Braz. J. Vet. Res. anim. Sci.,
São Paulo, v. 38, n. 6, p. 293-295, 2001.

Baypamun® action in hamsters experimentally infected with *Leptospira interrogans* serogroup Canicola

Ação do Baypamun® em hamsters experimentalmente infectados com *Leptospira interrogans*, sorogrupo *canicola*

Hélio LANGONI[1]; Rui SEABRA JÚNIOR[2]; Kenio Gouveia CABRAL[3]; Eva Laurice Pereira CUNHA[3]

CORRESPONDENCE TO:
Hélio Langoni
Departamento de Higiene Veterinária e Saúde Pública do Núcleo de Pesquisas em Zoonoses da Faculdade de Medicina Veterinária e Zootecnia UNESP
Campus de Botucatu
Distrito de Rubião Jr., s/n
18618-000 – Botucatu – SP
e-mail: hlangoni@fmvz.unesp.br

1- Departamento de Higiene Veterinária e Saúde Pública do Núcleo de Pesquisas em Zoonoses da Faculdade de Medicina Veterinária e Zootecnia da UNESP, Campus Botucatu – SP
2- Bolsista de Iniciação Científica Fapesp – Processo nº 96/06877-4
3- Área de Vigilância Sanitária da Faculdade de Medicina Veterinária e Zootecnia da UNESP, Botucatu – SP

SUMMARY

Baypamun® is an immunity modulator recommended as prophylactic and therapeutic use. In the present study, this product was used in the hamsters experimentally infected with *Leptospira interrogans* serogroup *canicola*. The animals were divided in 5 groups, with 20 animals each. This product was used as a therapeutic, prophylactic and as adjuvant. As a therapeutic use all the animals died. Despite of eight (40.0%) survivors, the prophylactic use wasn't also totally favorable due to leptospire recovering from kidneys of those animals. Based on the analysis of antibodies levels among animals that received vaccine and vaccine with Baypamun® as adjuvante, this product didn't conferred superior humoral answer. However, other studies should be accomplished utilizing this product in a longer period as well as verify the cellular answer by Baypamun® use as adjuvant in the infectious disease.

UNITERMS: Immunomodulators; *Leptospira*; Immunity.

INTRODUCTION

Baypamun® is an immune modulator, produced with *Parapoxvirus ovis* D1701 strain, that stimulates the unspecific immune response and is recommended for prevention and treatment of infectious diseases. This product, studied in several animal species, improved the concentration of antibodies in the milk of primiparous sows, and increased the survival of piggies[14]. In the same specie, the treatment of reproductive diseases and respiratory syndromes with Baypamun® reduced the mortality and increased the body weight rate[7].

In spite of the prophylactic and therapeutic action of Baypamun, it presented poor or completely negative results in the occurrence of respiratory diseases complex in felines[10] but the paraimunization was effective in leucemics cats[9]. Ovines with diarrhoea recovered in a few days after treatment[4]. Rabbits with traveling diseases caused by *Pasteurella multocida*, *Bordetella bronchiseptica* or *Mycoplasma* sp., and induced by different stress factors, had their occurrence reduced with Baypamun treatment[1].

There are no reports about the influence of Baypamun® over the course of Leptospirosis, however, Alves et al.[2] found that BCG enhanced the resistance of hamsters experimentally infected with serovar *pomona*, Ris and Hamel[12], observed that Freund's complete adjuvant was better than aluminum hydroxide in commercial vaccines produced with sorovar *pomona*. The present work aimed to verify the influence of Baypamun as a paraimmunizer in hamsters experimentally infected with *Leptospira interrogans* serogroup Canicola.

MATERIAL AND METHOD

One hundred female hamsters (*Mesocricetus auratus*) were used with weights varying 60 to 80 gm. The animals were conditioned in plastic boxes, 20 per group, observed twice a day, for 21 days, with daily annotations of the clinical alterations.

Before the inoculations, animals' serums were examined to verify the presence of antileptospira agglutinins. The blood was obtained through heart puncture and the serum was stored in flasks type "vaccines" maintained in freezer (-20ºC). Microscopic agglutination test (MAT) was used to serological analysis, at the screening dilution of 1:100[5].

The experimental infection was performed with a virulent strain of *Canicola* serogroup, cultivated in Flecther medium. For each animal the infectious inocula was 0,5 ml, with 10^6 microorganisms/ml by intra-peritoneal route[13]. The animals that reached the agonic state of the disease as well as the survivors were killed and examined searching for macroscopic alterations. Before the sacrifice, it was collected blood samples for anti-leptospire agglutinins determination study.

