Prevalence of Pet Periodontitis

- 80 percent of dogs and 70 percent of cats show signs of oral disease by age three, American Veterinary Dental Society (AVDS).
- Survey in USA 1999, only 7 % dog population considered healthy
- Untreated periodontal disease shorten a pets lifespan by 3-5 years.
- Most common problems for veterinarian during a pets annual physical exam is periodontal disease.
- One milligram of plaque alone contains over 1 trillion bacteria can cause disease.

Facts about Periodontitis

- Pathogens of pet periodontitis
  - Major pathogen in black-pigmentes anaerobic bacteria is *Porphyromonas sp*.
  - Most frequently isolated black-pigmented anaerobic bacteria from pet:
    - *Porphyromonas gulae*
    - *Porphyromonas gingivalis*
    - *Porphyromonas salivosa*
    - *Porphyromonas denticanis*

Facts about Periodontitis

- **Gingipain is the most important pathogenic factor of black-pigmented bacteria.**
  - Located on the surface of the bacteria and also released in the culture medium
  - Main virulent mechanisms:
    - Degrade proteins in host tissues
    - Destroy host defense mechanisms: cytokines, complement, macrophages
    - Form biofilm together with other bacteria in oral cavity

In vivo study

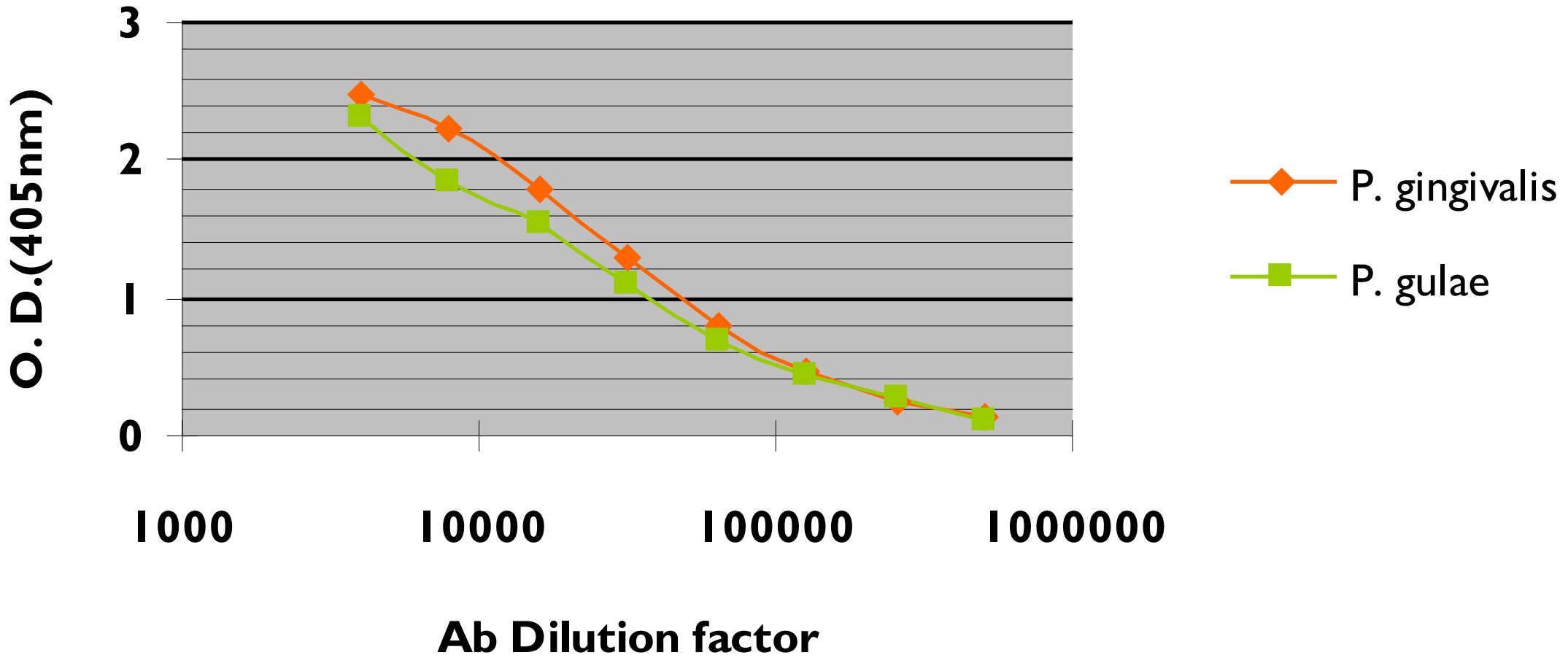
In vitro study: Specific IgY (PG) for pet

- Cross reactivity checked between *P. gingivalis and P. gulae* by the following methods:
  - ELISA
  - Enzyme Inhibition Assay
  - Cell adhesion / damage inhibition assay

In vitro study: Specific IgY (PG) for pet

- In-vitro Cross Reactivity Test: ELISA
  - Coating antigen (*P. gingivalis* & *P. gulae*)
  - Purified anti-PG Ab (2-fold serial diluted)
  - Measure by O-phenylene Diamine (OPD) program using $OD_{492/630}$

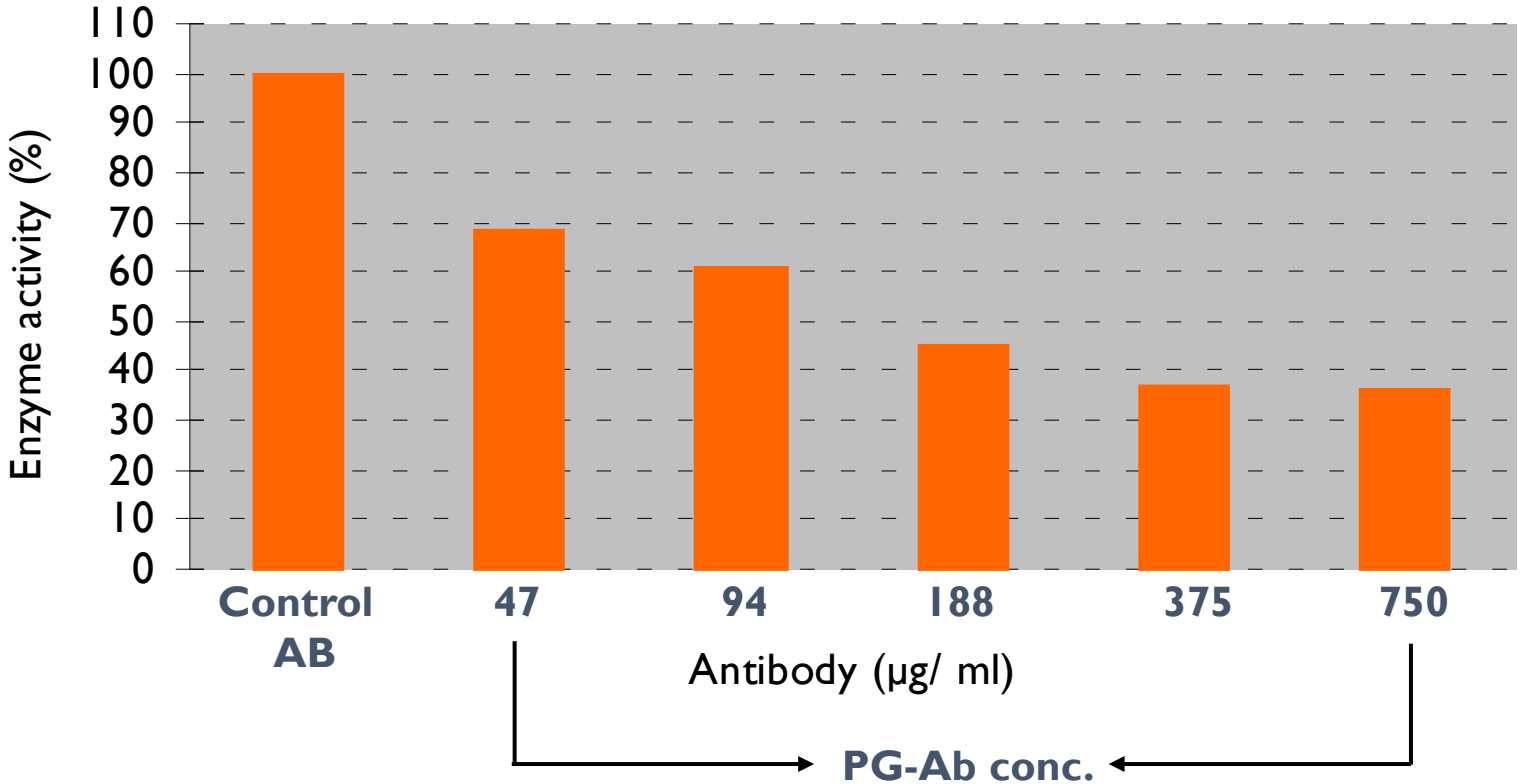
In vitro study: Specific IgY (PG) for pet



