IgY for Pets
Onset mechanism of Periodontal Disease

Dental plaque formation by saburra and bacteria

Hardening of plaque by the deposition of calcium leads to tartar formation

Growth of anaerobic bacteria in the tooth pockets which causes periodontitis

Progression of inflammation damages the gum tissue

Eventually teeth fall out

80% of pet dogs and 70% of pet cats in Japan is diagnosed with periodontal disease.
Pathogenic Factor of Periodontal Disease

Periodontal disease is caused by an infection of bacteria. The disease causing bacteria in pets are *Porphyromonas* genus.

*P. gulae, P. gingivalis, P. denticanis, P. salivosa*

The periodontopathic bacteria produces a strong protease like enzyme called gingipain, which plays a major role by degrading various host proteins.

**Gingipain is the pathogenic factor of periodontal disease.**

% of *Porphyromonas* genus against the total bacterial count in the dog oral microflora.
Gingipain is the most important pathogenic factor

- Forms biofilm (plaque)
- Oral bacteria
- Coagglutination

**Gingipain mechanism of action**

- Stroma protein
- Fibrinogen breakdown
- Fibrin

- Damage to oral cavity and gum tissue

- Macrophage, damage to neutrophil receptor
- Cytokine and complements breakdown

- Prevention of host immune defense
Anti-gingipain egg yolk immunoglobulin (IgY)

Application of passive immunisation system to produce eggs with accumulated anti-gingipain antibody by immunizing chicken with the pathogenic factor of periodontitis, gingipain partially purified from periodontopathic bacteria.

**Inactivation of pathogenic factor**
The antibody specifically binds to gingipain produced by the bacteria and inactivates the enzyme and toxicity.

**Extremely high specificity**
The antibody only inactivates gingipain, and does not affect with host’s biological system nor good bacteria.

**Extremely fast binding speed**
Due to the strong affinity of the antibody, antigen-antibody reaction occurs instantly.

**No resistance**
Creates no drug resistant bacteria, and effective against drug resistant bacteria.

**Safe and high palatability**
As the ingredient is powdered eggs, it has been used successfully in various products.
Basic Evaluation Test of Anti-gingipain Antibody

In vitro Effect confirmation test of anti-gingipain antibody against gingipain produced by *Porphyromonas* genus

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Department of Periodontology, Nihon University School of Dentistry

IRIG

[Image: IRIG_Immunology_Research_Institute_in_Gifu]
Gingipain Enzyme Activity Inhibition Test

Test method

Anti-gingipain IgY

Gingipain (250 mg/mL)

1hr Reaction

Control IgY

BapNA was used as a matrix and the enzyme activity was measured at OD\textsubscript{405}
Enzyme inhibition (P. gingivalis gingipain)

Enzyme activity (%)

Anti-gingipain IgY conc.

Cont. IgY

47
94
188
375
750 (µg/ml)

Enzyme inhibition (P. gulae gingipain)

Enzyme activity (%)

Anti-gingipain IgY conc.

Cont. IgY

47
94
188
375
750 (µg/ml)
Inhibition Test of Caused by Periodontopathic Bacteria

Test Method
Culture oral epithelial cells (FaDu cells) on a plate

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgY</td>
<td>Anti-gingipain antibody</td>
<td>Control antibody</td>
<td>PBS</td>
</tr>
<tr>
<td>Bacteria</td>
<td>P. gingivalis</td>
<td>P. gulae</td>
<td>PBS</td>
</tr>
</tbody>
</table>

At 37 °C for 1 hour

Measure the number of live FaDu cells
Inhibition effect against cell injury caused by *P. gulae*

- **Negative control** (PBS only)
- **Positive control** (*P. gulae* + PBS)
- **Control** (*P. gulae* + control IgY)
- **Test** (*P. gulae* + Anti-gingipain IgY)
The number of live cells after the inhibition test

<table>
<thead>
<tr>
<th></th>
<th>PBS Treatment only (Non infected)</th>
<th><em>P. gingivalis</em> (Infected)</th>
<th>Survivability of FaDu cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>17.5</td>
<td>3.9</td>
<td>22 %</td>
</tr>
<tr>
<td>Control antibody</td>
<td>16.5</td>
<td>4.5</td>
<td>27 %</td>
</tr>
<tr>
<td>Anti-gingipain antibody</td>
<td>16.0</td>
<td>12.5</td>
<td>78 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PBS Treatment only (Non infected)</th>
<th><em>P. gulae</em> (Infected)</th>
<th>Survivability of FaDu cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>17.5</td>
<td>4.5</td>
<td>28 %</td>
</tr>
<tr>
<td>Control antibody</td>
<td>16.5</td>
<td>5.5</td>
<td>24 %</td>
</tr>
<tr>
<td>Anti-gingipain antibody</td>
<td>16.0</td>
<td>10.8</td>
<td>68 %</td>
</tr>
</tbody>
</table>

(No. of live FaDu cells: $\times 10^4$/mL)
Coaggregation Inhibition Test of *P. gingivalis* with *A. naselundii*

**Test Method**

- Anti-gingipain antibody
- Control antibody

**P. gingivalis** strains
- FDC 381
- GAI 7802
- ATCC 33277
- ATCC 49417
- ATCC 53977

**Actinomyces. naselundii**
- WV 627

Reaction for 60 min

60 rpm 30 min

Determine coaggregation reaction
Coaggregation Inhibition Test of *P. gingivalis* with *A. naselundii*

<table>
<thead>
<tr>
<th><em>P. gingivalis</em> strain</th>
<th>Anti-gingipain antibody</th>
<th>Control antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDC 381</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>GAI 7802</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ATCC 33277</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ATCC 49417</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
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<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*(Numbers: Bacteria coaggregation score)*

Coaggregation score was determined by Kolenbrander, Cisar’s method between 0-4 as described below.