The animals were divided in five groups, with 20 animals each. The Group 1 was the infectious innoculum control, and received only leptospires culture. The Group 2 was treated with 0,5 ml doses of Baypamun® applied on four and two days before experimental infection. The Group 3 received two vaccine doses using Baypamun® as adjuvant, 0.5 ml of each one, into interval of 15 days, challenged 15 days after the last inoculation. Likely to Group 3, the Group 4 only didn't receive Baypamun®. In the Group 5, this product was applied four and six days after experimental infection, with doses of 0,5 ml each.

The vaccine was prepared according to Avila et al.[3] starting from leptospire liquid culture. The inactivation was done by 0.1% formalin solution, and the culture was centrifuged at 10,000 rpm during two hours and ressuspended in physiologic saline solution containing 0.18% red phenol, and adjusted for 4.0 x 10^8 leptospires/ml.

After performing the necropsies exams it were collected liver and kidneys samples, which were macerated separately. Saline solution with neomycin and nitrofurantoin in the concentration of 25 µgl/ml of each drug[11], was added in the proportion of 1/10. Serial decimal dilutions until 10^{-3} was cultivated using Fletcher medium. Pasteur pipette technique was also utilized for organs cultivation[8]. The flasks were incubated at 30ºC, during 60 days, for the observation of Dinger's zone.

The macerated tissue was examined under dark field microscopic of searching for leptospires. Serological results was statistical analysed by Mann-Whitney Test, with $\alpha = 0.05$ significant level.

RESULTS AND DISCUSSION

All the serum samples previously obtained were MAT negative. Antibodies titres ranged from negatives, in animals died until six days after infection, to 12,800 in the next days, in the Groups 1, 2 e 5.

In the Groups 3 and 4, respectively, titres ranged from 200 to 1,600 and 200 to 3,200. There was no statistical differences between two immunization schedules employed, with $p > 0.05$.

In the Group 1, seven (35.0%) animals died in the 6th day, eight (40.0%) in the 7th day, three (15.0%) in the 8th day, and two (10,0%) in the 9th day after infection. In the Group 2, eight (40.0%) animals survived, two (10.0%) died in the 6th day, four (20.0%) in the 7th day, two (10.0%) in the 8th day, two (10.0%) in the 9th day and two (10.0%) in the 12th day after the experimental inoculation. In the Group 5, 10 (50.0%) animals died in the 6th day, two (10.0%) in the 7th day, six (30.0%) in the 8th day, and two (10.0%) in the 9th day after infection. All the animals survived in the Groups 3 and 4.

The clinical signals observed were apathy, anorexia, dehydration, bristly hair, incoordination and moderate jaundice. The symptoms also were present in the eight (40.0%) survivors animals from Group 2, but they recovered and survived. Alterations were not observed in the Groups 3 and 4, which presented no clinical signs.

The reisolation was obtained from all the animals from Groups 1, 2 and 5, including the eight (40.0%) survivors from Group 2. Positive culture was not obtained in the Groups 3 and 4.

Liver and kidneys were congested and hemorrhagic at necropsy. Petechiaes and suffusions were observed in the lungs surface and moderate jaundice in epiplon and abdominal musculature.

Pasteur pipette technique was more effective for recovering leptospires, once the cultivation by serial dilution was positive in only one animal from Group 1. The first technique presents higher contamination risk, but with the necessary care can be avoided.

Baypamun® used as therapeutic form didn't give protection against leptospire infection in Group 5. In spite of eight (40.0%) animals that survived from Group 2, the prophylactic wasn't also totally favorable, due to recovering leptospire from kidneys of these animals, which means they became kidney carriers of leptospires.

Based on analysis of antibodies levels among animals from Groups 3 and 4, Baypamun® didn't increased humoral response. However, other studies should be accomplished utilizing this product in a longer period as well as verifying the cellular response.

RESUMO

Baypamun® é um imunomodulador recomendado na profilaxia e terapêutica de enfermidades. No presente estudo, este produto foi usado em hamsters experimentalmente infectados com *Leptospira interrogans*, sorogrupo *canicola*. Estes animais foram divididos em 5 grupos com 20 animais cada. O produto foi usado como terapêutico, profilático e como adjuvante. Quando usado de forma terapêutica, todos os animais vieram a óbito. Quando usado na profilaxia, 8 animais (40%) sobreviveram e foi possível recuperar o agente a partir dos rins desses animais. Baseado na análise do título de anticorpos nos animais que receberam ou não o Baypamun como adjuvante vacinal, o produto não elevou a resposta humoral. Os resultados sugerem, entretanto, outros estudos que utilizem o produto por períodos mais prolongados, além de se verificar a resposta celular para se avaliar o seu efeito como adjuvante nas infecções.