In vitro study: Specific IgY (PG) for pet

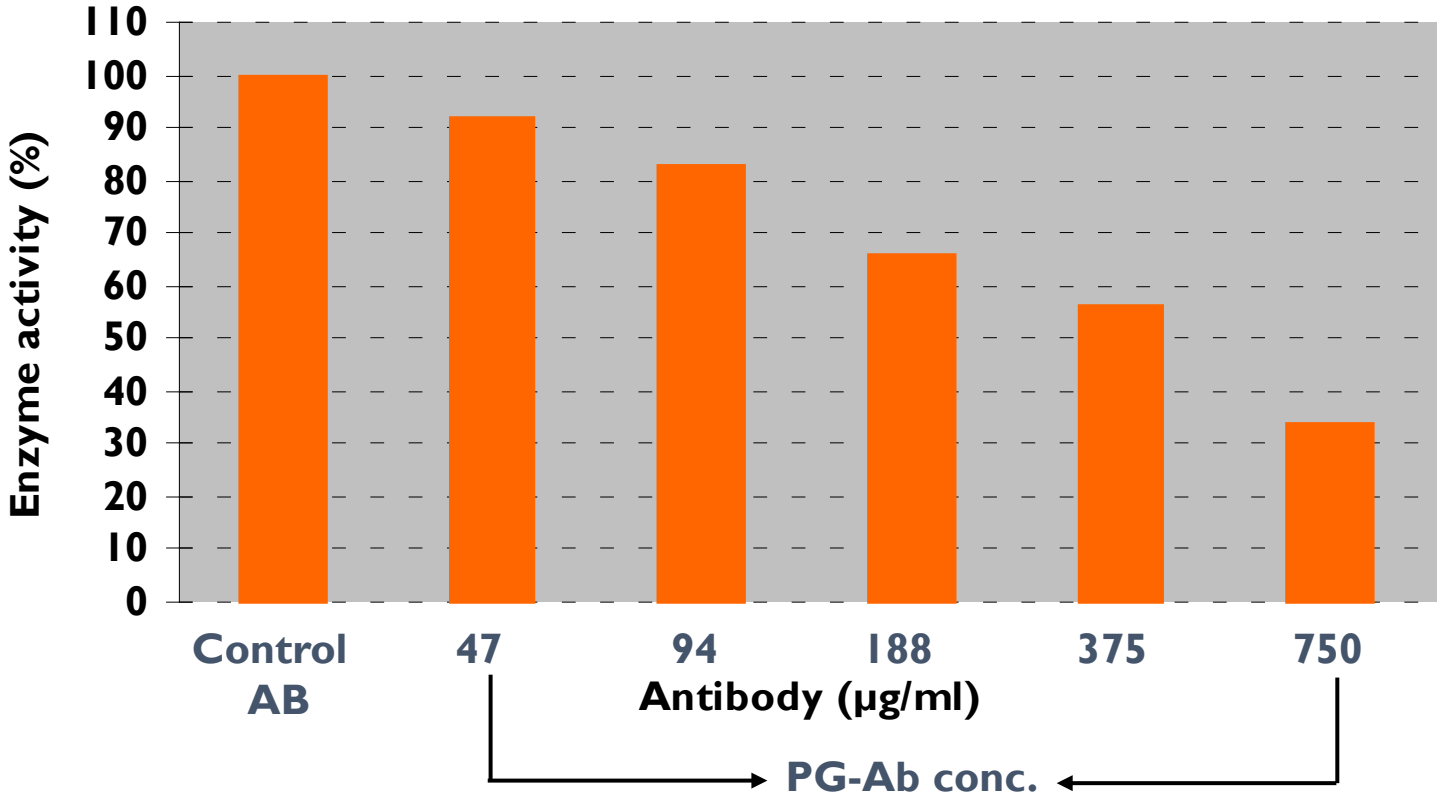
- In-vitro Cross Reactivity Test: Enzyme Inhibition Assay
  - *P. gingivalis* gingipain & *P. gulae* gingipain
  - Anti-gingipain Ab from *P. gingivalis* and Control Ab
  - Measure enzyme activity at OD_{405}

In vitro study: Specific IgY (PG) for pet



- Enzyme inhibition assay (*P. gingivalis* gingipain)

In vitro study: Specific IgY (PG) for pet



- Enzyme inhibition assay (*P. gulae* gingipain)

In vitro study: Specific IgY (PG) for pet

- In-vitro Cross Reactivity Test: Inhibition of Cell damage by enzyme
  - PBS only Enz only Enz+Cont Ab Enz+Test Ab
  - Incubate at 4° C, 1h
  - Add Premix into monolayered 6 well plate
  - 37° C, 5% CO_2, 1h inhibition
  - Count alive adhesive cells

Cross Reactivity Test : Cell damage inhibition assay

Results of Inhibition of cell damage

	PBS-treated	*P. gingivalis*	Protection (%)
PBS	17.5	3.9	22
Control Ab	16.5	4.5	27
Anti-PG Ab	16.0	**12.5**	**78**

	PBS-treated	*P. gulae*	Protection (%)
PBS	17.5	4.5	26
Control Ab	16.5	5.6	34
Anti-PG Ab	16.0	**10.8**	**68**

(No. live cells： $\times 10^4$ / ml)

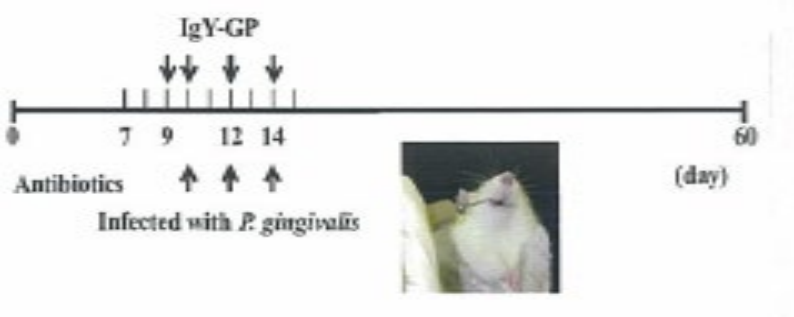
Summary: In-vitro Tests

- **Specific IgY (PG) inhibits the gingipain activity, and suppresses the damage to the cell by the *P. gingivalis* bacterium.**

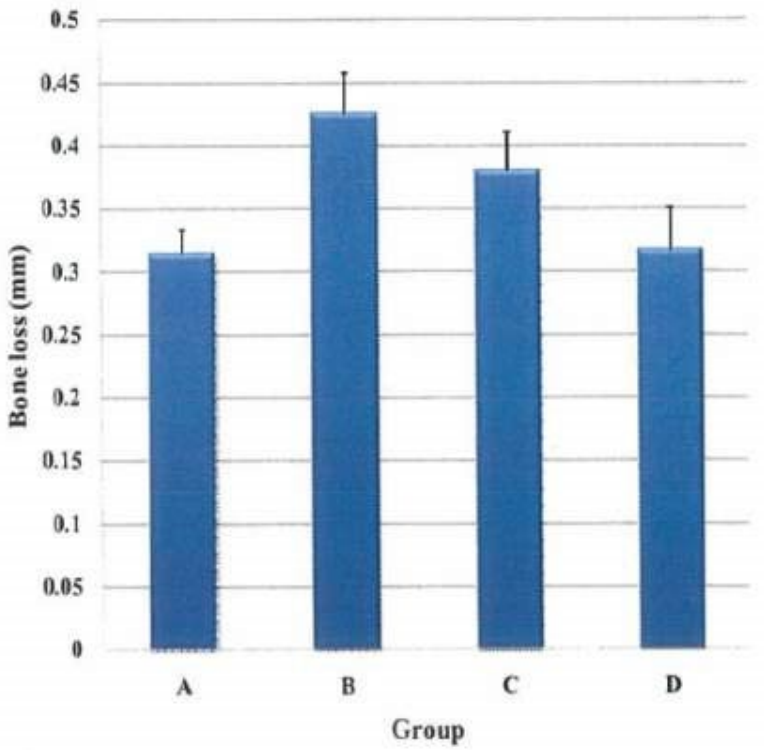
- **Specific IgY (PG) shows cross reactivity to gingipain of *P. gulae*, the common pathogen in pets.**

In vivo trials

Trial on Sprague-Dawley rats
(by Prof. Hamada, Kanagawa Dental College)



Measurement of bone loss in an upper jaw.



