

ACC&D's 5th International Symposium on Non-surgical Contraceptive Methods for Pet Population Control

Portland, Oregon, USA.
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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

Dog Rabies Vaccination and Dog Rabies Cases in Mexico

Dog Rabies Vaccination and Human Rabies Cases in Mexico

Lab Studied and Positive Samples in Mexico

Rabies PEP in Mexico

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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








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North America Rabies Management Plan

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

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GonaCon™ Study in Mexico

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.



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PROVEZA's Shelter Facilities. Villa de Tezontepec, Hgo



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Study Phases

- ✓ 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).
 - Group A: Just Rabies Vaccine (RPM).
 - Group B: Just GonaCon™ (LPM).
 - Group C: Rabies Vaccine (RPM) + Gonacon™ (LPM).
- ✓ 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)



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Formulation

- ✓ 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 luer-lock syringes (Air-Tite Products, Virginia Beach, VA, USA)



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Field Trial (61 Days Period)

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Field Observations (61 Days Period)

| Group A | Group B | Group C |
|--|---|---|
| <ul style="list-style-type: none"> ✓ 6 female dogs (in houses). ✓ Just rabies vaccine. ✓ 1 dog with serosanguinous discharge at D58. ✓ No Physical alterations, prostration, lesion or limping. ✓ 2 female dogs finished the study pregnant. ✓ Weight average: D0 - 21.22 kg. ± 10.54; D61 - 21.25 kg ± 10.86. | <ul style="list-style-type: none"> ✓ 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 - 15.29 kg ± 5.26; D61 - 13.86 kg ± 3.44. | <ul style="list-style-type: none"> ✓ 7 Female dogs (Confined), Rabies Vaccine + GonaCon™. ✓ 1st Week - 2 dogs with hyperthermia in LPM. ✓ 1 dog with serosanguinous discharge on D16* and other 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 ± 4.14. |

*Alive at D94, with a new serosanguinous discharge

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Field Trial (61 Days Period)

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

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Cell Blood Count (CBC)

(Hematocrit, Hemoglobin, Mean Corpuscular Value, Mean Corpuscular Hemoglobin, Leucocytes & Platelets)

| Lab Findings | Group A | | | Group B | | | Group C | | |
|--------------------|---------|-----|-----|---------|-----|-----|---------|-----|-----|
| | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Erythroblastopenia | 2 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Leukocytosis | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Leukopenia | 1 | 1 | 0 | 7 | 2 | 1 | 0 | 2 | 1 |
| Thrombocytopenia | 6 | 1 | 2 | 7 | 1 | 4 | 6 | 0 | 3 |
| Polycythemia | 0 | 0 | 2 | 0 | 1 | 3 | 1 | 0 | 2 |

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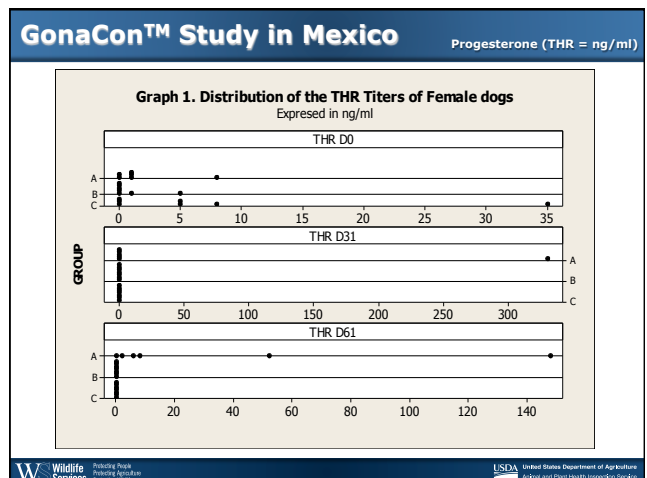
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Blood Chemistry Parameters (BCP)

