Concomitant Administration of GonaCon™ and Rabies Vaccine in Female Dogs (Canis familiaris) in Mexico

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Impact of the Rabies Program in Mexico

North America Rabies Management Plan

Stabilization of the Canine Population

- Dog and cat spaying and neutering
- Voluntary donation
- Euthanasia
- To reduce the risk of attacks on humans
- To create a culture of responsible owner

Human Rabies Cases in Mexico

Dog Rabies Vaccination and Human Rabies Cases in Mexico

Lab Studied and Positive Samples in Mexico

Rabies PED in Mexico

Signed at the 8th NARMP Meeting October, 2008, Atlanta, GA
OBJECTIVES

- To evaluate the safety of updated GonaCon™ formula in Mexico.
- To evaluate progesterone levels and the titers of antibodies against gonadotropin releasing hormone (GnRH).
- To document any effects of toxicity in domestic female dogs in Mexico.
- To see that simultaneous applications of GonaCon™ and the parenteral rabies vaccination used in Mexico does not limit the immune response to both treatments.
- To evaluate the risk of possible local adverse reactions on the application area of updated GonaCon™ formula.

HYPOTHESIS

- The number and kind of adverse local reactions is less and in a minor intensity that the reported in previous studies.
- The new GonaCon™ reduces the progesterone levels and develop antibodies titers against gonadotropin releasing hormone (GnRH).
- GonaCon™ has less adverse effects of toxicity in domestic female dogs in Mexico compared with previous versions.
- The simultaneous applications of GonaCon™ and the parenteral rabies vaccination in female dogs in Mexico do not limit the immune response for both products.
- The updated GonaCon™ formula is not generate local adverse reactions that could suggest discontinuing its use in Mexico.

GonaCon™ Study in Mexico

**Selection of female dogs for the study (Sep – Nov/2010).**
**Integration of groups – GonaCon™ importation (Oct/10 – Jan/11).**
**Field trial: 3 groups: 61 study days: Daily and weekly clinical evaluation: Sampling procedures on D0, D31 y D61 (Jan - Mar/11).**
**Laboratory Analysis (CBC, BCP, VNA, THR, GnRH), (Jan - Jul/11)**
**Macro and Microscopic findings evaluation (Mar - Sep/11).**
**Preliminary report integration (Sep - Oct/11).**
**Final Report (Dec/11)**

GonaCon™ Study in Mexico

**Conjugated to a large mollusk hemocyanin protein and emulsified with the adjuvant AdjuVac™**
- A 0.5 ml dose
- 500 µg of the GnRH conjugate
- 21µg of inactivated Mycobacterium avium (Adjuvant)
- Gentamicin (Life Technologies, Carlsbad, CA)
- Fungizone (Life Technologies, Carlsbad, CA)
- Formulation produced in a clean room to inhibit bacterial and fungal activity.
- Pre-loaded in 3ml Air-Tite luerc-lock syringes (Air-Tite Products, Virginia Beach, VA, USA)
**Field Observations (61 Days Period)**

**GonaCon™ Study in Mexico**

**Group A**
- 6 female dogs (in houses).
- Just rabies vaccine.
- 1 dog with serosanguineous discharge at D58.
- No physical alterations, prostration, lesion or limping.
- 2 female dogs finished the study prematurely.
- Weight average: D0: 21.23 kg ± 10.34; D61: 21.23 kg ± 10.96.

**Group B**
- 7 female dogs (Confined).
- Just GonaCon™.
- 1st Week - 4 dogs with pain and/or hyperthermia in LPM.
- 1 dog had puppies during the selection period (11/10).
- 2 more had puppies during the 1st week of the study (01/11).
- 1 dog with a small spot in the application site of GonaCon™ at D16.
- All female dogs conclude the study with muscle atrophy on the LPM.
- No prostration or limping.
- Weight average: D0: 15.74 kg ± 4.62; D61: 15.93 kg ± 4.16.