A: non-infected control
B: infected, non-treated
C: infected, treated with cont IgY
D: infected, treated with Specific IgY (PG)

Source: AAP/JSP 2010 Annual Meeting

Trial 1: Specific IgY (PG) in dog food

- KYODOKEN INSTITUTE FOR ANIMAL SCIENCE RESEARCH & DEVELOPMENT
  - Well known private institute for animal science research and development
- In vivo trial in dogs with Specific IgY (PG) in Pet food
- Standard pet food in pellet form named **“**Aijou Mongatari Series**”** from Japanese manufacturer YEASTER

Trial 1: Specific IgY (PG) in dog food

- Dogs used in this trial
  - Average Age 7 years
  - Average Body Weight 8 kg

- M. Duck

- Beagle

- Cavalier

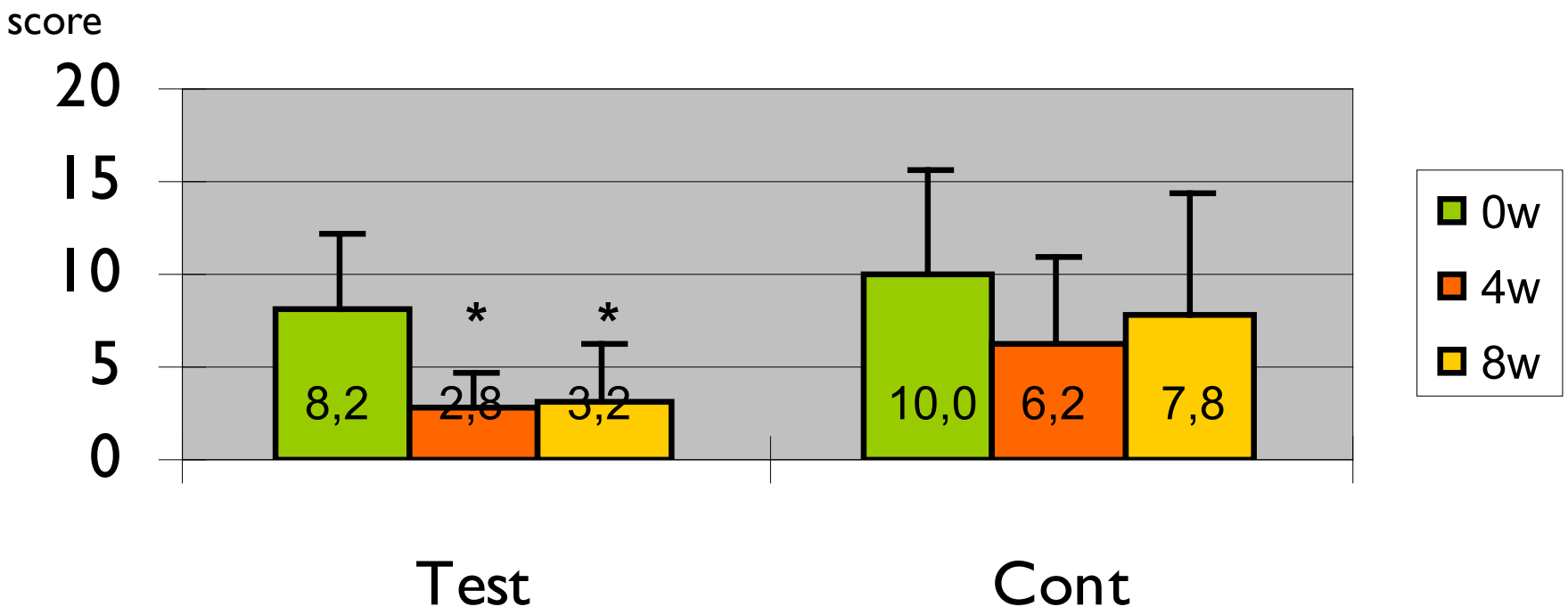
Trial 1: Specific IgY (PG) in dog food

- Group settings:
  - **Test Group** (n=10): 0.1% Specific IgY (PG) in dry feed
  - **Control Group** (n=5): Feed only no Specific IgY (PG) added
- Evaluation: at 0 week, 4 week, 8 week
- Parameters:
  - Inflammation score (foul breath, gum congestion, bleeding of gums, gum inflammation, gum ulcer, periodontal ligament inflammation)
  - Pocket depth (average PD)
  - Body weight

Trial 1: Specific IgY (PG) in dog food

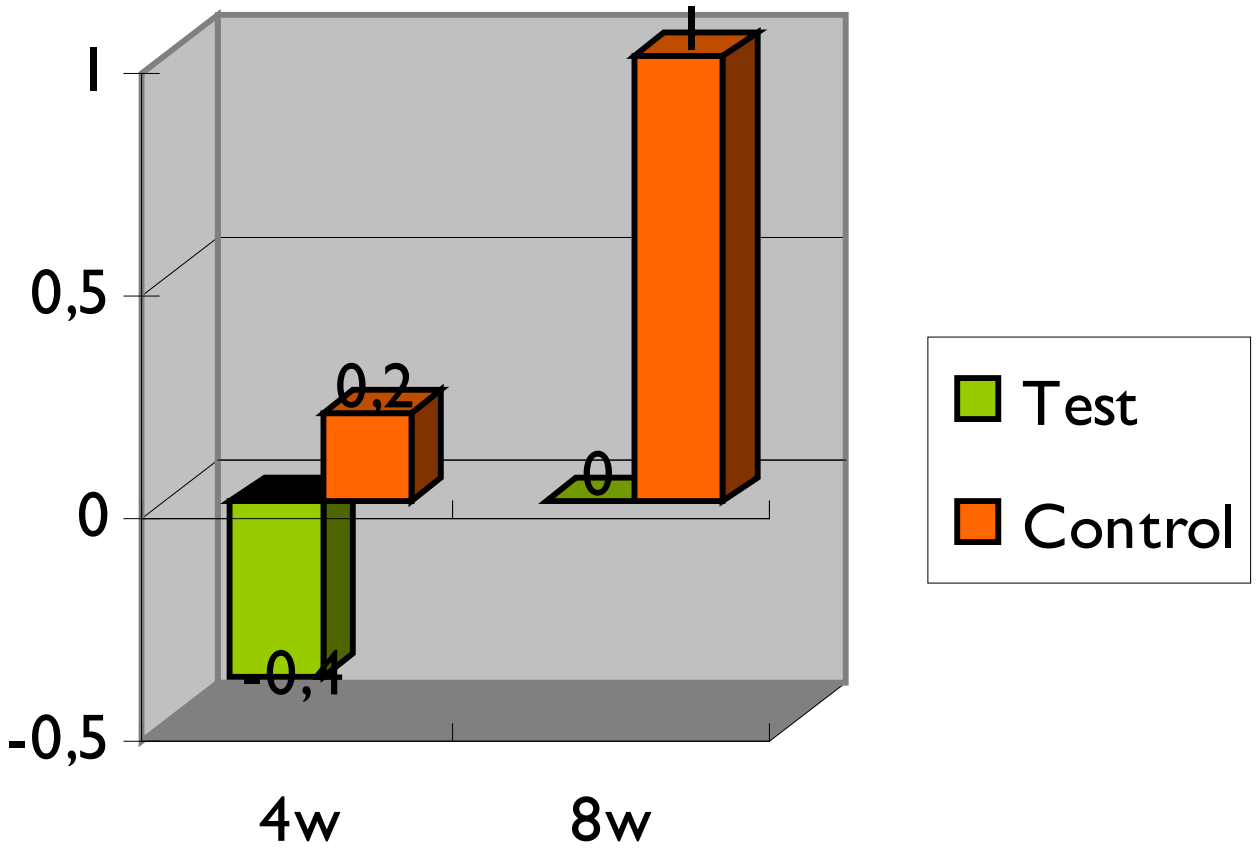
Score Parameters	0	1（Mild）	2（Moderate）	3（Severe）
Foul breath	No smell	Slightly smells	Obviously smells	Badly smells
Gums congestion	No red	Slightly red	Moderate red	Strong red
Gingival bleeding	Not bleed even if it strongly presses	Bleeds slightly when strongly pressing	Bleeds when lightly pressing	Only quietly pressing it bleeds or it bleeds naturally
Gums tumor	No	Slight tumor is admitted in near green gums	Tumor reaches the adhesion gums	Part of tumor and the corona dentis part is violently covered
Gums ulcer	No ulcer	Slight ulcer	Moderate ulcer	Severe ulcer
Hypersalivation	No	Slight	Medium	More
Parodontitis pocket	Depth of the gingival sulcus is 2 mm following.	Depth of gingival sulcus is deeper than that of 2 mm	Periodontal pocket get to alveolus bone top part in root of a tooth	Teeth came out

