Trop Anim Health Prod (2008) 40:607–613
DOI 10.1007/s11250-008-9139-0

ORIGINAL PAPER

Antibody seroprevalences against rabies in dogs vaccinated under field conditions in Bolivia

K. Suzuki · M. R. Pecoraro · A. Loza · M. Pérez · G. Ruiz · G. Ascarrunz · L. Rojas · A. I. Estevez · J. A. Guzman · J. A. C. Pereira · E. T. González

Accepted: 29 January 2008 / Published online: 16 February 2008

Abstract Bolivia currently has one of the highest numbers of cases for human and canine rabies and is thus clue to the elimination process. The objective of the present study was to assess antibody seroprevalences against rabies in dogs vaccinated under field conditions and other factors that might influence the success of the on-going rabies control programmes in an endemic area of the disease, Santa Cruz de la Sierra, Bolivia. All 240 study animals, selected using area-stratified random sampling, were investigated in April 2007. Test prevalences were adjusted for the imperfect test characteristics using the Rogan–Gladen estimator (deterministic and stochastic functions) and Bayesian inference. Ninety-four of the tested 240 vaccinated dogs were classified as test-positive for rabies-specific antibodies. With regard to adjusted overall antibody seroprevalence, Bayesian true prevalence estimates (41%, 95% CI: 37–46%) were lower than both of the Rogan–Gladen estimates. The effect of various epidemiological factors on post-vaccination response was also assessed.

Keywords ELISA · Field investigation · Immune response · South America · True prevalence estimation

K. Suzuki (✉) · M. R. Pecoraro · E. T. González
Facultad de Ciencias Veterinarias,
Universidad Nacional de La Plata,
Av. 60 y 118, La Plata,
B1900AVW Buenos Aires, Argentina
e-mail: pvs@provetsur.net

A. Loza · M. Pérez · G. Ruiz · G. Ascarrunz ·
A. I. Estevez · J. A. Guzman · J. A. C. Pereira
Facultad de Ciencias Veterinarias,
Universidad Autónoma Gabriel René Moreno,
Av. 26 de Febrero entre Av. Busch y Centenario,
Santa Cruz de la Sierra, Bolivia

L. Rojas
Laboratorio de Investigación y Diagnóstico Veterinario,
Av. Ejercito N° 153 y Casilla N° 29,
Santa Cruz de la Sierra, Bolivia

Abbreviations

AP	apparent prevalence
CI	confidence interval
CRL	cerebro de ratón lactante (suckling mouse brain)
CVS	challenge virus strain
ELISA	enzyme-linked immunosorbent assay
INLASA	Instituto Nacional de Laboratorios de Salud (National Health Laboratory, La Paz, Bolivia)
IU/ml	international units per millilitre
LIDIVET	Laboratorio de Investigación y Diagnóstico Veterinario (Veterinary Research and Diagnostic Laboratory, Santa Cruz, Bolivia)

OD	optical density
Se	sensitivity
Sp	specificity
TP	true prevalence

Introduction

Rabies is an incurable infection that kills 55,000 persons annually all over the world (WHO 2005). Most of the human deaths are found in developing countries where canine rabies is endemic and the main route of transmission is the bites of rabid dogs (Meslin et al. 1994). Prevention of human rabies is mostly dependent on controlling canine rabies. This can be achieved mainly by mass vaccination and elimination of the stray dog population (WHO 1987). In Latin America, some 44 million dogs are vaccinated annually and steps have been taken to protect the around 1 million persons at risk of contracting the disease. It is significant to represent that part of Latin America has already managed to terminate the transmission of the rabies virus in the dog population (Wunner 2005). However, cities in less developed countries such as Bolivia fall behind in control efforts, mainly because resources are insufficient. Bolivia currently has one of the highest numbers of cases for human and canine rabies and is thus clue to the elimination process (Belotto et al. 2005). Most of the human cases caused by dogs in recent years have appeared in the peripheral areas of large cities such as Santa Cruz de la Sierra in Bolivia mostly amongst low-income populations. This indicates that dog-transmitted human rabies could be connected with poverty and irrepressible city growth (Wunner 2005). Given the present situation in Santa Cruz de la Sierra, an assessment of antibody seroprevalences against rabies in dogs vaccinated under field conditions and other factors that might influence the success of the on-going rabies control programmes, in order to reduce risk of rabies in domestic dogs and humans, was needed. Our objectives were: (a) to evaluate the effect of various epidemiological factors on post-vaccination response in Santa Cruz de la Sierra, Bolivia, an endemic area of canine rabies, and (b) to estimate the true prevalences of seropositive individuals using the Rogan–Gladen estimator and Bayesian inference. In addition, the study only expresses information about the owned dogs.