UNITERMOS: Imunomoduladores; *Leptospira*; Imunidade.

REFERENCES

1- ALBUS, K.; ZIMMERMANN, E. Kurzmitteilung: bewetung eines paramunitatsinducers zur Verhinderung transportbedubgter Erkrankungen bei Zuchtkaninchen. **Kleintierpraxis**, v. 38, n. 2, p. 1-5, 1993.

2- ALVES, C. J.; VASCONCELLOS, S. A.; CAMARGO, C. R. A.; MACEDO, N. A.; MORAIS, Z. M.; NÜRMBERGER Jr, R.; PINHEIRO, S. R.; FERREIRA NETO, J. S. Influência da estimulação inespecífica com o BCG sobre a suscetibilidade do hamster à infecção experimental por *Leptospira interrogans* sorotipo *pomona*. **Brazilian Journal Veterinary Research Animal Science.**, v. 29, n. 2, p. 193-199, 1992.

3- ÁVILA, F. A.; ÁVILA, S. H. P.; BECHARA, G. H. Evaluation of the immune humoral response in swine experimentally vaccinated against *Leptospira interrogans* sorotipos *pomona* and *canicola*. **Ars Veterinária**, v. 1, n. 3, p. 51-55, 1985.

4- AYTUG, N.; SEZEN, Y.; TAVUKCUOGLU, F. Studies on the therapeutic and prophylatic effects of immunomodulation in diarrhoeic lambs. **Veteriner Fakultesi Dergisi**, v. 11, n. 4, p. 113-120, 1992.

5- BRASIL. Ministério da Saúde. **Manual de leptospirose**. Fundação Nacional de Saúde. Centro Nacional de Epidemiologia. Coordenação de Controle de Zoonoses e Animais Peçonhentos. 2.ed. Brasília: Fundação Nacional de Saúde, 1995. 98 p.

6- DUFFUS, W. P. H. Immunoprophylaxis. In: HALLWELL, R. E. W.; GORMAN, N. T. **Veterinary clinical immunology**. Philadelphia: W. B. Saunders, 1989, p. 205-211.

7- EGBERING, O. Moglichkeiten der Behandlung des Seuchenhaften Spatraborts des Schweines (SSS). **Praktische Tierarzt.**, v. 72, n. 3, p. 851-854, 1991.

8- GUIDA, V. O. Estudos sobre a leptospirose canina. IV. Pesquisas sobre os caracteres antigênicos de uma cepa isolada de cão. **Arquivo Instituto Biológico**, v. 19, n. 2, p. 149-152, 1949.

9- HORBER, D.; SCHNABEL, W.; MAYR, B. Praxiserfahrungen beider Paramunisierung FelV-positiver Katzen mit Baypamun HK. **Tierarztliche Umschau**, v. 47, n. 2, p. 558-560, 1992.

10- KLIMENTOWSKI, S.; KOLBL, S.; FISCHER, M. Untersuchungen uber die Wirksamkeit der Paramunisierung zur Bekampfung des Katzenschnupfens. **Berliner und Munchener Tierarztliche Wochenschrift**, v. 105, p. 253-259, 1992.

11- MYERS, D. M. Manual de métodos, para el diagnostico de laboratorio de la leptospirosis (nota tecnica n. 30. **Centro Panamericano de Zoonosis**, OPS/OMS, 1985.

12- RIS, D. R.; HAMEL, K. L. *Leptospira interrogans* serovar *pomona* vaccines with different adjuvants in cattle. **New Zealand Veterinary Journal**, v. 27, n. 4, p. 169-171, 1979.

13- SIDDIQUE, J. H.; SHAH, S. M. Evaluation of polyvalente leptospiral vaccine in hamsters. **Indian Veterinary Journal**, v. 67, n. 2, p. 1006-1010, 1990.

14- VALPOTIC, I.; VIJTIUK, N.; RADELJEVIC, D. Nonspecific immunization of primiparous sows with Baypamun enhances lacteal immunity in their offspring. **Veterinarski Arhiv**, v. 63, n. 5, p. 161-172, 1993.

Received: 18/06/2001
Accepted: 31/01/2002