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RABIES VIRUS NEUTRALIZING ANTIBODY PROFILE IN CATTLE VACCINATED WITH INACTIVATED VACCINE ADJUVANTED WITH EITHER ALUMINUM HYDROXIDE ALONE OR COMBINED WITH AVRIDINE

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ABSTRACT

A study was conducted in Brazil from November 1999 to November 2000 to evaluate the antibody response of cattle vaccinated with an inactivated rabies virus vaccine adjuvanted with aluminum hydroxide ($Al(OH)_3$) alone or combined with Avridine. Sixteen (16) female Holstein calves, 7 to 8 month old, with no history of vaccination against rabies were vaccinated (IM 2 mL) with the $Al(OH)_3$ adjuvanted vaccine on days 0, 90 and 360, and, 16 similar calves were vaccinated (IM 2 mL) with the Avridine containing vaccine. On days 0, 30, 90, 120, 180, 360 and 390 post primary vaccination, blood samples were collected from all calves for antibody titration by serum neutralization test in mice, with fixed virus / varying serum method, using 100 LD_{50} / 0.03 mL of the CVS rabies virus strain. The two vaccines provoked antibody response with titers that persisted above 2.264 Log_{10} SN_{50} throughout the study. The mean antibody titer at 30 days p.v. was numerically higher for the $Al(OH)_3$ adjuvanted vaccine, but the Avridine containing vaccine was numerically higher at day 90 although not significant. Following revaccination at day 90, the antibody level induced by the Avridine containing vaccine was higher and persisted higher until the end of the study. The difference was significant ($p<0.05$) on days 180, 360 and 390. Although the Avridine containing vaccine had a tendency to persist in higher level, both vaccines induced antibody titers that persisted >2.264 Log_{10} SN_{50} during the entire study period post initial vaccination.

KEY WORDS: Rabies, vaccine, adjuvant, avridine, aluminum hydroxide, cattle, neutralizing antibody.

RESUMO

ANTICORPOS NEUTRALIZANTES DO VÍRUS RÁBICO INDUZIDOS EM BOVINOS PELAS VACINAS DE VÍRUS INATIVADO FORMULADAS SOMENTE COM O ADJUVANTE HIDRÓXIDO DE ALUMÍNIO OU COMBINADA COM AVRIDINE. Avaliou-se a resposta de anticorpos em novilhas induzida pelas vacinas de vírus rábico inativado, formuladas somente com o adjuvante hidróxido de alumínio ($Al(OH)_3$) ou combinada com Avridine ($Al(OH)_3$ + Avridine). Dezesseis novilhas holandesas, 7 a 8 meses de idade, sem histórico de vacinação anterior foram vacinadas (IM 2 mL) nos dias 0, 90 e 360 com a vacina contendo somente $Al(OH)_3$ como adjuvante e 16 novilhas foram vacinadas (IM 2 mL) com a vacina adicionada de Avridine. Amostras de sangue foram coletadas nos dias 0, 30, 90, 120, 180, 360 e 390 e os anticorpos neutralizantes foram titulados pelo teste de soro-neutralização em camundongos usando-se quantidade fixa do vírus CVS (100 DL_{50} / 0,03 mL) e soro variável. As duas vacinas induziram títulos de anticorpos que persistiram acima de 2,264 Log_{10} SN_{50} durante todo o período do estudo. A média dos títulos de anticorpos aos 30 dias foi numericamente maior para a vacina com adjuvante $Al(OH)_3$, mas a vacina contendo Avridine apresentou média de títulos maior aos 90 dias, embora não significante. Após a revacinação aos 90 dias, as médias dos títulos de anticorpos foram maiores nos animais que receberam a vacina contendo Avridine. As diferenças foram significantes ($p<0,05$) nos dias 180, 360 e 390. Embora os títulos de anticorpos induzidos pela vacina contendo Avridine tenham mostrado tendência a se manter em níveis maiores, ambas as vacinas induziram respostas de anticorpos cujos títulos se mantiveram acima de 2,264 Log_{10} SN_{50} durante todo o período pós-vacinação.

PALAVRAS-CHAVE: Raiva, vacina, adjuvante, avridine, hidróxido de alumínio, bovino, anticorpo neutralizante.