(Glucose, Urea, Creatinine, Uric Acid, Cholesterol & Triglycerides)

| Lab Findings | Group A | | | Group B | | | Group C | | |
|----------------------|---------|-----|-----|---------|-----|-----|---------|-----|-----|
| | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Hypoglycemia | 4 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | 0 |
| Hyperazotemia | 6 | 6 | 5 | 5 | 6 | 7 | 7 | 7 | 7 |
| Hypercholesterolemia | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hypocholesterolemia | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| Hypopuremia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |

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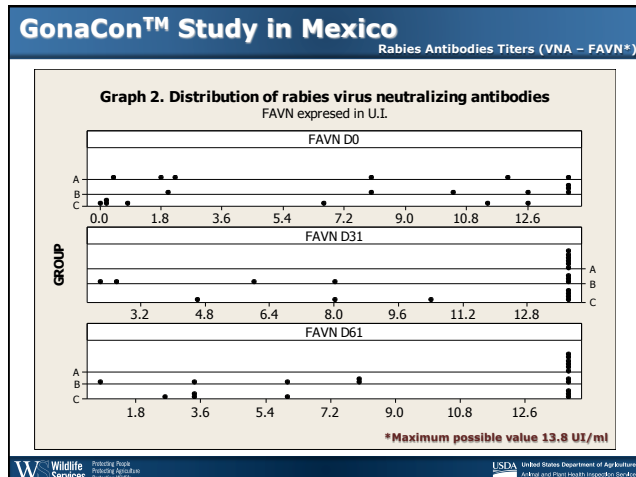


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Progesterone (THR = ng/ml)

Distribution, mean and standard deviation of female dogs by Progesterone Levels (THR in ng/ml)

| GROUP/DAY | GROUP A | | | GROUP B | | | GROUP C | | |
|-----------------------------|-----------|------------|------------|-----------|------------|------------|-----------|------------|------------|
| | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Lab Findings | | | | | | | | | |
| < 1.0 | 2 | 4 | 1 | 5 | 7 | 7 | 3 | 7 | 7 |
| = 1.0 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| > 1.0 | 1 | 1 | 5 | 1 | 0 | 0 | 4 | 0 | 0 |
| Statistical Findings | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Minimum | 0.20 | 0.40 | 0.9 | 0.0 | 0.0 | 0.0 | 0.10 | 0.10 | 0.0 |
| Maximum | 8.40 | 37.9 | 147.7 | 4.8 | 0.40 | 0.9 | 35.0 | 0.80 | 0.9 |
| Mean | 2.0 | 6.80 | 36.2 | 0.90 | 0.08 | 0.22 | 7.53 | 0.31 | 0.25 |
| St Dev | 3.16 | 15.24 | 58.0 | 1.75 | 0.14 | 0.30 | 12.48 | 0.23 | 0.31 |



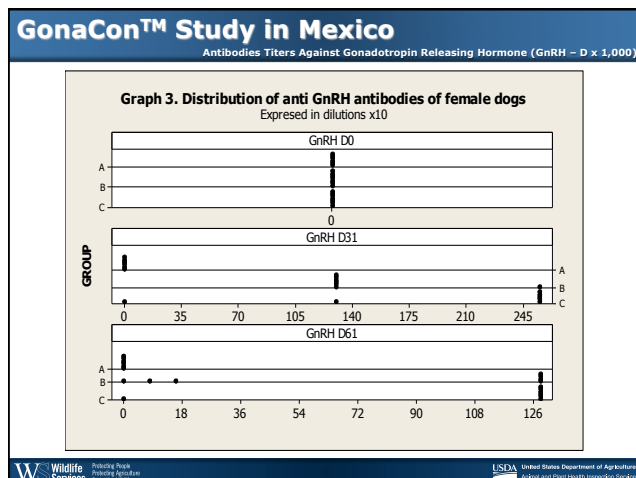
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Rabies Antibodies Titers (VNA – FAVN*)

