Efficiency of *Matricaria chamomilla* CH12 and number of doses of rabies vaccine on the humoral immune response in cattle

Luis Souza Lima de Souza Reis1,*, Neuza Maria Frazatti-Gallina2, Rosana de Lima Paoli2, Rogerio Giuffrida3, Avelino Albas4, Eunice Oba5, Paulo Eduardo Pardo1

1Universidade do Oeste Paulista-UNOESTE, Pós-graduação em Ciência Animal, Presidente Prudente-SP, CEP 19067-175, Brazil
2Seção de Raiva do Instituto Butantan, São Paulo-SP, CEP 05503-900, Brazil
3Universidade do Oeste Paulista, UNOESTE, Laboratório de Medicina Veterinária Preventiva, Presidente Prudente-SP, CEP 19067-175, Brazil
4Agência Paulista de Tecnologia dos Agronegócios, Presidente Prudente, São Paulo, CEP 19100-000, Brazil
5Universidade Estadual Paulista Júlio de Mesquita Filho-UNESP/FMVZ, Departamento de Reprodução Animal e Radiologia, Botucatu, São Paulo, CEP 18618-000, Brazil

This study evaluated the effect of *Matricaria chamomilla* and vaccination frequency on cattle immunization against rabies. Four groups (n = 15/group) were treated with or without *Matricaria chamomilla* CH12 and vaccinated with one or two doses of rabies vaccine (30 day interval). No effect of chamomile was found on cattle immunization against rabies; however, antibody titers were protective in cattle vaccinated twice, while 93.3% of cattle vaccinated only once had titers under 0.5 UI/ml after 60 days. In conclusion, the use of chamomile did not alter the humoral immune response in cattle, and two vaccine doses are suggested for achieving protective antibody titers.

Keywords: cattle, immune response, *Matricaria chamomilla*, rabies, vaccine

Rabies is one of the most serious zoonoses in the world because it consists of fatal encephalitis that may be found in mammals, and occurs within a wide geographical range [1,4]. It is caused by a virus belonging to the genus *Lyssavirus*, of the family *Rhabdoviridae* [7,8], and is transmitted mainly by the hematophagous bat *Desmodus rotundus* in Latin America [1,8,10]. The most effective and inexpensive procedure for rabies control is the regular vaccination of cattle [1,7,11]. Albas et al. [1] and Lodmell et al. [9] showed that an adequate immune response was not achieved with a single rabies vaccination in some animals, although the vaccine producers stated that the antigenic levels per vaccine dose were within the normal range.

There is evidence to show that *Matricaria chamomilla* extract has immunomodulatory [2,5] and allogeneic properties on lymphocyte proliferation and activation of T cells, although further elucidation is needed [2]. The aim of this work was to evaluate the effect of *Matricaria chamomilla* CH12 as well as the number of doses of rabies vaccine, on the humoral immune response in cattle.

Sixty Nelore calves (*Bos taurus indicus*), about 12 months old, belonging to a farm situated in Lutecia, SP, Brazil, were studied. These calves were fed on *Brachiaria decumbens* from an extensive pasture system and supplemented with commercial mineral salt in an ad libitum regime. The *Matricaria chamomilla* CH12, was produced by the homeopathic Veterinary Laboratory (Arenales Fauna & Flora, Brazil). This product was composed of *Matricaria chamomilla* CH12, milk CH12, *Bixa orellana* (0.75 g) and sucrose (100 g).

The experimental animals were randomly divided into four groups, FEV1, FEV2, V1, and V2 (15 animals per group). Cattle from FEV1 and FEV2 groups received *Matricaria chamomilla* CH12 mixed with mineral salt (Fosbov 15; Tortuga Cia Zootécnica Agrária, Brazil) for 90 days, and animals from groups V1 and V2 received only mineral salt.

The determination of *Matricaria chamomilla* CH12 consumption per animal was performed in the first month of the experiment as follows: the mineral salt supplemented with *Matricaria chamomilla* CH12 was weighed, put in the feeder and, after 24 h, removed to be weighed again. The
difference between the first and the second weighing divided by the number of animals that fed at the feeder was considered the average salt consumption per calf in 24 h. From these calculations, the amount of Matricaria chamomilla consumed by each cattle was determined to be about 2 g a day.

We used a commercial liquid rabies vaccine (Rai-Vac; Fort Dodge Saúde Animal, Brazil) containing a suspension of fixed rabies Pasteur Virus cultured on baby hamster kidney (BHK)-21 cells, inactivated by beta-propiolactone, adsorbed to an adjuvant aluminum hydroxide and preserved with thimerosol at 1 : 10,000. The vaccine had antigen levels about 2 g a day.