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INTRODUCTION

Rabies, a fatal infectious disease caused by a neurotropic virus of the *Lyssavirus* genus, *Rhabdoviridae* family affects animals and men (TURNER, 1984). The virus is maintained in the nature by three distinct epidemiological patterns: the urban rabies maintained by the canine population affecting the domestic pets, dogs and cats, the rural and sylvatic rabies maintained by all species of bats and wild carnivores, such as skunks, wolves, foxes and racoons in the USA and Canada, or red foxes in Europe. In Latin America the vampire bats (*Desmodus rotundus, Diaemus youngii* and *Diphylla ecaudata*) are the main source of transmission to bovine and other animals (ACHA & SZYFRES, 1987).

Rabies was known since antiquity, described for the first time in 500 B.C. (STEELE, 1975), and the vaccine against rabies was the first vaccine obtained by artificial means by Pasteur in 1885 (TURNER, 1984). Although many countries or regions have successfully controlled the disease incidence (MORRIS & GEERING, 1981), rabies infection continues to pose a significant risk to the human and animal population in many other countries. For example, in Brazil, in 1999, cases of rabies were registered in several States affecting 970 canines, 2.628 bovines, 331 other domestic animals, and 31 wild animals (BOLETIM DE VIGILÂNCIA EPIDEMIOLÓGICA DA RAIVA NAS AMÉRICAS, 1999). Human rabies occurred in 9 States with a total of 18 cases during 2000 (ARAÚJO, 2000).

The urban rabies control program includes the vaccination of dogs and cats, and the stray dog population control, restriction of free movement, selective euthanasia recommended in special cases for example, non vaccinated dogs living near slaughterhouses, supermarkets or food manufacturing establishments (OMS, 1992). The urban control program is conducted by the public health authorities due to its zoonotic significance, while the control of rural rabies is conducted under the responsibility of the agriculture authorities, where its economic importance plays a major role. The rural rabies control program includes sanitary education to farmers, rabies diagnostic activities, control of vampire bats, vaccine quality control, etc., and vaccination of livestock and farm animals conducted by the owners.

Many vaccine types have been used in Brazil for the control of urban or rural rabies, including Flury HEP (high embryo passage), Fuenzalida & Palacios (mouse brain passage) and tissue culture produced with either attenuated live virus (ABELSETH, 1967) or inactivated ones (GUIDOLIN et al., 1983; LARGHI et al., 1976). The inactivated rabies virus vaccines adjuvanted with aluminum hydroxide alone or combined with other adjuvants such as saponine have been largely used for the protection of cattle (RIBEIRO-NETO et al., 1973). The inclusion of the Avridine (a synthetic lipoidal amine adjuvant) additionally to aluminum hydroxide demonstrated a significant improvement on the primary antibody response of cattle following a 5-mL vaccination dose. Three hundred (300) days after revaccination the antibody profile and the percent protection against virulent rabies virus challenge was similar in both groups (CÔRTES et al., 1993).

The objective of the present study was to compare the antibody profile (determined by the mouse serum neutralization test) of cattle vaccinated with inactivated rabies virus vaccines adjuvanted with either aluminum hydroxide alone or combined with Avridine using 2-mL dose.

MATERIAL AND METHODS

Vaccines

The antigen of PV rabies virus strain grown in BHK deep suspension culture and inactivated by BEI (Binaryethyleneimine) was adsorbed onto aluminum hydroxide gel and formulated as a commercial Avridine vaccine (Rabivac - Laboratorios Pfizer Ltda) or without Avridine (vaccine with aluminum hydroxide alone). Four liters of each formulation were bottled for the experimental purpose. The vaccine containing Avridine was recorded as Exp. Vaccine Lot 0599001 ($Al(OH)_3$ + Avridine) and the vaccine without Avridine as Exp. Vaccine Lot 0599002 ($Al(OH)_3$). Both formulations contained the same amount of antigen and other components with the only difference being the addition of Avridine formulated as described elsewhere (CÔRTES et al., 1993). The vaccines passed the mouse potency test performed by the Habel Method (Table 1).