Materials and methods

Study area

Santa Cruz de la Sierra is the capital city of the Department of Santa Cruz, located in the eastern part of Bolivia (17°45′ S, 63°14′ W) at 416 metres above sea level. The city has a sunny and semi-tropical climate, with an average temperature of 21°C in winter and 32°C in summer (FAO 2001). The total land area is about 370 square kilometres. The population is estimated at 1.4 million people (Ministerio de Salud y Prevísion Social 2007). The Government of Bolivia issued a regulation of special sanitary measures for rabies control in November 2005, owing to the increasing incidence of dog and human rabies cases in recent years (Ministerio de Salud y Deportes 2005). During 2002–2006, 184 to 872 dog rabies suspect samples were tested annually in the city; 5–58% of them were rabies positive by fluorescent antibody test (1,029 confirmed rabid dogs). In the same period, human rabies cases occurred occasionally (0–5 cases) (Laboratorio de Investigación y Diagnóstico Veterinario, 2007, personal communication). There had been free vaccination campaigns in dogs (mass and intensive perifocal) by the Municipal Health Service, as follow-up approaches to reports of victims by rabies. The last mass vaccination campaign, which was more organised than ever, was implemented on 26 and 27 August 2006 (Gobierno Municipal Autónomo de Santa Cruz de la Sierra 2006). The CRL (cerebro de ratón lactante; suckling mouse brain) vaccine for the campaigns was produced on suckling mouse brains inoculated with fixed virus strains (CVS 91 and 51), inactivated with ultraviolet radiation and isotonised with glucose containing phenol and thimerosal. It was manufactured at the National Health Laboratory (Instituto Nacional de Laboratorios de Salud; INLASA) in La Paz, the capital of Bolivia. Dogs aged ≥1 month are eligible for vaccination during the campaigns and the owner receives a vaccine certificate following vaccination.

Selection of study animals

The Municipal Government of Santa Cruz de la Sierra had divided the city into four zones [Centro (centre), Sur (south), Norte (north) and Este (east)] as areas-of-responsibility of each of the four municipal health service centres, being in charge of rabies control. In general, socio-economic status drops as distance increases from the city centre (Widdowson et al. 2002). Therefore, the study adopted the zoning rule with a modification of combining Sur, Norte and Este as Santa Cruz's outskirts area comparing to Centro as Central Santa Cruz area, in order to select the animals (Fig. 1). The method of selection used was an area-stratified random sampling. It is an effective method for reducing variance, if a known factor causes significant variation in the outcome variable, but is not the target of the analysis (Thrusfield 2005). The Municipal Government of Santa Cruz de la Sierra provided access to the estimates of the dog population and data of dog owning households in each zone based on the results of the last vaccination campaign mentioned above. Allocation of individuals to the different strata was proportional to the estimates of the dog population in each area. Sixty-four and 176 vaccinated dogs were randomly recruited from Central Santa Cruz area and Santa Cruz's outskirts area, respectively, resulting in a total of 240 animals. The required sample size was estimated using the statistical power analysis software PASS 2005 (Number Cruncher Statistical Systems, Kaysville, UT, USA). The sample size of 240 from a dog population of 400,000 was sufficient to produce a 95% confidence interval (CI) with a desired accuracy of ±5% when the estimated antibody seroprevalence was 80%.

Data collection

The field work was conducted by teams comprising of the authors and staff from the Municipal Government of Santa Cruz de la Sierra. All 240 study animals were investigated in April 2007. The work consisted of data collection through questionnaire interviews for each dog owning household, together with blood sample collections for each animal. The questionnaires for obtaining owned dog general characteristics (age, sex, breed and function) were prepared in Spanish by the authors. The household head or another adult person in the household was asked for information. Confirmation of vaccination status of dogs was implemented by individual vaccination certificates or according to information given by respondents. Dogs identified as vaccinated against rabies within the last one year prior to the interview were classified as vaccinated. Blood samples were taken from the cephalic or saphenous veins of each dog, collected into tubes and allowed to clot at room temperature. The samples were then centrifuged and the separated serum was stored frozen at –20°C until analysis.

