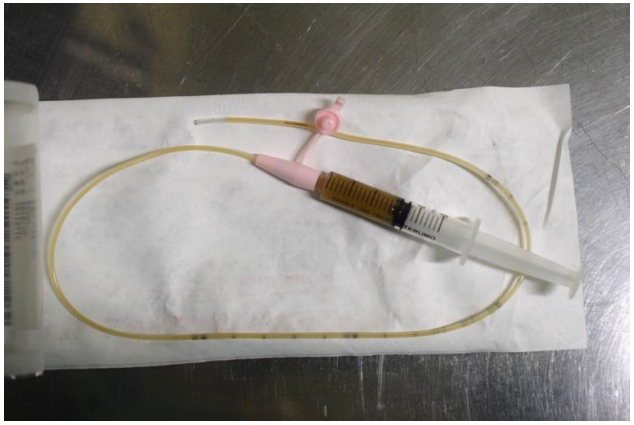



In treatment cases, other care such as fluid and nutritional support was also done.

# A trial of fecal microbiota transplantation (FMT) for recurrent *C. difficile* infection in marmosets

FMT is an effective remedy for recurrent *C. difficile* infection (CDI) in humans.
A successful case report was reported also in a marmoset (Yamazaki et al., 2017)

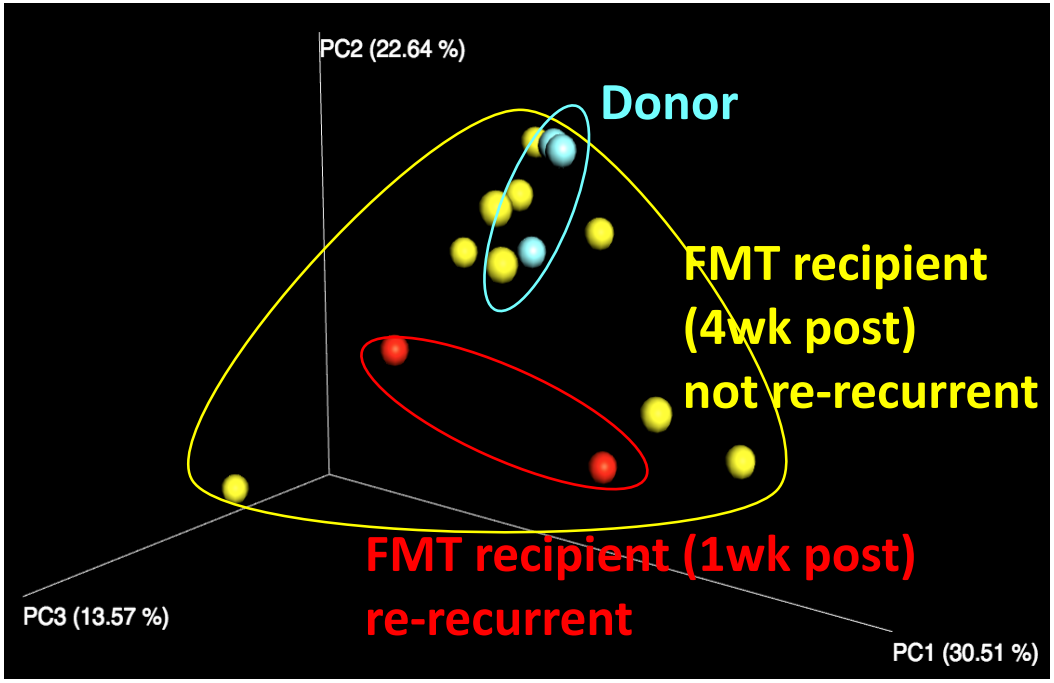




| | |
|---|---|
| Donor | A healthy individual (3y, female, 468g)<br>Negative for *Salmonella*, *Shigella*, *Yersinia*, EPEC, *C. difficile* |
| Recipient | 22 recurrent CDI cases (1-15y)<br>Diarrhea and CD toxin positive 2-10 times |
| Donor feces/FMT | Fresh or frozen (-80°C)<br>Fecal suspensions of a healthy were administered intragastrically to recurrent CDI cases a day after final vancomycin administration. |

# A trial of fecal microbiota transplantation (FMT) for recurrent *C. difficile* infection in marmosets

| Donor feces | No. of cases | No relapse within 12 weeks post FMT |
|---|---|---|
| Fresh | 13 | 11 (77%) |
| Frozen | 9 | 7 (78%) |
| Total | 22 | 17 (77%) |




PCoA analysis with weighed Unifrac distances of fecal microbiota based on the bacterial 16S rRNA gene dataset.