**Group C**
- 7 female dogs (Confined).
- Rabies Vaccine + GonaCon™.
- 1st Week - 2 dogs with hyperthermia in LPM.
- 1 dog with serosanguineous discharge at D16 and others in D59.
- 1 dog with a small spot in the application site of GonaCon™ at D16.
- All female dogs conclude the study with muscle atrophy on the LPM.
- No prostration or limping.
- Weight average: D0: 15.74 kg ± 4.62; D61: 15.93 kg ± 4.16.
**GonaCon™ Study in Mexico**

**Blood Sampling Procedures**
(5 samples set for each dog/day)

**Blood Chemistry Parameters (BCP)**
(Decrease, Urea, Creatinine, Uric Acid, Cholesterol & Triglycerides)

- Hypoglycemia
- Hyperazotemia
- Hypercholesterolemia
- Hypocholesterolemia
- Hypouricemia

**Cell Blood Count (CBC)**

<table>
<thead>
<tr>
<th>Lab Findings</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroblastopenia</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>1 0 0</td>
<td>1 0 0</td>
<td>1 0 0</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>1 1 0</td>
<td>1 1 0</td>
<td>1 1 0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>6 1 2</td>
<td>7 1 4</td>
<td>6 0 3</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>0 0 2</td>
<td>0 1 3</td>
<td>1 0 2</td>
</tr>
</tbody>
</table>

**Graph 1. Distribution of the THR Titers of Female dogs**

<table>
<thead>
<tr>
<th>Group</th>
<th>THR D0</th>
<th>THR D31</th>
<th>THR D61</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 5 10</td>
<td>0 5 10</td>
<td>0 5 10</td>
</tr>
<tr>
<td>B</td>
<td>0 5 10</td>
<td>0 5 10</td>
<td>0 5 10</td>
</tr>
<tr>
<td>C</td>
<td>0 5 10</td>
<td>0 5 10</td>
<td>0 5 10</td>
</tr>
</tbody>
</table>
GonaCon™ Study in Mexico

Rabies Antibodies Titers (VNA – FAVN*)

Distribution, mean and standard deviation of female dogs by Rabies Virus Neutralizing Antibodies (VNA) for IU class by Groups and Day

<table>
<thead>
<tr>
<th>GROUP/DAY</th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Findings</td>
<td>D0</td>
<td>D31</td>
<td>D61</td>
</tr>
<tr>
<td>&lt; 0.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5 to 3.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 3.0</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Statistical Findings</td>
<td>D0</td>
<td>D31</td>
<td>D61</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>St Dev</td>
<td>3.56</td>
<td>4.68</td>
<td>6.28</td>
</tr>
</tbody>
</table>

*Maximum possible value 13.8 IU/ml
### GonaCon™ Study in Mexico

**Antibodies Titors Against Gonadotropin Releasing Hormone (GnRH – D x 1,000)**

<table>
<thead>
<tr>
<th>GROUP/DAY</th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Findings</td>
<td>Mean</td>
<td>Std Dev</td>
<td>Maximum</td>
</tr>
<tr>
<td>Day 1</td>
<td>146.3</td>
<td>0.0</td>
<td>128.0</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.0</td>
<td>0.0</td>
<td>109.7</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Statistical Findings**

- GnRH immune response was detected. At Day 31, anti-GnRH titers in Groups B and C were lower than Group A (F=2.46, P=0.116). At Day 31, rabies VNA titers in Group B were lower than Group A. Also, Group C titers were lower than Group B (F=3.01, P=0.076). At Day 61, rabies VNA titers in Groups B and C were lower than Group A (F=3.92, P=0.040).

### Rabies VNA titers

- All dogs were reported to have been rabies vaccinated; dogs in Group B were not revaccinated against rabies. At Day 0, rabies VNA titers in Groups A and C were similar, but lower than Group B (F=3.92, P=0.040). At Day 31, rabies VNA titers in Group B were lower than Group A. Also, Group C titers were lower than Group B (F=3.01, P=0.076). At Day 61, rabies VNA titers in Groups B and C were lower than Group A (F=3.92, P=0.040).

### Anti-GnRH antibody titers

- GonaCon™ was never used in these dogs before this study. At Day 9, no GnRH immune response was detected. At Day 31, anti-GnRH titers among dogs in Groups B and C were similar, but higher than Group A (F=18.19, P=0.001). The same results were observed on Day 61 (F=10.27, P=0.001).

### Macropscopic Findings

- **Controls**
  - Studies in 6 animals.
  - Hypophysitis: not apparent findings.
  - Hypophysitis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells). More acidophilic cells (1).
  - Ovary: Increased number of atretic follicles. Irregular reduction of second, preantral and third follicles. Basophilic aspect into the tunica albuginea.
  - Muscle: 10 samples.
  - Group B: No apparent findings (3). Moderate and focal chronic granulomatous myositis (1). Diffuse coagulative necrosis (1). Group C: Slight focal chronic granulomatous myositis (1). Slight to severe multifocal necrosis (3).