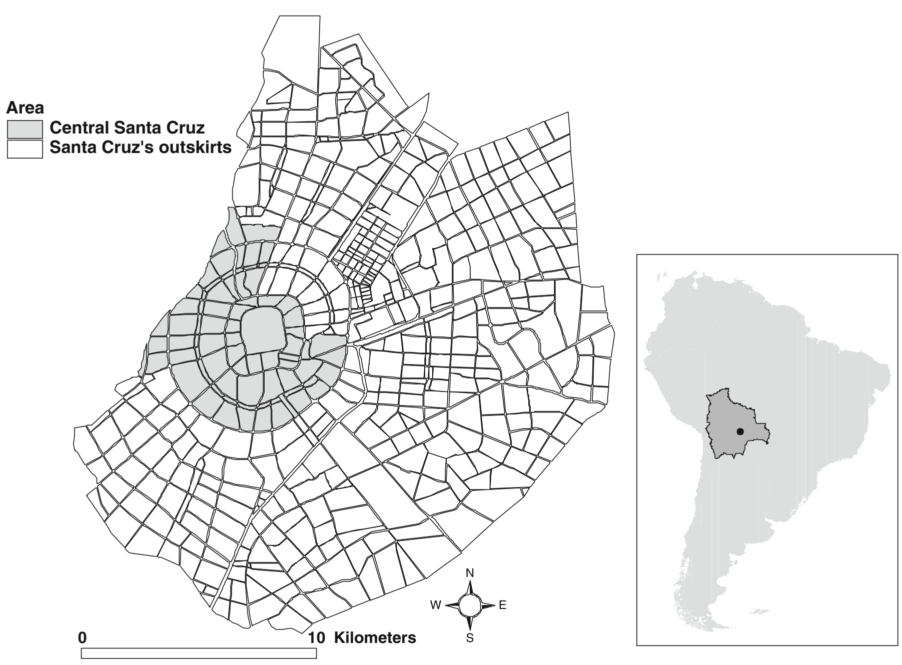

Fig. 1 Map of the study area. Inset: dot indicates position of Santa Cruz de la Sierra in Bolivia (shaded) in South America

Laboratory examinations

Blood samples were used for diagnostic investigations at the Veterinary Research and Diagnostic Laboratory (Laboratorio de Investigación y Diagnóstico Veterinario; LIDIVET). Sera were analysed using a commercial indirect enzyme-linked immunosorbent assay (ELISA) that employed the glycoprotein extracted from inactivated and purified virus membrane as antigen (Platelia Rabies II Kit, Bio-Rad Laboratories, France). Positive and negative controls were included for each series of samples analysed. Absorbance was read on an ELISA reader at 450–620 nm. A level of antibody equal to or greater than 0.5 IU/ml was considered by WHO and OIE experts as adequate protection from the risk of contamination (WHO 1987). Therefore, the results were expressed as protected or not protected status after optical density (OD) of each sample was compared to the threshold OD corresponding to the cut-off value at 0.5 IU/ml.

Data analysis

Data collected were entered into a database using the Base in the OpenOffice.org software version 2.2.0 (Sun Microsystems, Santa Clara, CA, USA). Maps were produced using the geographical information system software ArcGIS version 9 (ESRI Inc., Redlands, CA, USA). The relationship between geographical area (central vs. outskirts) and dog char-acteristics was analysed with Pearson's chi-squared statistic for comparison of these categorical factors, using the R software version 2.5.0 (Ihaka and Gentleman 1996). A P-value of 0.05 was used to indicate statistical significance and all statistical hypothesis tests were two-sided. Based on the published ELISA sensitivity (88.4%) and specificity (98.8%) values (Anon 2005; Feyssaguet et al. 2005), estimated true prevalences of antibodies among study dogs were calculated as follows.

(1) True prevalences (TP) for each geographical area were derived from the apparent prevalences (AP) using the Rogan–Gladen estimator and information about the sensitivity (Se) and specificity (Sp):

$$\mathrm{TP} = (\mathrm{AP} + \mathrm{Sp} - 1)/(\mathrm{Se} + \mathrm{Sp} - 1)$$

For estimation of true prevalence (deterministic function with 95% CI) above, Survey Toolbox software version 1.04 (Cameron 1999) was used.

