Seroepidemiology of sheep brucellosis in the microregion of Feira de Santana, BA, Brazil

Soroepidemiologia de brucelose ovina na microrregião de Feira de Santana, Bahia, Brasil

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Abstract

The aim of the present study was to perform a seroepidemiological survey of *Brucella ovis* in ovine flocks in the microregion of Feira de Santana, State of Bahia (BA), Brazil. Ten municipalities with the largest sheep flocks were selected for this survey: Antonio Cardoso, Feira de Santana, Ipecaetá, Ipirá, Itatim, Pintadas, Rafael Jambeiro, Santa Teresinha, Santo Estévão and Serra Preta. The sample size was established on the basis of three parameters: significance level (99%), sampling error (5%), and estimated prevalence (50%). The total sample was divided proportionally to the sheep population found in the respective municipalities. The flocks examined in each municipality were randomly selected. The animals were older than six months and were distributed among 49 properties in the municipalities. Samples of blood from 793 male and female sheep were analyzed. During visitations, an epidemiological questionnaire was applied for collection of information and analysis of possible risk factors. All sera samples were analyzed by agar gel immunodiffusion (AGID) and the antigen was a mixture of soluble proteins and lipopolysaccharides from *B. ovis* (strain Reo 198). Seropositive animals (6.94%, 55/793) to *B. ovis* were detected. However, significant statistical difference (p>0.05) was not found for age and sex. Risk factors that might be associated with cases of seropositive animals for the variables analyzed were not found. In 61.22% (30/49) of the examined farms at least one seropositive animal was detected. Only two of the ten municipalities above had no seropositive animal.

Keywords: *Brucella ovis.* Risk factors. AGID. Occurrence. Serology.

Resumo

O objetivo do presente trabalho foi a realização de um inquérito soroepidemiológico de *Brucella ovis* em rebanhos de ovinos da Microrregião de Feira de Santana, Estado da Bahia, Brasil. Foram selecionados os dez municípios de maior efetivo ovino: Antônio Cardoso, Feira de Santana, Ipecaetá, Ipirá, Itatim, Pintadas, Rafael Jambeiro, Santa Teresinha, Santo Estévão e Serra Preta,. O tamanho da amostra foi calculado com base nos parâmetros: nível de significância, 99%; erro amostral, 5%, prevalência estimada, 50%. A amostra total foi fracionada segundo a população de ovinos do respectivo município. Foram examinados 793 animais, machos e fêmeas, com idade superior a seis meses, distribuídos em 49 propriedades dos municípios visitados. Por ocasião das visitas, foi aplicado um questionário epidemiológico destinado à análise de possíveis fatores de risco. Todos os soros foram submetidos ao teste da imunodifusão em gel de ágar (IDGA) com o antígeno constituído por proteínas e lipopolissacarídeos solúveis de *B. ovis* amostra Reo198. Foram obtidos 6,94% (55/793) de animais positivos e não houve diferença significativa (p>0,05) para idade e sexo. Para as variáveis analisadas, não foram encontrados fatores de risco que pudessem estar associados aos casos de animais reatores positivos. Dentre as propriedades trabalhadas, 61,22% (30/49) apresentaram pelo menos um animal reagente e dos dez municípios visitados, apenas dois não apresentaram animais positivos.

Palavras-chave: *Brucella ovis.* Fatores de risco. IDGA. Ocorrência. Sorologia..

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Introduction

Ovine brucellosis caused by *Brucella ovis* is a chronic infectious disease, responsible for reproductive impairment, with orchitis and epididymitis in males, abortion in females, and birth of weak lambs[1].

Sexual transmission between males and females is the most common mechanism of infection of ovine brucellosis. However, it can occur in sheep through sodomy, a usual behavior among sheep. Infected females can infect lambs through lactation besides eliminating the bacteria in the genital secretion[2].

Diagnosis based only on animal history and clinical examination of the reproductive system is not conclusive as there are infected animals without clinical signs[3,4]. Moreover, there are other bacteria which cause reproductive alterations such as *Corynebacterium pseudotuberculosis*, *Histophilus* spp., *Actinobacillus* spp., *Pasteurella* spp, e *Streptococcus* spp.[2,5,6]. Thus, definitive diagnosis can be obtained by either isolation or identification of the bacteria or analysis of the immune response to infection using a serological test. The agar gel immunodiffusion (AGID) test is fast and easy to perform. It produces reliable results and favors the development of programs to control the disease[7].

In a study on brucellosis among sheep in the Recôncavo Baiano (BA, Brazil), Silva et al.[8] used the antigen produced by the Desiderius Finamor Institute of Veterinary Research in the AGID test and found seropositive animals (3.27 %, 6/183). In the microregion of Juazeiro (BA, Brazil), Souza et al.[9] also analyzed 694 samples using AGID and showed that local flocks had seropositive sheep (8.62%, 5/58) and 0.72% (5/694) of the examined sheep present antibodies to *B. ovis*. The low prevalence of brucellosis was attributed to the method of sheep farming that prevails in the region, which minimized the possibility of introducing the agent. Marinho and Mathias[3] and Salaberry et al.[10] analyzed serum samples from sheep flocks in the states of São Paulo and Minas Gerais, respectively. They used complement fixation as serologic testing and they did not observe seropositive animals. In the state of Santa Catarina, Schäfer et al.[11] used AGIDand they did not observe seropositive animals. The AGID test is recommended by the Office International des Epizooties (OIE) for the diagnosis of brucellosis in sheep. Association of serological techniques, such as AGID and enzyme-linked immunosorbent assay (ELISA), should be encouraged as it allows obtaining more reliable results[12].