Animals, vaccinations and blood sampling

Thirty- two (32) female Holstein calves, 7 to 8 months old, with no history of vaccination against rabies were randomly assigned to a T1 or T2 group of 16 calves each. Calves in T1 group were vaccinated on days 0 and 90 with the Exp. Vaccine Lot 0599002 ($Al(OH)_3$) and calves in T2 group were vaccinated on those same days with the Exp. Vaccine Lot 0599001 ($Al(OH)_3$ + Avridine). The vaccinations were given by IM injections with a 2 mL dose. On day 360 the calves received a third vaccination dose (2 mL, IM). Blood samples were collected from all animals on days 0, 30, 90, 120, 180, 360 and 390 according to the study design outlined in Table 2.

Table 1 - Potency test results.

Exp. Vaccines	Volume	Habel Test
Lot 0599001 ($Al(OH)_3$ + Avridine) Manufacturing date: 20 May 1999	4,000 mL	4.5 Log_{10} Pass
Lot 0599002 ($Al(OH)_3$ Manufacturing date: 20 May 1999	4,000 mL	5.5 Log_{10} Pass
Pass level		4.0 Log_{10}

Serological method

The serum samples obtained were assayed by serum neutralization test in mice using a fixed amount of virus / varying serum, according to the method described by FITZGERALD (1996). Each serum sample was serially diluted in distilled water containing 2% normal horse serum, by two-fold steps starting from 1:5. Each serum dilution was mixed with an equal volume of the CVS rabies virus strain pre-diluted to contain approximately 200 LD_{50} / 0.03 mL and the mixtures were incubated at 37°C for 90 minutes. Each virus-serum dilution mixtures were inoculated intracerebrally in 5 mice (21 day old Swiss albino mice, weighing 11-14 g), with 0.03 mL of the mixture. After inoculation, the mice were examined during 21 days for signs of rabies or death. The number of survival or death were recorded and the virus neutralizing titers were calculated according to the method of REED & MÜENCH (1938) and expressed as- Log_{10} SN_{50}.

Statistical analysis

The general linear repeated measures mixed model analysis of variance was used for statistical analysis of the Log_{10} SN_{50}. Least square means were used for Pairwise comparisons between treatments at each sampling day. The significance level was set at alpha = 0.05.

RESULTS AND DISCUSSION

The primary onset of antibodies was higher than 2 Log_{10} SN_{50} in both vaccinated groups. At 30 days post vaccination the mean antibody titer was numerically higher for the calves receiving the vaccine adjuvanted with aluminum hydroxide alone, but at day 90, the titers in the group injected with the vaccine containing Avridine was higher although not significant (Table 3 and Fig. 1). Following revaccination on day 90, both vaccinated groups increased the antibody titers that were maintained above 2.65 Log_{10} SN_{50} until day 360 post primary vaccination. At day 390, following revaccination on day 360, the antibody titers achieved 3.163 Log_{10} SN_{50} and 3.446 Log_{10} SN_{50} for the vaccine adjuvanted with $Al(OH)_3$ alone and for the vaccine containing Avridine, respectively. The antibody level induced by the Avridine containing vaccine following revaccination on day 90, was higher and persisted higher until the end of the study compared to the antibody titers induced by the vaccine adjuvanted with $Al(OH)_3$ alone. The difference was significant ($p<0.05$) on days 180, 360 and 390.

The serological results reported here are similar to those obtained by CÔRTES et al. (1993) and demonstrate that although the antibody profile following the primary vaccination in this study was examined only until day 90, there was a tendency for the vaccine adjuvanted with Avridine to maintain higher level as reported by Côrtes and co-workers. In the present study, the antibody titers induced by both vaccines were higher than 2.264

Table 2 - Study design.

		Blood sample (B) and Vaccination (V) Day						
Groups / Treatment	No. Animals	DOT** 0	DOT 30	DOT 90	DOT 120	DOT 180	DOT 360	DOT 390
T1 Lot 0599002 ($Al(OH)_3$)	16	(B) (V)*	(B)	(B) (V)*	(B)	(B)	(B) (V)*	(B)
T2 Lot 0599001 ($Al(OH)_3$ + Avridine)	16	(B) (V)*	(B)	(B) (V)*	(B)	(B)	(B) (V)*	(B)

* Vaccination: 2 mL by IM route. ** Day On Test

Note: On days 0, 90 and 360, blood samples were collected before vaccination.

Table 3 - Rabies virus neutralizing antibody titers in cattle following vaccination with the vaccine adjuvanted with aluminum hydroxide alone.