(2) The calculations per geographical area were done with the following distributions for AP, Se and Sp:

$$\mathrm{AP} \sim \mathrm{Beta}\,(\mathrm{d_a} + 1; \mathrm{n_a} - \mathrm{d_a} + 1),$$

$$\mathrm{Se} \sim \mathrm{Beta}\,(\mathrm{d} + 1, \mathrm{n} - \mathrm{d} + 1),$$

$$\mathrm{Sp} \sim \mathrm{Beta}\,(\mathrm{d} + 1, \mathrm{n} - \mathrm{d} + 1)$$

where d=the number of desired (positive or negative) outcomes, n=the number of samples tested per geographical area, and the subscript 'a' indicates an area-specific value. For estimation of true prevalence (stochastic function with 95% CI) above, the spreadsheet software Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) in combination with the simulation add-in software @Risk for Windows version 4.5.7 Pro (Palisade Corporation, Newfield, NY, USA) with 100,000 iterations were used.

(3) A Bayesian model was used to derive posterior Bayesian estimates (denoted TP_B, Se_B and Sp_B) from prior distributions and the data from each geographical area in the study. The same prior beta distributions for Se and Sp as in the Rogan–Gladen approach were used. The Markov chain–Monte Carlo (MCMC) simulation was run for 120,000 iterations of which the first 20,000 iterations were discarded as 'burn-in'. The posterior means and 95% CI (sometimes called "Bayesian credibility interval") were recorded for the area-specific TP_B estimates and for posterior estimates of the test characteristics, Se_B and Sp_B. The models were run in WinBUGS 1.4.3 (Lunn et al. 2000). The source code for WinBUGS to estimate the prevalence with one test in a population was made available by Branscum et al. (2004).

Results

The 240 dogs studied accounted for about 0.07% of the total owned dogs in the study area. Of all, 123 were male (51%), 114 were female (48%) and three animals had no questionnaire records. Dogs under one year of age accounted for eight percent only of the total. Proportions of crossbred dogs (vs. purebred) and dogs owned for guarding (vs. pet) were 79% and

Table 1 Apparent Seroprevalences against rabies among vaccinated dogs in the central and outskirts of Santa Cruz de la Sierra, Bolivia in April 2007, based on an ELISA cut-off level of 0.5 EU/ml, categorised by dog characteristics

	Central Santa Cruz				Santa Cruz's outskirts			
	No. of immunised	No. of sampled	%	95% CI	No. of immunised	No. of sampled	%	95% CI
Age	(χ^2=2.43, 3 df, P=0.49)				(χ^2=1.45, 3 df, P= 0.69)			
<1 year	1	4	27	(4–50)	7	15	53	(39–66)
1–2 years	13	32	46	(37–55)	29	67	48	(42–54)
3–4 years	4	12	36	(22–51)	19	53	40	(33–47)
>4 years	3	16	20	(10–31)	18	38	53	(44–61)
Not recorded*	–	–			0	3	0	
Sex	(χ^2=0.51, 1 df, P=0.47)				(χ^2=1.01, 1 df, P=0.31)			
Male	8	30	30	(21–38)	43	93	51	(46–57)
Female	13	34	42	(33–51)	30	80	42	(37–48)
Not recorded*	–	–			0	3	0	
Breed	(χ^2=0.05, 1 df, P=0.83)				(χ^2=0.001, 1 df, P=0.97)			
Purebred	16	51	34	(27–41)	56	139	44	(40–49)
Crossbred	4	12	36	(22–51)	13	31	47	(38–56)
Not recorded*	1	1	100		4	6	75	(55–96)
Function	(χ^2=0.17, 1 df, P=0.68)				(χ^2=3.11, 1 df, P=0.08)			
Pet	7	26	30	(20–39)	32	64	56	(49–63)
Guard	13	37	39	(31–47)	36	103	39	(34–44)
Not recorded*	1	1	100		5	9	63	(46–80)

*Excluded from each χ^2 test

58%, respectively. Two-hundred and thirty-five animals (98%, 95% CI: 95–99%) were vaccinated for rabies at the last mass vaccination campaign implemented in August 2006 (including one animal vaccinated twice by a private veterinary practitioner as well as the campaign within the last one year). The other five animals had missing questionnaire records. Overall, 94 (39%, 95% CI: 33–45%) of the tested 240 vaccinated dogs were classified as test-positive for rabies-specific antibodies. With respect to apparent antibody seroprevalences related to dog characteristics, the percentage of seropositivity did not tend to increase as the dogs got older. Although there was no difference about sex or breed classifications comparing seropositivity status in both areas, the evidence that dogs owned as pets had greater antibody seroprevalence than the dogs owned for guard in Santa Cruz's outskirts area was close to significance (Table 1). Apparent antibody seroprevalence was smaller for dogs owned within central Santa Cruz than in the dogs owned in the outskirts, although there was no statistical significance (χ^2=1.14, 1 df, P=0.29). With regard to adjusted antibody seroprevalence, Bayesian TP estimates were consistently lower than both of the Rogan–Gladen