# *Clostridium difficile* infection and Fecal Microbiota Transplantation in common marmosets

Summary

- *C. difficile* should be noted as one cause of marmoset diarrhea and severe colitis.
- *C. difficile* infection relates with imbalance intestinal microbiota and FMT would be effective for the therapy.
- The emergence of C. difficile infection problem make us remind the importance of microbiological control and intestinal microbiota in marmoset husbandry.

## Trials of microbiological control in common marmosets

# Strict microbiological control for immunodeficient marmosets and germfree marmosets

# Strict microbiological control through Caesarean section-delivery in marmosets

In the past, a trial of establishment of a SPF marmoset colony was reported (Hobbs et al., 1977) .

*Present address: OLAC (1976) Ltd, Shaw's Farm, Blackthorn, Bicester, OX6 0TP.

**Table 1. Summary of breeding records of conventional colony of *Callithrix jacchus***
May 1969—January 1973

| *Animal* | *Pregnancies* | *Abortions* | *Resorptions* | *Full term*† | *Pregnancy success*† | | | *Foetuses per pregnancy* | | | *Young born* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *all live* | *live and dead on delivery* | *all dead* | 3 | 2 | 1 | *alive* | *dead* |
| 2 | 8 | 3 | 1 | 4 (1) | 2 (1) | 2 | 0 | 1 | 4 | 2 | 6 (3) | 2 |
| 4 | 3 | 0 | 1 | 2 | 1 | 1 ¶ | 0 | 0 | 2 | 0 | 3 | 1 |
| 6 | 4 | 0 | 0 | 4 (1) | 4 (1) | 0 | 0 | 1 | 3 | 0 | 9 (3) | 0 |
| 8 | 4 | 1 | 0 | 3 | 2 | 1 | 0 | 1 | 2 | 0 | 6 | 1 |
| 12* | 1 | 0 | 0 | 1 (1) | 0 | 0 | 1 (1) | 0 | 1 | 0 | 0 | 2 (1) |

**Strict microbiological control is needed for special purposes.**

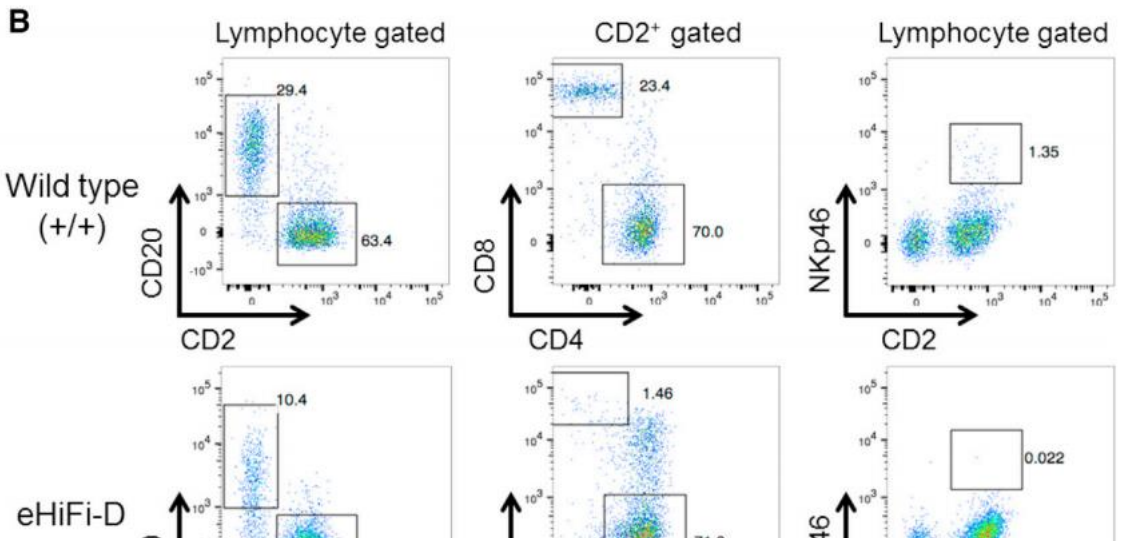
Cell Stem Cell
Resource

Sato and Sasaki et al., 2016

CellPress

# Generation of a Nonhuman Primate Model of Severe Combined Immunodeficiency Using Highly Efficient Genome Editing

Kenya Sato,[1] Ryo Oiwa,[1] Wakako Kumita,[1] Rachel Henry,[2] Tetsushi Sakuma,[3] Ryoji Ito,[1] Ryoko Nozu,[1] Takashi Inoue,[1] Ikumi Katano,[1] Kengo Sato,[4] Norio Okahara,[1] Junko Okahara,[1] Yoshihisa Shimizu,[1] Masafumi Yamamoto,[1] Kisaburo Hanazawa,[5] Takao Kawakami,[6] Yoshie Kametani,[7] Ryuji Suzuki,[8] Takeshi Takahashi,[1] Edward J. Weinstein,[2] Takashi Yamamoto,[3] Yasubumi Sakakibara,[4] Sonoko Habu,[9] Jun-ichi Hata,[1] Hideyuki Okano,[10,*] and Erika Sasaki[1,11,*]