Calf Number	Antibody titers (Log_{10} SN_{50})						
	DOT 0*	DOT 30	DOT 90*	DOT 120	DOT 180	DOT 360*	DOT 390
5468	0.903	1.999	2.204	2.919	2.644	2.429	3.100
5480	0.778	2.317	1.753	2.656	2.129	3.090	3.190
5481	0.778	2.508	2.555	2.669	2.505	2.650	2.920
5494	<0.699	2.328	2.317	2.806	3.360	3.010	2.890
5496	0.778	3.182	3.659	2.806	2.505	1.995	3.310
5517	<0.699	2.413	2.137	2.618	2.758	2.740	3.300
5520	0.845	3.470	2.505	2.592	2.739	2.950	2.860
5523	0.778	2.328	1.602	2.713	2.254	2.620	3.290
5528	<0.699	2.366	1.702	2.333	2.505	1.840	3.100
5529	<0.699	2.154	2.054	2.430	2.166	2.505	3.310
5542	<0.699	3.822	2.108	2.346	3.107	2.550	2.940
5545	0.778	2.505	2.279	2.806	2.505	2.980	3.050
5546	0.903	2.873	1.807	2.629	3.709	3.172	3.770
5547	<0.699	2.714	2.656	2.862	2.667	2.990	3.100
5551	0.778	3.759	2.555	2.881	2.881	2.980	3.220
5553	<0.699	3.157	2.325	2.413	2.333	2.016	3.260
Mean	<0.763	2.743	2.264	2.655	2.673	2.657	3.163
SD	0.071	0.571	0.493	0.191	0.428	0.416	0.225
Minimum	<0.699	1.999	1.602	2.333	2.129	1.840	2.860
Maximum	0.903	3.822	3.659	2.919	3.709	3.172	3.770

* Vaccination days.

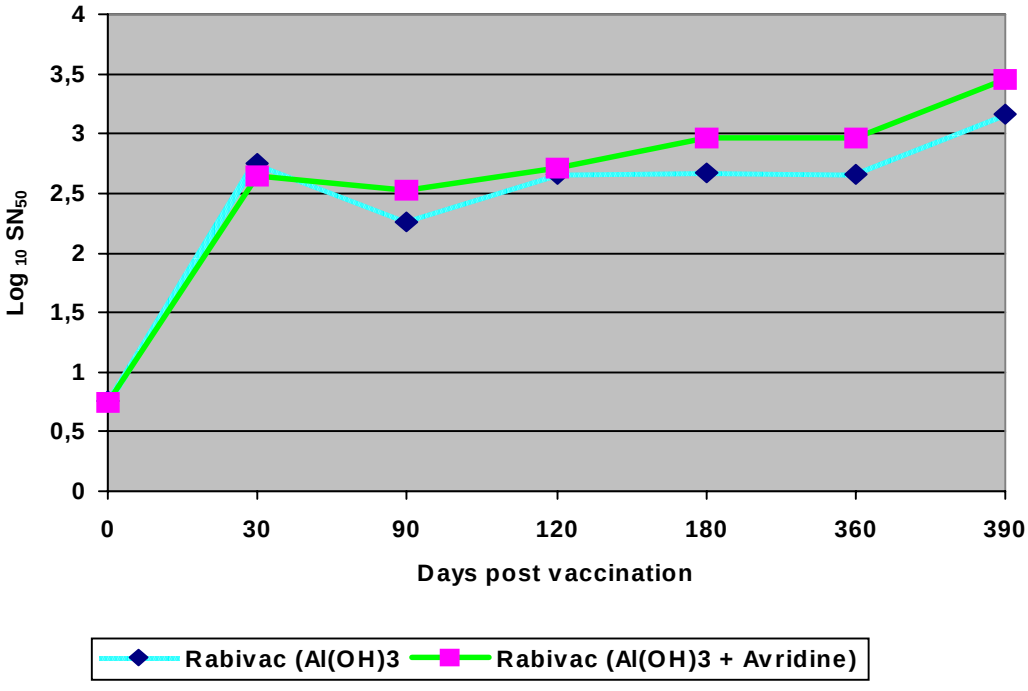

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Fig. 1 - Mean rabies virus neutralizing antibody response profile of cattle vaccinated with inactivated vaccine adjuvanted with aluminum hydroxide alone or combined with Avridine.