Trial 1: Specific IgY (PG) in dog food



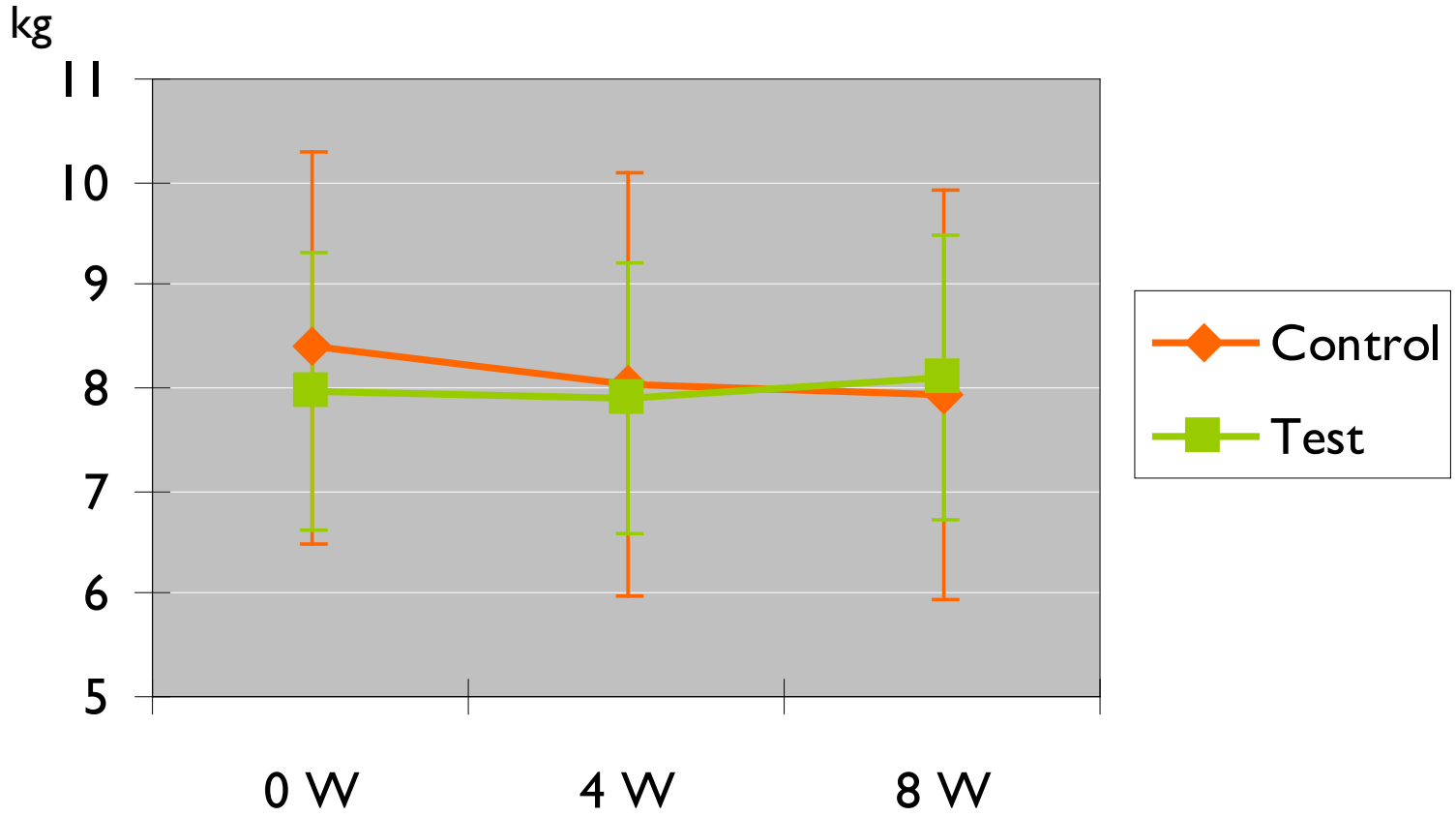
- Changes of total inflammation scores
- * Compared to 0w, P < 0.01 (Anova + Tukey test)

Trial 1: Specific IgY (PG) in dog food



- Change of average pocket depth

Trial 1: Specific IgY (PG) in dog food



- Relative changes of body weight

Trial 2: Clinical trial with dental gel

Trial 2: Specific IgY (PG) in dental gel

- KYODOKEN INSTITUTE FOR ANIMAL SCIENCE RESEARCH & DEVELOPMENT
  - Well known private institute for animal science research and development

- Periofil gel from SHOWA pharmaceuticals, Japan
  - Gel containing 20% Specific IgY (PG) powder

Trial 2 : Specific IgY (PG) in dental gel

- Dogs used in this trial (n=5)
  - Average body weight 6,6 kg

- Shiba

- Pug

- M. Duck

- Beagle

- Cavalier

Trial 2 : Specific IgY (PG) in dental gel

- Test settings:
  - **Test Site** (n=10 sites): Right half of mouth
    20% Specific IgY (PG) mixed in Periofil gel
  - **Control Site** (n=10 sites): Left half of mouth
    Periofil gel without Specific IgY (PG)

Trial 2 : Specific IgY (PG) in dental gel

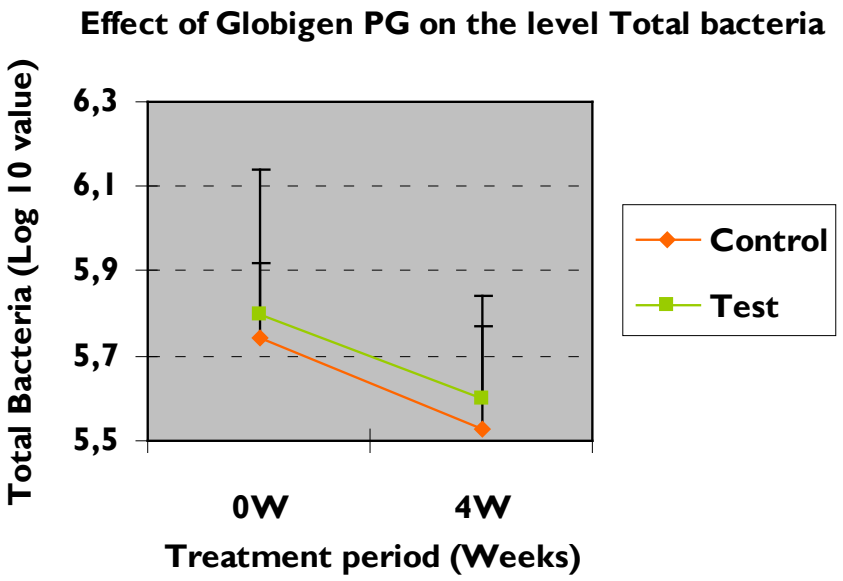
- Application: Dental gel administered 4 times locally and directly by root canal syringe to periodontal pocket at weekly intervals.