The World Health Organization recommends rabies-neutralizing antibody titers of at least 0.5 IU/ml for effective prevention in humans against rabies virus contamination. Some studies have stated that this neutralizing antibody titer is the minimal level required to protect cattle [1,6] against rabies. However, a descriptive analysis showed that 93.3% of the cattle that received a single vaccine dose (FEV1 and V1) had antibody titers under 0.5 IU/ml after 60 days of vaccination, independent of the treatment with thimerosol at 1%

Rabies neutralizing antibody titers are typically used to evaluate the humoral immune response in cattle after rabies vaccination [1,8,11,12]. Moreover, it is recommended by the Centers for Disease Control and Prevention (USA). The first serum samples collected from all cattle tested on day 0 (FEV1, FEV2, V1 and V2) were not reactive for rabies, indicating that these animals had no prior contact with rabies virus or the vaccine. Thus, all the antibody titers found here were induced by the rabies vaccination during the study.

In the present study, Matricaria chamomilla CH12 did not stimulate the production of rabies neutralizing antibodies (Fig. 1). On day 30 the antibody titers were similar between cattle that received Matricaria chamomilla CH12 and the respective treatment without supplementation (FEV1 × V1; FEV2 × V2). This suggested that the immunomodulatory effect of Matricaria chamomilla, found by Amirghofram et al. [2] and Gharagozloo and Ghaderi [5] in humans, did not occur in cattle for rabies immunization.

The results found in the present study lead to the conclusion that the use of Matricaria chamomilla CH12 added to mineral salt did not affect the humoral immune response. In addition, two doses of rabies vaccine were
shown to be required for rabies protection (≥ 0.5 UI/ml) in cattle.

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References

1. Albas A, Pardo PE, Bremer-Neto H, Gallina NMF, Mourão Fuches RM, Sartori A. Vacinação anti-rábica em bovinos: comparação de cinco esquemas vacinais. Arq Inst Biol 2005, 72, 153-159.
2. Amirghofran Z, Azadbakht M, Karimi MH. Evaluation of the immunomodulatory effects of five herbal plants. J Ethnopharmacol 2000, 72, 167-172.
3. Banzatto DA, Kronka SN. Experimentação Agrícola. 3rd ed. p. 247, Funep, Jaboticabal, 1995.
4. Coleman PG, Fèvre EM, Cleaveland S. Estimating the public health impact of rabies. Emerg Infect Dis 2004, 10, 140-142.
5. Gharagozloo M, Ghaderi A. Immunomodulatory effect of concentrated lime juice extract on activated human mononuclear cells. J Ethnopharmacol 2001, 77, 85-90.
6. Giometti J, Chiacchio SB, Albas A, Pardo PE, Bremer-Neto H, Giometti AI, Reis LSLS. Influência da suplementação com crômio na resposta imune humoral anti-rábica em Bovinos. Arq Inst Biol 2006, 73, 421-427.
7. Hankins DG, Rosekrans JA. Overview, prevention, and treatment of rabies. Mayo Clin Proc 2004, 79, 671-676.
8. Kotait I, Gonçalves CA, Peres NF, Souza MCAM, Targueta MC. Controle da Raiva dos Herbívoros. Manual Técnico 1. pp. 1-11, Instituto Pasteur, São Paulo, 1998.
9. Lodmell DL, Smith JS, Esposito JJ, Ewalt LC. Cross-protection of mice against a global spectrum of rabies virus variants. J Virol 1995, 69, 4957-4962.
10. Piza AT, Pieri KMS, Lusa GM, Caporale GMM, Terreran MT, Machado LA, Zanetti CR. Effect of the contents and form of rabies glycoprotein on the potency of rabies vaccination in cattle. Mem Inst Oswaldo Cruz 2002, 97, 265-268.
11. Queiroz da Silva LH, Cardoso TC, Perri SHV, Pinheiro DM, Carvalho C. Pesquisa de anticorpos anti-rábicos em bovinos vacinados da região de Araçatuba, SP. Arq Inst Biol 2003, 70, 407-413.
12. Sihvonen L, Kulonen K, Neuvonen E. Immunization of cattle against rabies using inactivated cell culture vaccines. Acta Vet Scand 1994, 35, 371-376.
13. Smith JS, Yager PA, Baer GM. A rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralizing antibody. In: Meslin FX, Kaplan MM, Koprowski H (eds.). Laboratory Techniques in Rabies. 4th ed. pp. 181-192, World Health Organization, Geneva, 1996.
14. Zalan E, Wilson C, Pukitis D. A microtest for the quantitation of rabies virus neutralizing antibodies. J Biol Stand 1979, 7, 213-220.