Table 2 Estimated seroprevalences against rabies among vaccinated dogs in the central and outskirts of Santa Cruz de la Sierra, Bolivia in April 2007, based on an ELISA cut-off level of 0.5 EU/ml

	Central Santa Cruz	Santa Cruz's outskirts	Total
No. of dogs sampled	64	176	240
Apparent seroprevalence (%)	33	41	39
True seroprevalence estimation [% (95% CI)]			
Rogan-Gladen (deterministic)	36 (30–42)	46 (42–50)	44 (40–47)
Rogan-Gladen (stochastic)	37 (24–50)	46 (38–55)	44 (36–51)
Bayesian inference	35 (26–43)	44 (38–49)	41 (37–46)

True seroprevalences were estimated using the Rogan-Gladen estimator as well as by Bayesian modelling with distributions for sensitivity ~ Beta (541, 72) and specificity ~ Beta (1018, 13) estimates. All Rogan–Gladen point estimates were well within the Bayesian credibility intervals (Table 2). The Bayesian posterior sampling means for the Se_B and Sp_B, estimated from the study, were 88.4% (95% CI: 85.8–90.8%) and 98.7% (95% CI: 97.9–99.3%), respectively.

Discussion

Although the routine diagnosis of rabies suspect samples in dogs is basically made on brain tissue at LIDIVET, there is no framework for serologic surveillance systems against canine rabies in Santa Cruz de la Sierra. This is the first report of canine rabies antibody seroprevalence in the study area. An appropriate sampling method was used to secure a certain degree of external validity. However, it must be kept in mind that the non-acceptance of the dog owners to have their animals bled would be likely to be at risk of bias in the data collected. Fortunately, the study accomplished 100% participation among the owners selected. The pet dogs studied in Santa Cruz's outskirts area, which were usually given better individual attention, had more antibody seroprevalence than the guard dogs, which were basically in outdoor environment. It could be possible that factors involving nutrition or parasitic infections might cause the poor immune response that was detected (Macpherson et al. 2000). It was reported that the percentage of positive samples of canine rabies raised notably with distance from the city centre of Santa Cruz de la Sierra and as socio-economic status dropped (Widdowson et al. 2002). Lower vaccination coverage was also reported to be associated with poorer areas in the Philippines (Beran et al. 1972). However, some of the rise in percentage positive as distance increased from the centre might be owing to reporting bias. This could be explained by the differences of accessibility for reporting to the official between the central area and outskirts area. In the current study, there was no difference of canine rabies seroprevalence between these two areas. The last vaccination campaign of an unprecedented scale during the last decade would contribute to reduce the disparities. It is important to adjust the apparent prevalences for the imperfect test characteristics of ELISA. The authors used both a Rogan–Gladen estimation (deterministic and stochastic functions) and Bayesian inference. The approaches yielded comparable true-prevalence estimates, with those of the Bayesian model being lower and having narrower CIs. The Rogan–Gladen approach has the advantage that it is widely known and also can be used as a simple deterministic function (entering fixed values for AP, Se and Sp) which is not required any specific software for calculation. One disadvantage is that the estimator can yield negative results in the case of certain combinations of AP, Se and Sp. The Bayesian model approach is more complicated but comparatively easily can be implemented in software WinBUGS which is freely available. Its advantage is to provide posterior distributions (estimates) for Se and Sp as well as TP. However, knowledge and assumptions on the prior distributions, value range and starting values of the model inputs are required for implementing stochastic process. The overall seroprevalence in the study was 41% (Bayesian inference). In addition, the seroprevalence of 41% would overestimate the true seroprevalence throughout the study area. It was not doubtful that the study owned dogs were more likely to have been vaccinated than stray dogs. It is recommended for public health authorities to take account of capturing stray dogs only once for vaccination and identification as well as mass maintaining vaccination programmes for owned dogs. In the study area, rabies vaccination was offered free of charge in the last mass campaign, which has had an estimate coverage consistency about 60% of the target dog population (The Municipal Health Service of Santa Cruz de la Sierra, personal communication 2006). Based on this estimation in conjunction with the results of the current study, the authors would estimate that less than a quarter of the total number of dogs was only immunised against rabies in the study area. The WHO recommends that 70% of the dog population in a community should be immunised to eliminate or prevent outbreaks of rabies infection (WHO 1987, 2005). It is also recommended to monitor immune response, i.e. antibody seroprevalence following a vaccination campaign against rabies in dogs, as well as the vaccination coverage periodically.