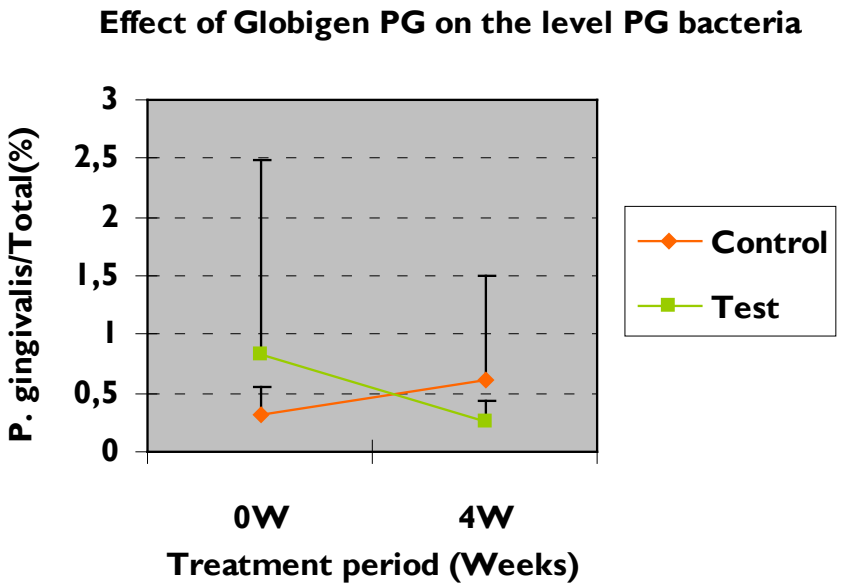
- Evaluation: at 0, 1, 2, 3 and 4 weeks

- Parameters:
  - Bacterial count
  - Inflammation score (pocket depth, body weight, bleeding of gums, gum inflammation, gum ulcer, gum congestion, periodontal ligament inflammation)
  - Pocket depth (average PD)
  - Body weight

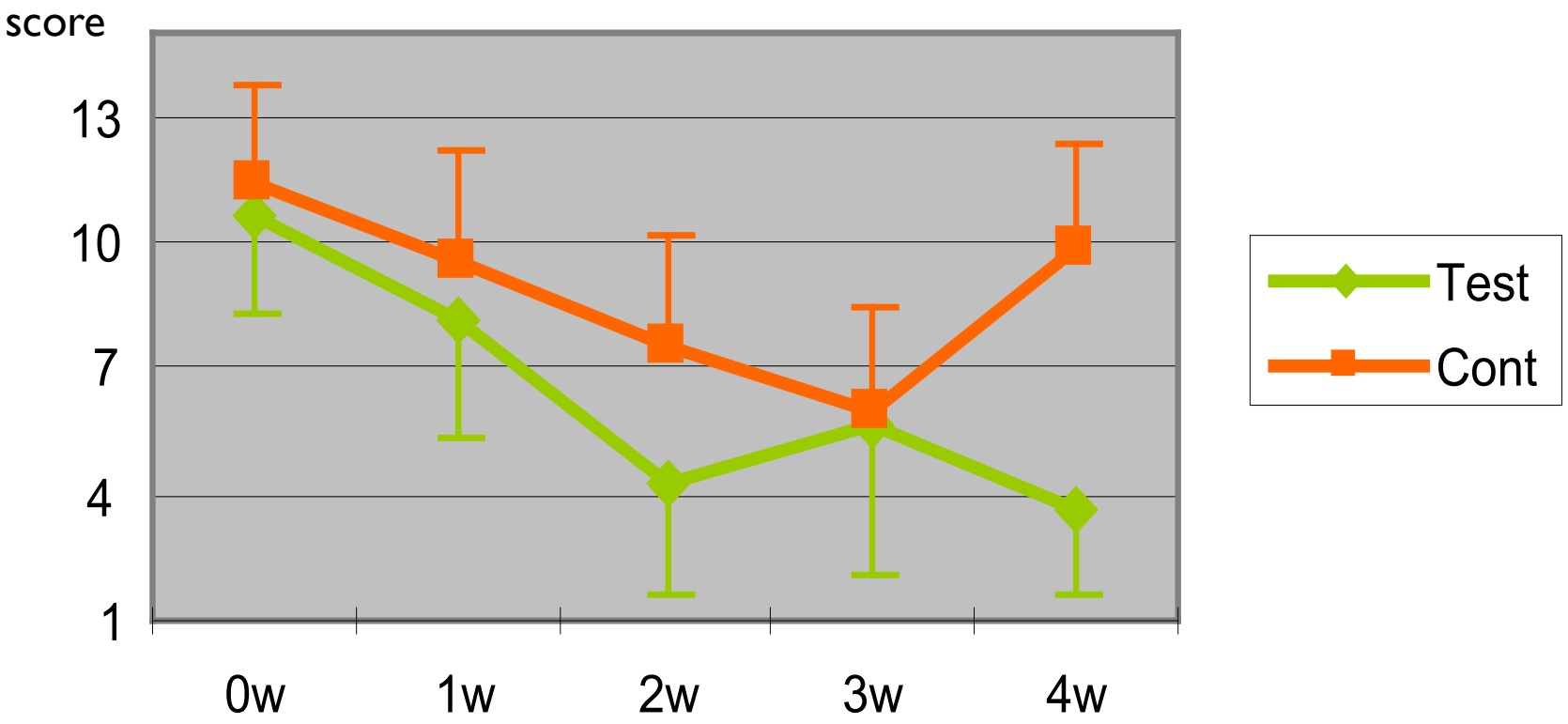
Trial 2: Specific IgY (PG) in dental gel

	Total Bacteria (Log 10 value)		PG/ total bacteria (%)	
Group	Week 0	Week 4	Week 0	Week 4
Globigen PG	5.8 ± 0,12 (n=5)	5.6 ± 0.24 (n=10)	0.83 ± 1.65 (n=5)	0.26 ± 0.17 (n=10)
Control	5.74 ± 0.40 (n=10)	5.53 ± 0.24 (n=10)	0.32 ± 0.24 (n=10)	0.61 ± 0.89 (n=10)



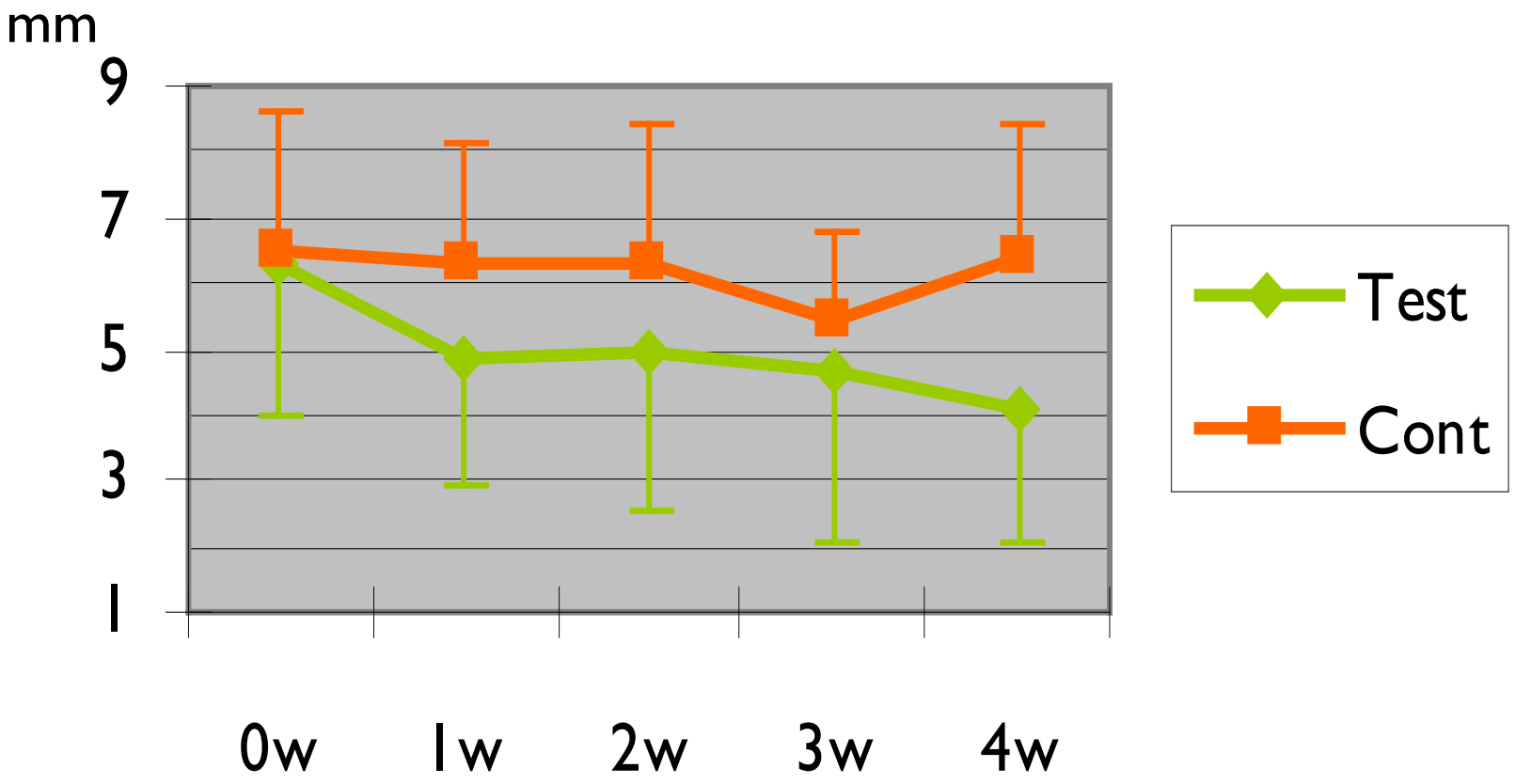


Trial 2: Specific IgY (PG) in dental gel



- Changes of total inflammation score

Trial 2: Specific IgY (PG) in dental gel



- Changes of average pocket depth

Trial 3: Effect of Specific IgY (PG) used in dental gum

Protocol:

Animal： Beagle（Average Age = 7 years, Average Body Weight = 8 kgs）

Group settings:

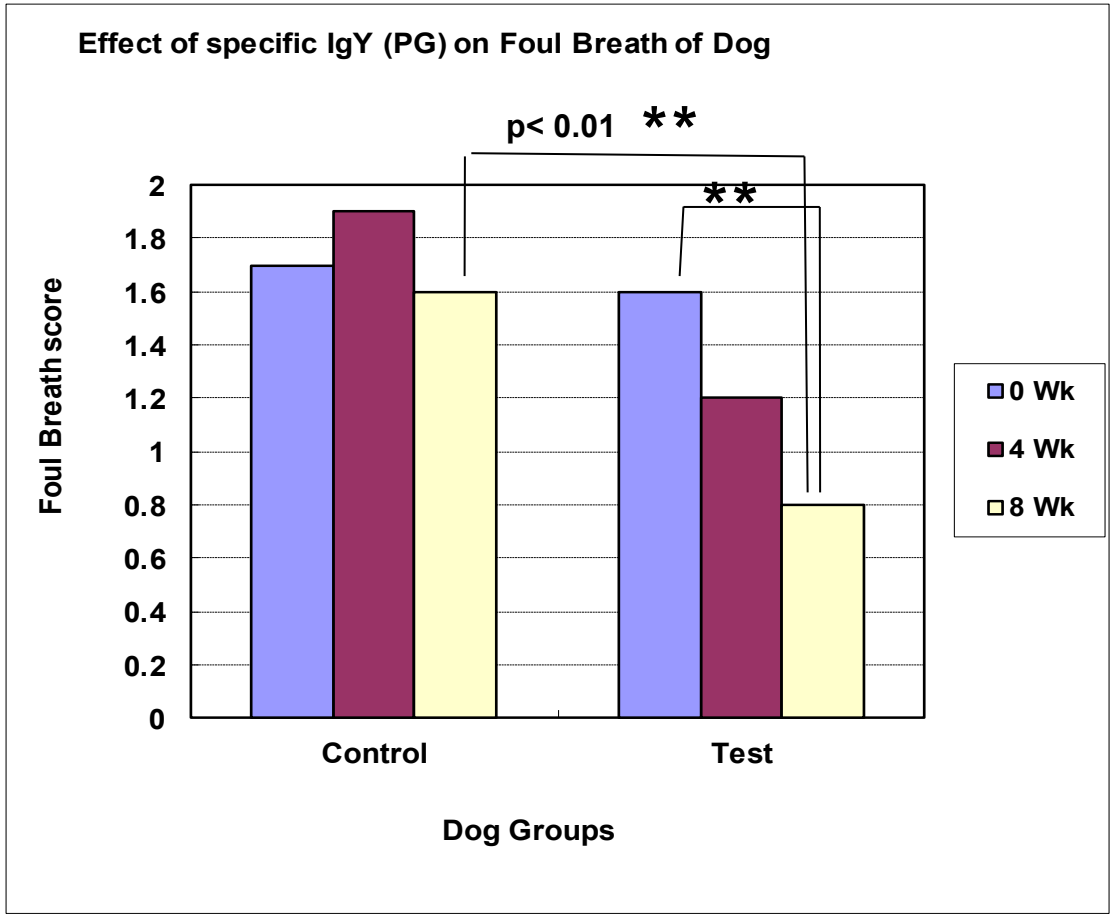
Test Group (n = 10) ： 250 mg Specific IgY (PG) -dental gum /dog/day
Control Group (n = 10)：Feed only .

Evaluation：at 0 W, 4W, 8W

Parameters :

- Inflammation Score: (Foul breath, Gum congestion, Bleeding of gums, Gum inflammation, Gum ulcer, Periodontal ligament inflammation)
- Pocket depth (Average PD)
- Body Weight

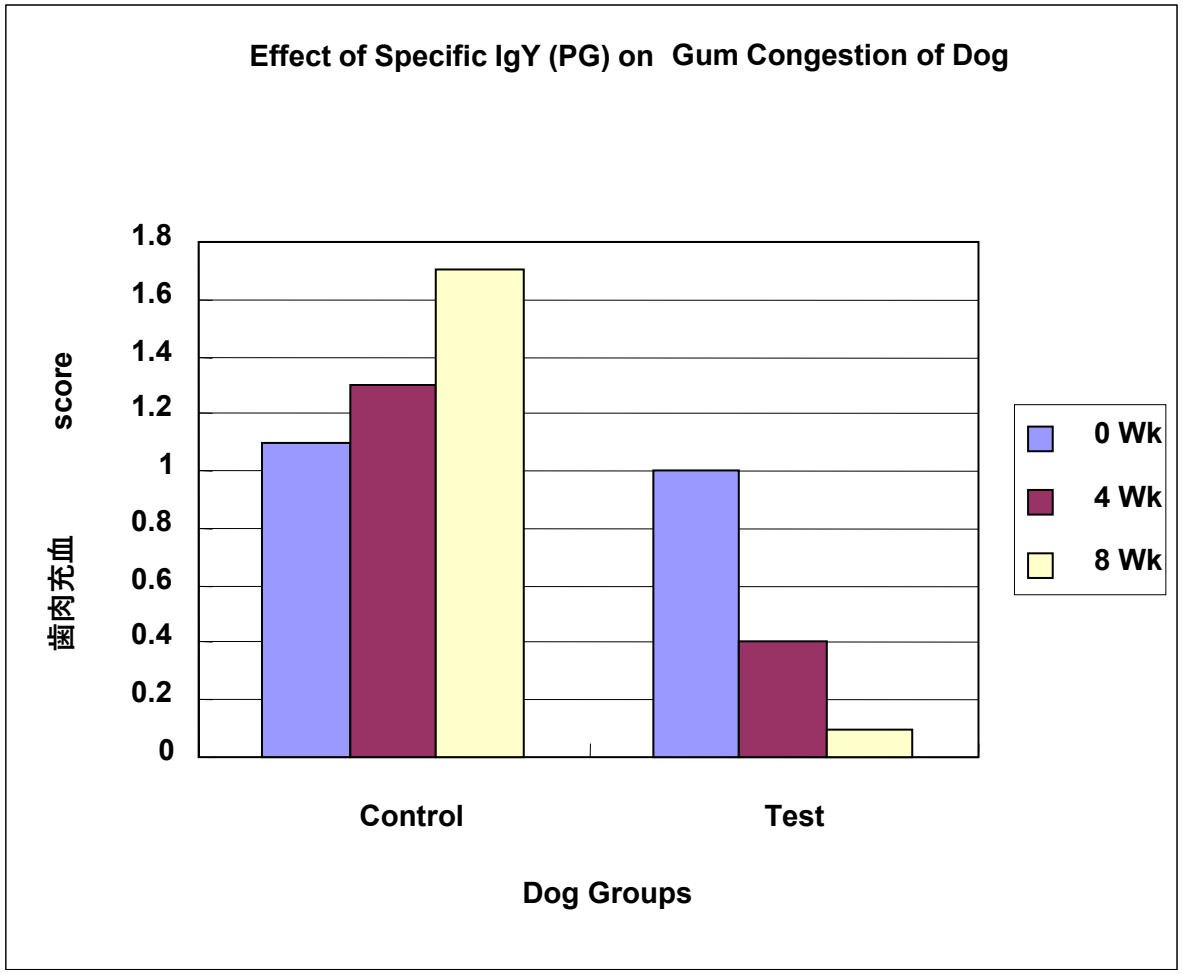
Effect of Dental gum on Foul Breath of Dogs