Table 4 - Rabies virus neutralizing antibody titers in cattle following vaccination with the vaccine adjuvanted with aluminum hydroxide plus Avridine.

Calf Number	Antibody titers (Log_{10} SN_{50})						
	DOT 0*	DOT 30	DOT 90*	DOT 120	DOT 180	DOT 360*	DOT 390
5461	0.778	2.409	1.774	2.927	3.107	2.806	3.300
5469	<0.699	3.822	2.902	2.409	2.505	3.010	3.230
5484	<0.699	2.325	1.953	2.392	2.505	2.990	3.230
5485	0.778	3.258	3.057	2.854	3.258	2.870	4.160
5497	0.903	2.271	2.601	2.978	3.483	2.900	3.400
5501	0.778	2.555	2.505	2.881	2.618	2.930	3.800
5504	0.778	2.204	3.203	2.505	2.994	2.550	3.100
5508	0.778	2.409	2.137	2.927	2.968	3.030	3.230
5518	0.845	2.413	2.656	2.650	3.015	3,107	3.300
5526	0.778	3.347	2.366	2.806	3.107	2.900	3.510
5535	<0.699	2.317	2.054	2.311	3.057	3.270	3.230
5537	<0.699	3.228	2.806	2.378	2.806	3.005	4.260
5539	<0.699	2.366	2.260	2.756	3.504	2.950	3.400
5540	<0.699	2.409	2.693	2.907	2.693	3.005	3.220
5541	<0.699	2.455	2.204	2.769	2.806	2.994	3.320
5548	<0.699	2.374	3.107	2.968	2.927	2.980	3.450
Mean	<0.750	2.635	2.517	2.714	2.960	2.956	3.446
SD	0.062	0.487	0.433	0.237	0.302	0.150	0.339
Minimum	<0.699	2.204	1.774	2.311	2.505	2.550	3.100
Maximum	0.903	3.822	3.203	2.978	3.504	3.270	4.260

* Vaccination days.

Table 5 - Mean antibody titers of cattle vaccinated with rabies vaccine adjuvanted with aluminum hydroxide alone or combined with Avridine.

Days post vaccination	Vaccine with $Al(OH)_3$			Vaccine with $Al(OH)_3$ + Avridine		
	No. of calves	Mean Log_{10} SN_{50}	SD	No. of calves	Mean Log_{10} SN_{50}	SD
0*	16	<0.763 [a]	0.071	16	<0.750 [a]	0.062
30	16	2.743 [a]	0.571	16	2.635 [a]	0.487
90*	16	2.264 [a]	0.493	16	2.517 [a]	0.433
120	16	2.655 [a]	0.191	16	2.714 [a]	0.237
180	16	2.673 [a]	0.428	16	2.960 [b]	0.302
360*	16	2.657 [a]	0.416	16	2.956 [b]	0.150
390	16	3.163 [a]	0.225	16	3.446 [b]	0.339

* Vaccination days.

[a, b] = Between treatments, means with different superscripts are significantly different ($P<0.05$).

Log_{10} SN_{50} during the entire study period. Interestingly enough, the sharply and high antibody increase following booster vaccination at 180 or 360 days obtained by CÔRTES et al. (1993) was not observed in the present study when animals were revaccinated at 90 days and 360 days post primary vaccination. It is also noteworthy that, in their study, there was a rapid decrease in the antibody level after the primary vaccination that was not observed in the present study.

The high titer at the moment of revaccination in this study may have limited the antibody response, but after revaccination the antibody titers were maintained at a plateau higher than 2.65 Log_{10} SN_{50} until day 360. Although impairment of passively acquired immunity to vaccination response is well known, interference of actively induced immunity to booster response shortly after revaccination has not been demonstrated (ITO et al., 1991).

Nevertheless, the results reported here demonstrate that both formulations have a high onset and duration of immunity, and indicate that revaccination at 90 days post primary vaccination is beneficial and

represents an additional precaution to improve protection when the epidemiological risks of the disease infection are high. Thereafter revaccination at on an annual basis is recommended.