Acknowledgements The study was carried out as part of the project for the capacity development for improvement of livestock hygiene in the southern part of South America through regional cooperation [in Spanish: Proyecto de desarrollo profesional continuo para los veterinarios del Sur (PROVETSUR)], funded by the Japan International Cooperation Agency. The authors wish to thank the staff of the Municipal Government of Santa Cruz de la Sierra for their support. We are extremely grateful to the dog owners who allowed us to work with them during the study. Valuable and insightful comments by the reviewers are gratefully acknowledged.

References

Anon, 2005. *Platelia Rabies II Kit User's manual, Ref. 355 1180*, (Bio-Rad Laboratories, Marnes-la-Coquette)

Belotto, A., Leanes, L.F., Schneider, M.C., Tamayo, H. and Correa, E., 2005. Overview of rabies in the Americas, *Virus Research*, 111, 5–12

Beran, G.W., Nocete, A.P., Elvina, O., Gregorio, S.B., Moreno, R.R. and Nakao, J.C., 1972. Epidemiological and control studies on rabies in the Philippines, *Southeast Asian Journal of Tropical Medicine and Public Health* 3, 433–445

Branscum, A.J., I.A. Gardner and W.O. Johnson, 2004. Bayesian modeling of animal- and herd-level prevalences, *Preventive Veterinary Medicine*, 66, 101–112

Cameron, A.R., 1999. *Survey toolbox - a practical manual and software package for active surveillance of livestock diseases in developing countries*, (Australian Centre for International Agricultural Research, Canberra)

FAO, 2001. *FAOCLIM 2: world-wide agroclimatic database*, (FAO, Rome)

Feyssaguet, M., Morize, J.L., Todorova, V., Blanchard, I. and Bourgeios, J.P., 2005. Platelia Rabies II, a new ELISA kit for the detection of rabies antibodies in animal and human serum or plasma, Kiev, (OIE First International Conference on "Rabies in Europe")

Gobierno Municipal Autónomo de Santa Cruz de la Sierra, 2006. *La campaña de vacunación contra la rabia alcanzó una cobertura del 80%, Boletin N° 125*, 29 August 2006, Santa Cruz de la Sierra (in Spanish)

Ihaka, R. and Gentleman, R., 1996. R: a language for data analysis and graphics, *Journal of Computational and Graphical Statistics*, 5, 299–314

Lunn, D.J., A. Thomas, N. Best and D. Spiegelhalter, 2000. WinBUGS - a Bayesian modelling framework: concepts, structure, and extensibility, *Statistics and Computing*, 10, 325–337

Macpherson, C.N.L., Meslin, F.X. and Wandeler, A.I., 2000. *Dogs, zoonoses, and public health*, (CABI Publishing, Wallingford)

Meslin, F.X., Fishbein, D.B. and Matter, H.C., 1994. Rationale and prospects for rabies elimination in developing countries. In: Ruprecht, C.E., Dietzschold, B. and Koprowski, H. (eds), *Lyssaviruses*, (Springer Verlag, Berlin), 1–26

Ministerio de Salud y Deportes, 2005. *Reglamento de medidas sanitarias especiales para el control de la rabia, Nota CITE: RABIA/141/11/05*, 8 November 2005, La Paz (in Spanish)

Ministerio de Salud y Prevísion Social, 2007. *Población total y grupos etareos*, http://www.sns.gov.bo/bolsns/pob1.asp, accessed 5 May 2007 (in Spanish)

Thrusfield, M., 2005. *Veterinary epidemiology*, (Blackwell Science Ltd Oxford)

WHO, 1987. *Guidelines for dog rabies control, WHO/VPH/83.43 Rev.1*, Geneva

WHO, 2005. *WHO expert consultation on rabies. First report (WHO Technical Report Series No. 931)*, Geneva

Widdowson, M.A., Morales, G.J., Chaves, S. and McGrane, J., 2002. Epidemiology of urban canine rabies, Santa Cruz, Bolivia, 1972–1997, *Emerging Infectious Diseases*, 8, 458–461

Wunner, W.H., 2005. Rabies in the Americas, *Virus Research*, 111, 1–4