Foul Breath

	Control	Test
0 Wk	1.7	1.6
4 Wk	1.9	1.2
8 Wk	1.6	0.8

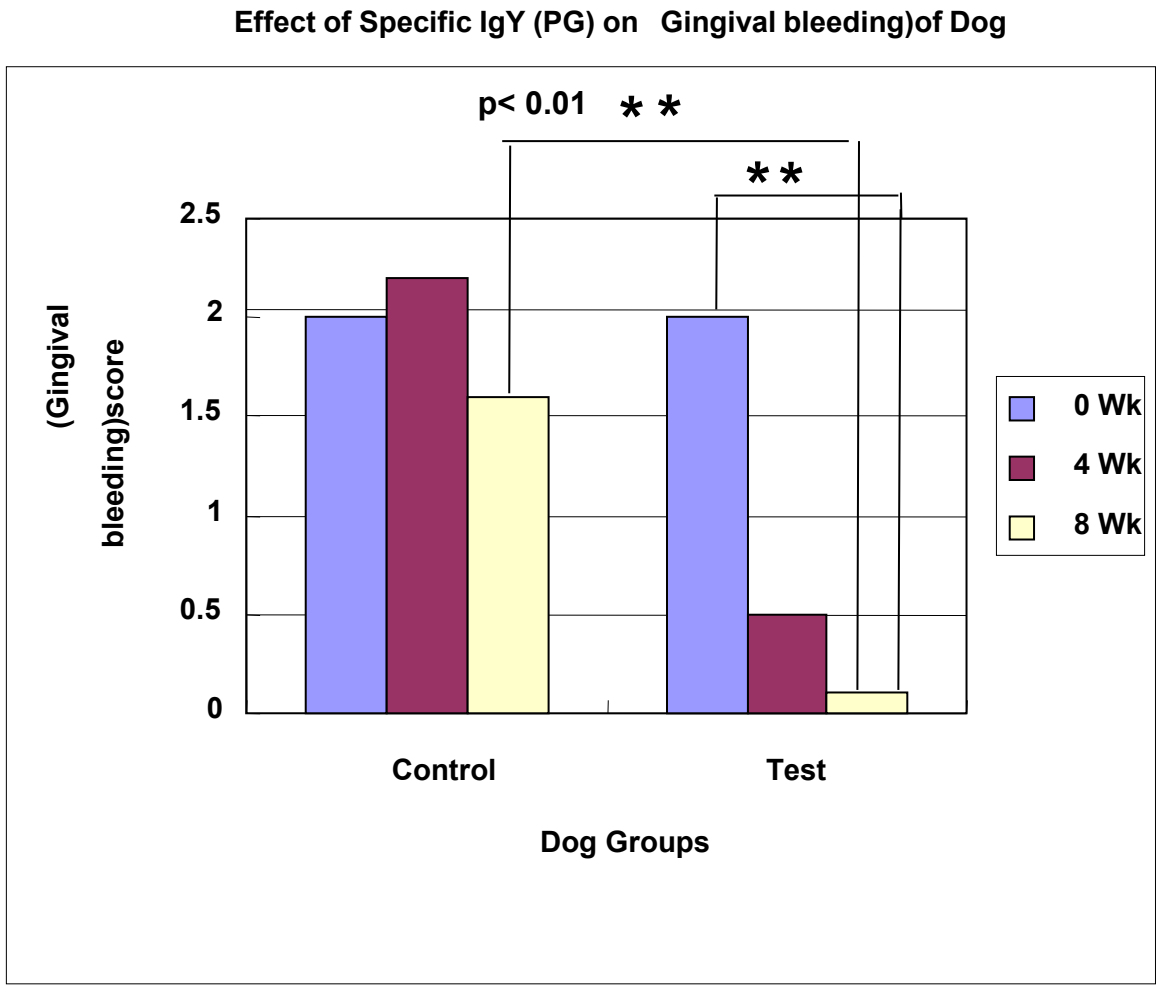
Effect of Dental gum on gum congestion of Dogs



Gum Congestion of Dog

	Control	Test
0 Wk	1.1	1
4 Wk	1.3	0.4
8 Wk	1.7	0.1

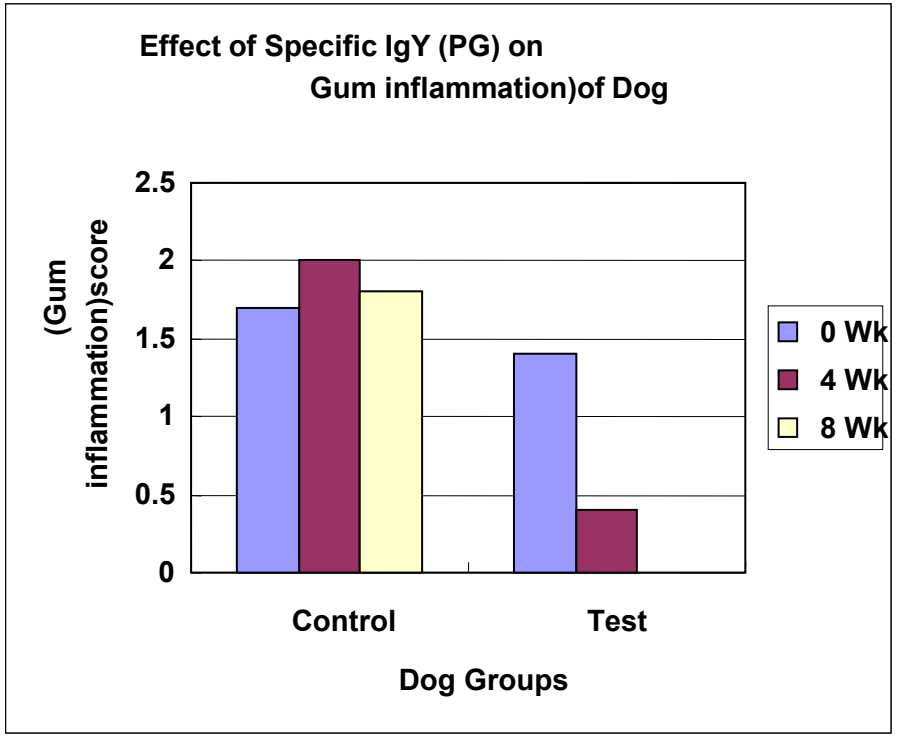
Effect of Dental gum on Gingival bleeding of Dogs