0 : No coaggregation was seen  
1 : Scattered small aggregates were observed in the cloudy suspension.  
2 : Small lumps of aggregates were observed in the cloudy suspension.  
3 : Big lumps of aggregates were observed in the clear suspension.  
4 : The suspension was clear and the aggregate was one big lump in the middle.
Basic Evaluation Test - Summary

- Anti-gingipain antibody inhibited the enzyme activity of the protease enzyme, gingipain produced from *P. gingivalis* and *P. gulae*.

- Anti-gingipain antibody inhibited the oral epithelial cell injuries caused by *P. gingivalis* and *P. gulae*.

- Anti-gingipain antibody inhibited the coaggregation of *P. gingivalis* with general bacteria in the mouth cavity.

In vitro basic evaluation test revealed that the anti-gingipain IgY has the ability to inhibit the activity and the action of gingipain, which can lead to the prevention of periodontal disease.
Evaluation test on dogs diagnosed with periodontal disease

Effectiveness and safety evaluation test of dry pet food containing anti-gingipain antibody given to dogs diagnosed with periodontal diseases for 8 weeks.

Testing Agency : Kyodoken Institute for Animal Science Research & Development
Test Protocol

Animals
Dog species: Beagle, Miniature Dachshund, Cavalier King Charles Spaniel
Age: 5-7 years, and dogs of indeterminate age but kept for more than 5 years.
Weight: 6.6～10.2kg
Selection criteria: Dogs diagnosed with periodontal disease that have the confirmed presence of teeth pockets in the both gingival sulcus and obvious halitosis.

Groups
Test: 10 dogs (8 Beagles, 1 Cavalier, 1 Dachshund); Average weight 7.9±1.3 kg
Control: 5 dogs (4 Beagles, 1 Dachshund); Average weight 8.3±1.9 kg

Food
Test group: 0.1% anti-gingipain antibody was added to commercial dry food for dogs.
Control group: Only commercial dry food for dogs.
Amount: Given amount per day was calculated from each individual weight
Duration: 8 weeks

Evaluation
Oral cavity score: Halitosis, congestion, bleeding, swelling and ulceration of the gums, salivation, periodontitis, and periodontal pocket depth.
Observation and measurement at the beginning, after 4 and 8 weeks

General score: Vitality, faeces characteristics, appetite (Weigh leftover feed)
Observed everyday during the trial
Weighed at the beginning, after 4 and 8 weeks
# Oral cavity evaluation and its criteria

<table>
<thead>
<tr>
<th>Evaluated points</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Halitosis</td>
<td>None</td>
</tr>
<tr>
<td>Congestion of gum</td>
<td>None</td>
</tr>
<tr>
<td>Bleeding of gum</td>
<td>No bleeding with strong pressure</td>
</tr>
<tr>
<td>Swelling of gum</td>
<td>None</td>
</tr>
<tr>
<td>Ulceration of gum</td>
<td>None</td>
</tr>
<tr>
<td>Salivation</td>
<td>None</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Depth of gingival sulcus less than 2mm</td>
</tr>
</tbody>
</table>
The change in the oral cavity score observed

The total oral cavity score is the sum of the individual scores on the points shown below.

Halitosis, congestion, bleeding, swelling and ulceration of the gums, salivation, periodontitis.

Blue letter indicates that there was a significant difference between evaluation points.
**Gum congestion clinical score**

- **Clinical Score**
  - Test: 1.6, 1.2
  - Control: 0.8, 0.4
  - Differences: -1.0, -0.8

- **Differences in gum congestion clinical score**
  - $P=0.03$ (T-test)

**Gum bleeding clinical score**

- **Clinical Score**
  - Test: 2.0, 1.6
  - Control: 1.2, 0.8
  - Differences: -0.2, 0.0

- **Differences in gum congestion clinical score**
  - $P=0.003$ (T-test)
臨床スコア変化値

試験区 対照区
⊿8w(8w-0w)

臨床スコア値

試験区 対照区

0w 8w

P=0.002 （Wilcoxon）

P=0.05 （T-test）
Tartar removal effect

At the beginning of trial

Test group No.2

PD: 3mm  
Score: 5

Test group No.5

PD: 3mm  
Score: 8

Test group No.7

PD: 5mm  
Score: 9

At 4 weeks

PD: 1mm  
Score: 2

PD: 3mm  
Score: 2

PD: 4mm  
Score: 2

At 8 weeks

PD: 1mm  
Score: 0

PD: 3mm  
Score: 2

PD: 4mm  
Score: 4

Was only observed in 3 out of 10 dogs in the test group.

PD: Pocket depth
Results of General Observations

No abnormality was observed in Vitality, faeces characteristics, appetite (No leftover feed) of 15 dogs in the test and control group during the trial period of 56 days.
Summary - Evaluation test on dogs diagnosed with periodontal disease

- Administration of dry food with additional anti-gingipain antibody showed effectiveness as shown below.
  (1) Oral clinical symptoms of dogs diagnosed with periodontal disease was significantly improved.
  (2) Congestion and bleeding of the gums, periodontitis was significantly improved.
  (3) Firm tartar removal effect was observed in 3 out of 10 dogs.

- Safety and acceptability of anti-gingipain antibody for dogs was acknowledged.