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REFERENCES

ABELSETH, M.K. Further studies on the use of ERA vaccine in domestic animals. *Can. Vet. J.*, v.8, p.221-227, 1967.

ACHA, P.N. & SZYFRES, B. *Zoonoses and communicable diseases common to man and animals*. 2.ed. Pan Am. Health Organ. Sci. Publ., n.503, p.1-963, 1987.

ARAÚJO, F.A.A. Situação da raiva no Brasil. In: SEMINÁRIO INTERNACIONAL DE RAIVA, 1., 2000, SãoPaulo. *Anais*. p.22.

CRICK, J. & BROWN, F. An inactivated baby hamster kidney cell rabies vaccine for use in dogs and cattle. *Res. Vet. Sci*., v.12, p.156-161, 1971.

CÔRTES, J.A.; RWYEMAMU, M.M.; ITO, F.H.; UMEHARA, O.; MEDEIROS NETO, R.R.; DE LUCCA-NETO, D.; BALTAZAR, M.C.; VASCONCELLOS, S.A.; VASCONCELLOS, M.E.P. Immune response in cattle induced by inactivated rabies vaccine adjuvanted with aluminium hydroxide either alone or in combination with avridine. *Rev. Sci. Tech. Off. Int. Epizoot*., v.12, n.3, p.941-955, 1993.

FITZGERALD, D.A. Potency test for antirabies serum and immunoglobulin. In: MESLIN, F.X.; KAPLAN, M.M.; KOPROWSKY, H. *Laboratory techniques in rabies*. 4.ed. World Health Organization, 1996. p.417-422.

GUIDOLIN, R.; BALTAZAR, M.C.; ZELANTE, F. Produção de vacina anti-rábica veterinária em suspensão de células BHK. *Rev. Microbiol*., v.14, p.27-35, 1983.

ITO, F.H.; CÔRTES, J.A.; RWEYEMAMU, M.M.; UMEHARA, O.; MEDEIROS NETO, R.R.; DE LUCCA-NETO, D.; BALTAZAR, M.C.; VASCONCELLOS, S.A.; VASCONCELLOS, M.E.P. Course of secondary humoral immune response shortly after revaccination with bhk-21 cell culture inactivated rabies vaccine adjuvanted with aluminum hydroxide. *Braz. J. Vet. Res. Anim. Sci*., v.28, n.1, p.51-57, 1991.

LARGHI, O.P.; SAVY, V.L.; NEBEL, A.E.; RODRIGUEZ, A. Ethyleneimine-inactivated rabies vaccine of tissue culture origin. *J. Clin. Microbiol*., v.3, n.1, p.26-33, 1976.

MORRIS, R.S. & GEERING, W.A. Surveillance and control of virus diseases: East Asia and Autralasia. In: GIBBS, E.P.J. *Virus diseases of food animals*. New York: Academic Press, 1981. v.1., 101-133.

ORGANIZAÇÃO MUNDIAL DE LA SALUD, COMITÉ DE EXPERTOS DE LA OMS SOBRE RABIA. Octavo Informe. Ginebra, Organización Mundial de la Salud, 1992. 88p. (OMS, Serie de informes Técnicos, 824).

REED, L.J. & MÜENCH, H. Simple method of estimating fifty percent end points. *Am. J. Hyg*., v.27, p.493-497, 1938.

RIBEIRO-NETO, A.; NILSSON, M.R.; CÔRTES, J.A.; MIZUNO, M.; MIGUEL, O. Comparative study of cattle antirabies vaccines. II. Protection conferred by Alurabiffa, ERA and Formidogel vaccines. *Zentralbl. Veterinaer Med., B*., v.20, p.398-404, 1973.

STEELE, J.H. History of rabies. In: BAER, G.M. (Ed.) *The natural history of rabies*. New York: Academic Press, 1975. v.1, p.1-29.

TURNER, G.S. Rabies. In: TOPLEY & WILSON'S. *Principles of bacteriology, virology and immunity*, 7. ed. Baltimore: WILLIAMS & WILKINS, 1984. v.4, p.472-486.

VIGILÂNCIA EPIDEMIOLÓGICA DA RAIVA NAS AMÉRICAS *Bol. Vigilância Epidemiol. Raiva Américas*. Rio de Janeiro: Organização Pan-Americana da Saúde, v.31, 1999. 34p.

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