**Severe combined immunodeficient (SCID) nonhuman primates (NHPs) would have various applications for advance in biomedical research.**

# Rearing newborns of immunodeficient marmosets


Newborn room


Newborns through Caesarean section-delivery have been reared with hand milk feeding in a clean room using Biobubble®☐.

Hand rearing

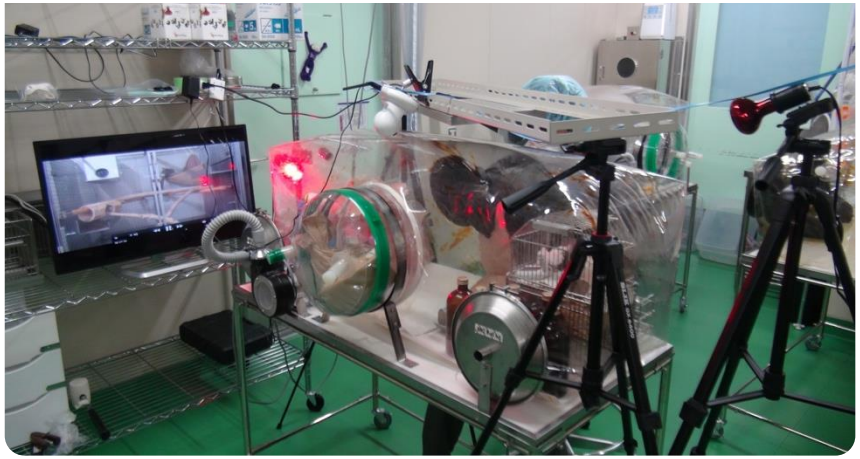
# Keeping of immunodeficient marmosets


Cages in biobubble ®

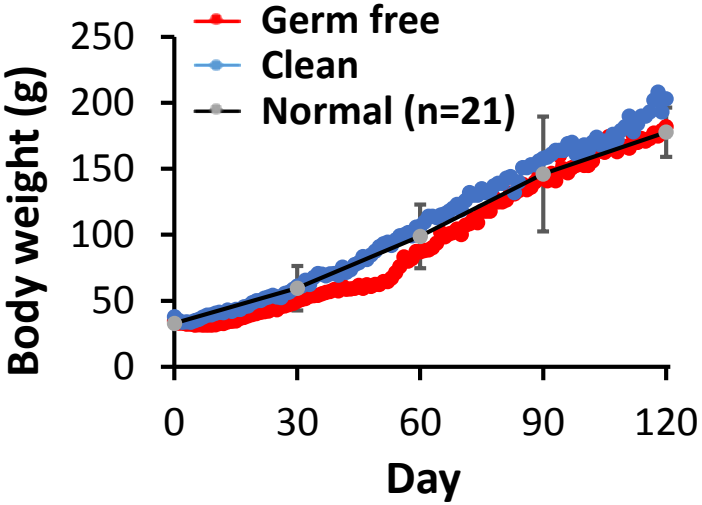
- Immunodeficient marmosets have been kept for 4 years.
- *C. difficile* infection also becomes a health problem despite a barriered environment.
- Metronidazole or vancomycin treatment is helpful but suitable intestinal bacterial flora will improve their health.

# Germ-free marmosets for microbiota research

**Germfree and gnotobiotic marmosets have a potential for dynamically developing microbiome research.**



Isolators for germfree




Growing up of a germ free marmoset

# High-level microbiological control for immunodeficient marmosets and germfree marmosets

Summary

- Strict microbiological control through Caesarean section is specially needed for SCID marmosets and germfree marmosets, and that is possible.

- “Good” intestinal bacterial flora is needed to improve health of SCID marmosets.

- Husbandry of these special animals should be improved to succeed in research.

# Acknowledgement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**Central Institute for Experimental Animals**

Erika Sasaki
Yoko Kurotaki
Ryoko Nozu
Masami Ueno
Nobuhito Hayashimoto
Masahiko Yasuda
Kenya Sato
Takayuki Mineshige
Terumi Yurimoto
Emi Sasaki
Lee Chia-Ying
Norio Okahara

Keio University 1858 CALAMVS GLADIO FORTIOR

**Keio University**

Prof. Hideyuki Okano

Prof. Kenya Honda

and other all members of marmoset research group