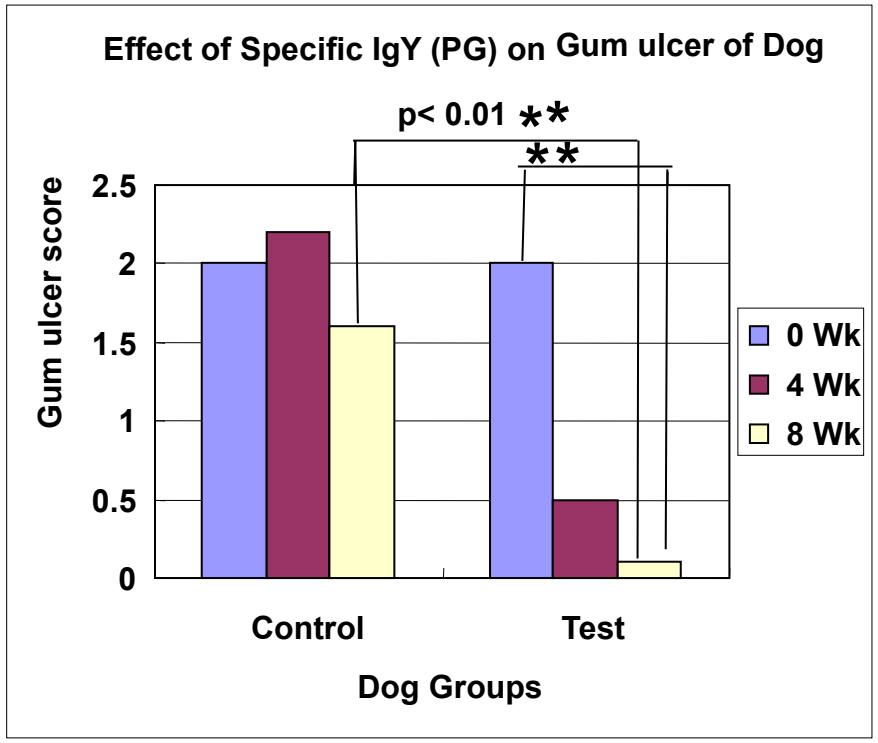


Gingival bleeding Score

Test period	Control	Test
0 Wk	1.1	1.3
4 Wk	2.1	0.6
8 Wk	1.7	0.4

Effect on gum inflammation of Dogs





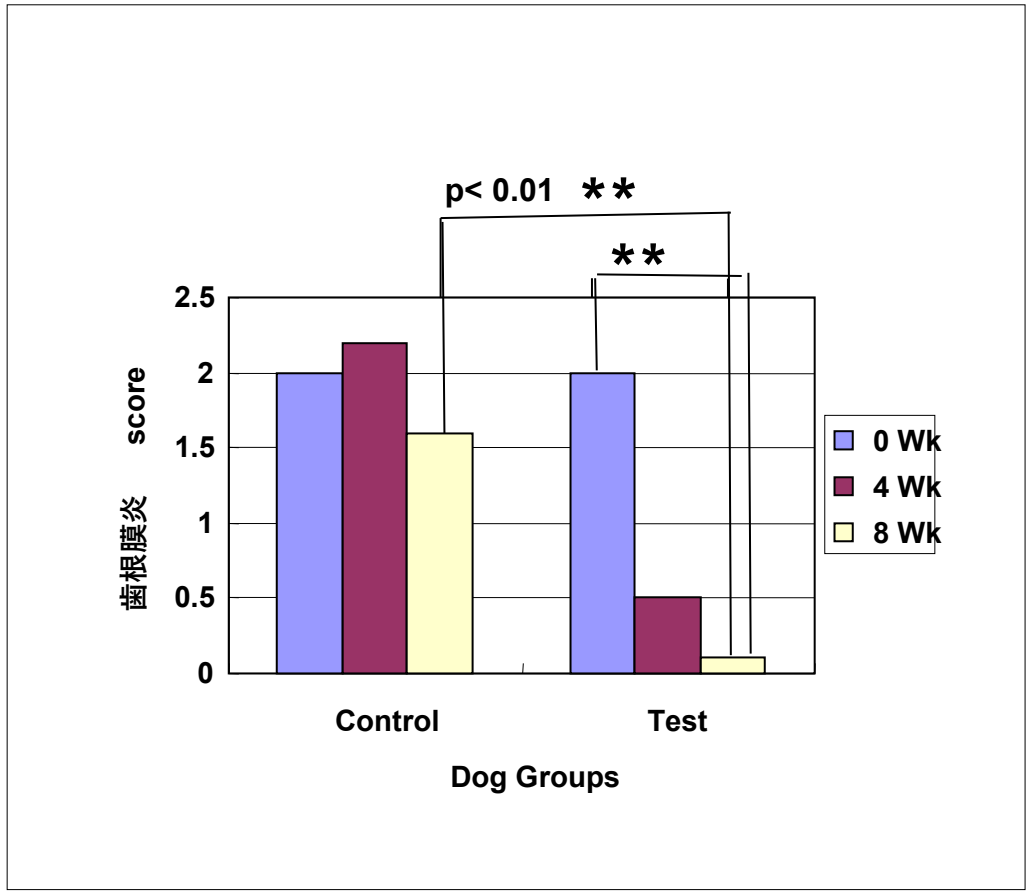
Gum inflammation

	Control	Test
0 Wk	1.7	1.4
4 Wk	2	0.4
8 Wk	1.8	0

Gum ulcer

	Control	Test
0 Wk	0.4	0.2
4 Wk	0.3	0
8 Wk	1.1	0

Effect on Apical Periodontitis



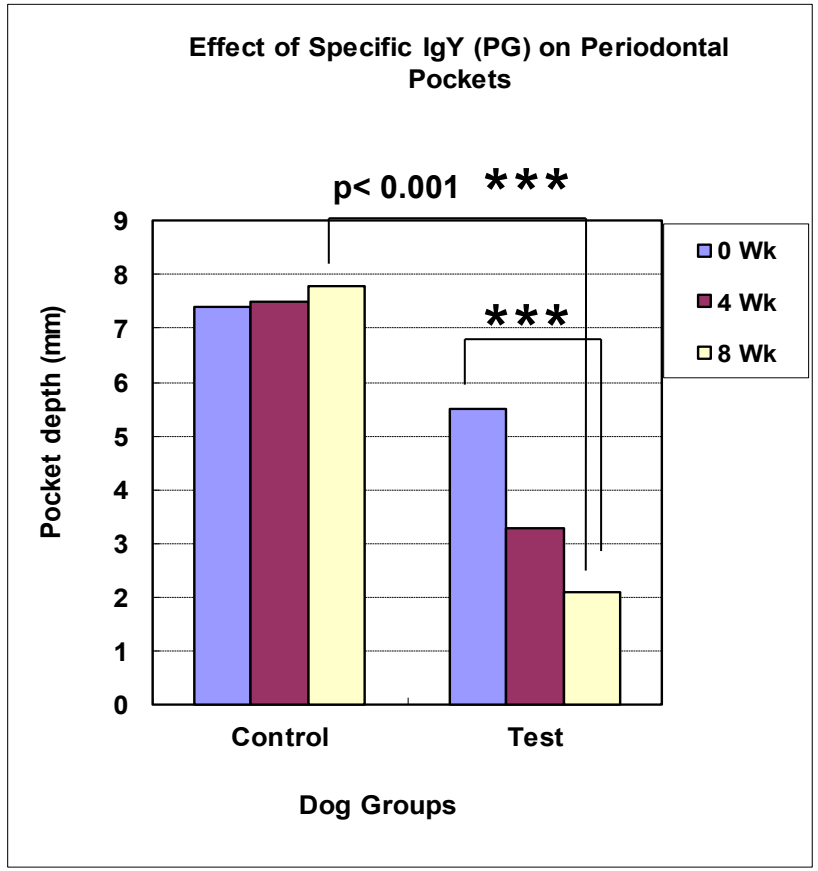
Apical periodontitis score

Test period	Control	Test
0 Wk	2	2
4 Wk	2.2	0.5
8 Wk	1.6	0.1

Effect on Periodontal Pocket Depth (mm)

Test Dog	0 Wk	4 Wk	8 Wk
1	3	2	2
2	5	3	2
3	8	2	0
4	3	1	0
5	3	2	2
6	5	5	2
7	10	2	2
8	5	3	3
9	8	5	4
10	5	8	4
AVG	5.5	3.3	2.1
SDV	2.42	2.11	1.37

Contr Dog	0 Wk	4 Wk	8 Wk
11	3	2	5
12	5	5	4
13	5	5	7
14	8	10	10
15	5	5	5
16	8	10	10
17	10	10	7
18	10	8	10
19	10	10	10
20	10	10	10
AVG	7.4	7.5	7.8
SDV	2.67	2.99	2.48



Effect on Tarter removal

	Treatment with Gum (Test) at			Tarter removal (%)	
Test Dog	0 Wk	4Wk	8Wk	4Wk	8Wk
1	3	2	2	0	0
2	5	3	2	0	0
3	8	2	0	100	100
4	3	1	0	50	50
5	3	2	2	0	30
6	5	5	2	50	90
7	10	2	2	40	60
8	5	3	3	30	50
9	8	5	4	0	0
10	5	8	4	0	0
AVG	5.5	3.3	2.1	27	38
SDV	2.415	2.110	1.370	33.7	38.2

	Treatment with Gum (Test) at			Tarter removal (%)	
Control Dog	0 Wk	4 Wk	8 Wk	4Wk	8Wk
11	3	2	5	0	0
12	5	5	4	0	0
13	5	5	7	0	0
14	8	10	10	0	0
15	5	5	5	0	0
16	8	10	10	0	0
17	10	10	7	0	0
18	10	8	10	0	0
19	10	10	10	0	0
20	10	10	10	0	0
AVG	7.4	7.5	7.8	0	0
SDV	2.674	2.990	2.485	0	0

Effect on pocket depth and sub-gingival bacteria

Test Dog No.	Parameters	0 Week	8 Weeks
1	Pocket depth (mm)	8	0
	P. gingivalis (Log10)	2.3	1.9
	Total Bacteria (Log10)	4.3	4.2
2	Pocket depth (mm)	10	2
	P. gingivalis (Log10)	3.1	1
	Total Bacteria (Log10)	5.1	3.7
3	Pocket depth (mm)	8	4
	P. gingivalis (Log10)	2.0	1.9
	Total Bacteria (Log10)	4.6	4.9
Control Dog			
1	Pocket depth (mm)	8	10
	P. gingivalis (Log10)	2.5	3.2
	Total Bacteria (Log10)	4.5	5.2
2	Pocket depth (mm)	10	10
	P. gingivalis (Log10)	1.7	2.1
	Total Bacteria (Log10)	4.4	4.5
3	Pocket depth (mm)	10	10
	P. gingivalis (Log10)	3.0	3.2
	Total Bacteria (Log10)	5.1	5.3