Distribution, mean and standard deviation of female dogs by rabies virus neutralizing antibodies (VNA) for I.U. class by Groups and Day

| GROUP/DAY | GROUP A | | | GROUP B | | | GROUP C | | |
|-----------------------------|-----------|------------|------------|-----------|------------|------------|-----------|------------|------------|
| | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Lab Findings | | | | | | | | | |
| < 0.5 | 1 | 0 | 0 | 1 | 2 | 1 | 3 | 0 | 1 |
| 0.5 to 3.0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| > 3.0 | 3 | 6 | 6 | 6 | 5 | 6 | 3 | 7 | 7 |
| Statistical Findings | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Minimum | 0.39 | 13.77 | 13.77 | 1.99 | 2.28 | 0.87 | 0.06 | 4.56 | 2.62 |
| Maximum | 13.77 | 13.77 | 13.77 | 13.77 | 13.77 | 13.77 | 12.56 | 13.77 | 13.77 |
| Mean | 6.34 | 13.77 | 13.77 | 10.6 | 8.59 | 7.67 | 4.54 | 11.14 | 8.12 |
| St Dev | 5.71 | 0.0 | 0.0 | 4.38 | 5.21 | 4.85 | 5.6 | 3.69 | 5.38 |

*Maximum possible value 13.8 UI/ml



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Antibodies Titers Against Gonadotropin Releasing Hormone (GnRH - D x 1,000)

Distribution, mean and standard deviation of female dogs by Anti GnRH antibodies class by Groups and Day

| GROUP/DAY | GROUP A | | | GROUP B | | | GROUP C | | |
|-----------------------------|-----------|------------|------------|-----------|------------|------------|-----------|------------|------------|
| | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Lab Findings | | | | | | | | | |
| 0 | 6 | 6 | 6 | 7 | 0 | 1 | 7 | 1 | 1 |
| 1/8 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1/16 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1/128 | 0 | 0 | 0 | 0 | 6 | 4 | 0 | 1 | 6 |
| 1/256 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 | 0 |
| Statistical Findings | D0 | D31 | D61 | D0 | D31 | D61 | D0 | D31 | D61 |
| Minimum | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Maximum | 0.0 | 0.0 | 0.0 | 0.0 | 256 | 128 | 0.0 | 256 | 128 |
| Mean | 0.0 | 0.0 | 0.0 | 0.0 | 146.3 | 76.6 | 0.0 | 201.1 | 109.7 |
| St Dev | -- | -- | -- | -- | 48.4 | 64.3 | -- | 100.7 | 48.4 |



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Statistical Findings

Progesterone

✓ At Day 0, THR levels in Groups A and B were lower than Group C (F=1.64, P=0.222). At Day 31 THR levels in Groups B and C were significantly lower than Group A (F=1.11, P=0.0351), which did not receive GonaCon™. At Day 61, THR levels in Groups B and C were equal and lower than Group A (F=2.22, P=0.137).

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=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).

Anti-GnRH antibody titers

✓ GonaCon™ was never used in these dogs before this study. At Day 0, no anti-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=0.0001). The same results were observed on Day 61 (F=10.27, P=0.001).



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Macroscopic Findings

Controls

- ✓ Studies in 6 animals.
- ✓ Left cardiac hypertrophy (4). Valvular endocarditis (2).
- ✓ Slight to severe diffuse hepatosis (1). Chronic perihepatitis (3).
- ✓ Peyer's patch hyperplasia (1).
- ✓ Slight to severe chronic interstitial nephritis (3). Nephrosis (1).
- ✓ Several gastrointestinal parasites diseases in different clinical status: Dipilidium, Strongiloides, Ascariasis, Ancylostomiasis (Mucous enteritis in several sections of the intestine).

Group B

- ✓ Studies in 5 of 7 animals.
- ✓ Left cardiac hypertrophy (5).
- ✓ Slight to severe diffuse hepatosis (4). Chronic perihepatitis (2).
- ✓ Peyer's patch hyperplasia (5).
- ✓ Slight to severe chronic interstitial nephritis (2). Nephrosis (2).

Group C

- ✓ Studies in 6 of 7 animals.
- ✓ Left cardiac hypertrophy (2).
- ✓ Slight diffuse hepatosis (1). Chronic Perihepatitis (1).
- ✓ Peyer's patch hyperplasia (2).
- ✓ Slight to severe chronic interstitial nephritis (4).