- **Group B**
  - Studies in 6 animals.
  - Hypophysitis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells). More acidophilic cells (1).
  - Ovary: Increased number of atretic follicles. Irregular reduction of second, preantral and third follicles. Basophilic aspect into the tunica albuginea.
  - Muscle: 10 samples.
  - Group B: No apparent findings (3). Moderate and focal chronic granulomatous myositis (1). Diffuse coagulative necrosis (1).
  - Group C: Slight focal chronic granulomatous myositis (1). Slight to severe multifocal necrosis (3).

- **Group C**
  - Studies in 6 animals.
  - Hypophysitis: not apparent findings.
  - Hypophysitis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells). More acidophilic cells (1).
  - Ovary: Increased number of atretic follicles. Irregular reduction of second, preantral and third follicles. Basophilic aspect into the tunica albuginea.
  - Muscle: 10 samples.
  - Group B: No apparent findings (3). Moderate and focal chronic granulomatous myositis (1). Diffuse coagulative necrosis (1). Group C: Slight focal chronic granulomatous myositis (1). Slight to severe multifocal necrosis (3).

**Preliminary Microscopic Findings**

- **Controls**
  - Studies in 6 dogs.
  - Hypophysitis: not apparent findings.
  - Hypophysitis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells). More acidophilic cells (1).
  - Ovary: Increased number of atretic follicles. Irregular reduction of second, preantral and third follicles. Basophilic aspect into the tunica albuginea.
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  - Group C: Slight focal chronic granulomatous myositis (1). Slight to severe multifocal necrosis (3).

- **Group B**
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  - Hypophysitis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells). More acidophilic cells (1).
  - Ovary: Increased number of atretic follicles. Irregular reduction of second, preantral and third follicles. Basophilic aspect into the tunica albuginea.
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  - Group B: No apparent findings (3). Moderate and focal chronic granulomatous myositis (1). Diffuse coagulative necrosis (1).
  - Group C: Slight focal chronic granulomatous myositis (1). Slight to severe multifocal necrosis (3).

- **Group C**
  - Studies in 6 dogs.
  - Hypophysitis: not apparent findings.
  - Hypophysitis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells). More acidophilic cells (1).
  - Ovary: Increased number of atretic follicles. Irregular reduction of second, preantral and third follicles. Basophilic aspect into the tunica albuginea.
  - Muscle: 10 samples.
  - Group B: No apparent findings (3). Moderate and focal chronic granulomatous myositis (1). Diffuse coagulative necrosis (1).
  - Group C: Slight focal chronic granulomatous myositis (1). Slight to severe multifocal necrosis (3).
**GonaCon™ Study in Mexico**

**Microscopic Findings**

**Hypophysis (Pituitary Gland)**
- Normal
- Diffuse congestion and edema
- Coagulative necrosis

**Ovary**
- Normal
- Reduction of second, preantrium and third follicles

**Muscle**
- Normal
- Chronic granulomatous myositis
- Diffuse coagulative necrosis
- Severe multifocal necrosis

**Less and minor intensity of adverse local reactions than in previous formulation used.**

**GonaCon™ reduces progesterone levels and develop antibodies titers against GnRH.**

**GonaCon™ had not generated adverse effects of toxicity.**

**Simultaneous application of GonaCon™ and rabies vaccine did not limited the immune response.**

**Conclusions and Recommendations**
GonaCon™ Study in Mexico

Conclusions and Recommendations

- GonaCon™ generated NOT measured muscle atrophy.
- Observed chronic granulomatous myositis and the diffuse coagulative necrosis were not limping source.
- No dejection, ulceration or paralysis were observed.
- A new study in owned female dogs during a long period (2 years) could be useful to measure the real possibilities to use the product in Mexico.

GonaCon™ Study in Mexico

Special Considerations – Lessons Learned

- All dogs were clinically evaluated and reported in several stages of metabolic alterations (hepatic and renal function).
- It looks like these metabolic problems are not related with the study but is needed more analysis about this condition.
- A full report of macro and microscopic findings in the collected organs and tissues will be useful.
- All animals into the study had previously received rabies vaccine. We have no chances to get non vaccinated dogs in the field.

GonaCon™ Study in Mexico

Special Considerations – Lessons Learned

- The FAVN test were adjusted for the top of 13.8 UI/ml. It is needed to compare FAVN and RIFFT titers to better understand rabies antibodies responses.
- On the last days was detected the muscle atrophy. We had not a previously established strategy to make systematic measurements, but observations were recorded.
- During the study 2 female dogs into the Group B, and 1 into the Group C, that were pregnant and had puppies in that time are kept alive and they are clinically healthy.

GonaCon™ Study in Mexico

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GonaCon™ Study in Mexico

REPORT UNDER PUBLICATION PROCESS

Thank You!

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