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Preliminary Microscopic Findings

Controls

- ✓ Studies in 6 dogs.
- ✓ Hypophysis: Not apparent findings.
- ✓ Ovary: Not apparent findings. Several phases of follicle development and reduced number of atresic follicles.
- ✓ Muscle: Not apparent findings.
- *Samples from application site

Group B

- ✓ Studies in 5 of 7 dogs.
- ✓ Hypophysis: Slight diffuse congestion and edema (4). Coagulative necrosis (Basophilic Cells), More acidophilic cells (1).
- ✓ Ovary: Increased number of atresic follicles. Important reduction of second, preantrum and third follicles. Basophilic aspect into the tunica albuginea.
- ✓ Muscle: 10 samples.

Group C

- ✓ Studies in 6 of 7 dogs.
- ✓ Hypophysis: Slight diffuse congestion (4). Diffuse edema (3). Coagulative necrosis (Basophilic cells), More acidophilic cell (4).
- ✓ Ovary: Important reduction of second, preantrum 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 (2). Slight to severe multifocal necrosis (3).



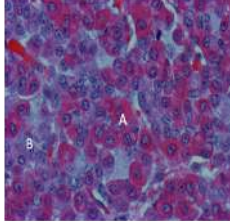
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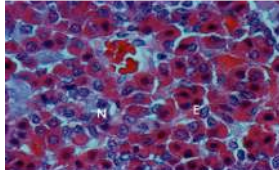
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GonaCon™ Study in Mexico Microscopic Findings

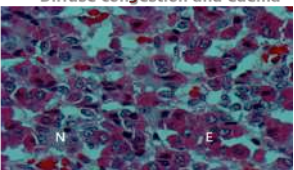
Hypophysis (Pituitary Gland)



Normal



Diffuse congestion and edema

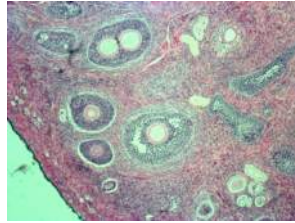


Coagulative necrosis

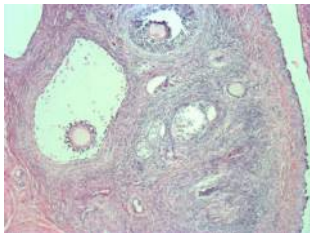
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GonaCon™ Study in Mexico Microscopic Findings

Ovary



Normal

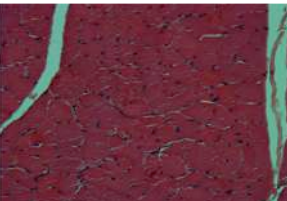


Reduction of second, preantral and third follicles

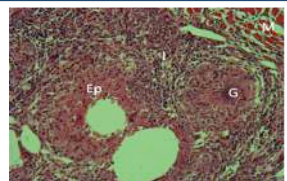
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GonaCon™ Study in Mexico Microscopic Findings

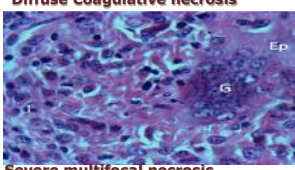
Muscle



Normal



**Chronic granulomatous myositis
Diffuse Coagulative necrosis**



Severe multifocal necrosis

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GonaCon™ Study in Mexico Conclusions and Recommendations

- ✓ **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.**

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GonaCon™ Study in Mexico

Conclusions and Recommendations

- ✓ **GonaCon™ generated NOT measured muscle atrophy.**
- ✓ **Observed chronic granulomatose 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.**



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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.**



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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.**



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REPORT UNDER PUBLICATION PROCESS

Effects of Transdermal Intrauterine Injection of GonaCon™ and Rabies Vaccine in Domestic Female Free-Living Spotted Foxes

71st International Conference on Dog Population Management

1st International Symposium on Dog Population Management

71st Conferencia Internacional de Robis en las Américas


LIAMAM 21da Conferencia Internacional de Robis en las Américas 16-18 de Octubre de 2011 San Juan, Puerto Rico

7th International Conference on Fertility Control in Wildlife

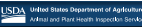
August 29-31st August 21, 2012 Jackson Hole, Wyoming

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Thank You!



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