In the Recôncavo Baiano (BA, Brazil), the relationship between age group of sheep and occurrence of infection by *B. ovis* was analyzed and the authors found no significant difference between seropositive sheep with ages higher (5%, 5/100) and lower (1.2%, 1/83) than three years[8]. Some studies showed that prevalence of seropositivity in adult animals was higher than in young animals[13, 14]. Studies on the occurrence of *B. ovis* in the state of Bahia, where the second largest sheep flock in the country is raised, are scarce. Therefore, the purpose of this study was to conduct a seroepidemiological survey of brucellosis caused by *B. ovis* and assess its risk factors for sheep flocks in the municipalities of the micro region of Feira de Santana (BA, Brazil).

Materials and Methods

The survey was conducted in the micro region of Feira de Santana, a part of the Center-North meso region in the state of Bahia, which comprises 24 municipalities[15]. Ten municipalities with the largest sheep flock in the state, Antônio Cardoso, Feira de Santana, Ipecaetá, Ipirá, Itatim, Pintadas, Rafael Jambeiro, Santa Teresinha, Santo Estévão, and Serra Preta, were selected among those that compose the micro region under study. Blood samples were collected in the period from Sep 2010 to Nov 2011.

The minimum number of animals to be examined was calculated according to Thrusfield[16], and the confidence interval (99%) and sampling error (5%)

were also established. As the estimated prevalence was not known, a value of 50% was utilized to maximize the sample size (n = 663). The sample size corresponding to each municipality was proportional to its fraction in the total flock of the micro region. In each property, additional samples were preemptively collected to compensate for any loss due to hemolysis. Thus, 793 samples were collected. The properties (49) were selected in a non-random may because the available list of properties in the rural municipalities was incomplete (Table 1)

Questionnaires on general aspects, information about the origins of the flock and of the nutritional, reproductive, and sanitary management, besides the main sheep diseases were applied in the properties visited in order to characterize the farming systems prevailing in the region and to assess the risk factors related to ovine brucellosis.

Blood samples were randomly collected from male and female sheep aged at least six months. Their approximate ages were determined by examining their teeth. These animals were thus classified into three age groups: until one year (milk tooth; without molt), from one to four years (first, second, and third molts), and over four years (fourth molt and those with full molt, known as "full mouth").

The sheep breed was not a pre-established requirement, but a farm-dependent variable. Sheep of the Santa Ines, Dorper, and mixed breeds (including those of undefined breed, UDB) participated in this study.

The selected animals were clinically evaluated. Physical examination of the males included palpation of testes and epididymis, with measurement of scrotal circumference, regarding the aspects of symmetry, consistency, and presence of adhesions.

After antisepsis, blood samples were collected by jugular venipuncture using vacuum tubes. After clot formation, the tubes were centrifuged (1500 g; 10 min) and the sera were placed in *Eppendorf* tubes, identified, and then stored (-20 °C) until serological testing was performed.

The AGID method was used for detection of antibodies to *B. ovis*. The tests were performed in

Table 1 - Population and number* of animals used in serological evaluation of brucellosis by *B. ovis* in sheep flocks in the micro region of Feira de Santana (Bahia, Brazil)

Municipalities	Sheep population	Frequence (%)	Minimum number required	Number of sheep examined
Antônio Cardoso	5,166	4.2	28	35
Feira de Santana	11,700	9.6	64	84
Ipecaetá	6,015	4.9	33	48
Ipirá	45,775	37.6	249	286
Itatim	4,500	3.7	24	32
Pintadas	11,047	9.1	60	70
Rafael Jambeiro	15,598	12.8	85	94
Santa Teresinha	5,242	4.3	29	34
Santo Estevão	5,789	4.8	32	42
Serra Preta	10,990	9.0	60	68
TOTAL VALUES	121,822	100	663	793

* Parameters used: significance level (99%), sampling error (5%), and estimated prevalence (50%)

the Laboratory of Infectious Diseases (School of Veterinary Medicine, UFBA), using kits produced in the Institute of Technology of Paraná (TECPAR), and Noble agar **(Difco®)** following the manufacturer's recommendations. The antigen consisted of soluble proteins and lipopolysaccharides from *B. ovis* (strain REO198).

Differences between frequencies for seropositive animals were determined using the chi-square test. To evaluate the risk factors for *B. ovis*, univariate analysis was performed using the Odds ratio (OR) punctual and interval estimates. Both calculations were performed using the PAST software.[16, 18, 19]

Results and Discussion

In the analysis of antibodies to *B. ovis*, 6.94% (95% CI: 5.17-8.70%) of the animals showed to be seropositive (Table 2). Among the municipalities selected, 61.22% (95% CI: 47.58-74.87%) of the rural properties showed to have seropositive animals. It was pointed out that only two municipalities (Itatim and Santo Estévão) among those evaluated (10) had no seropositive animals (Table 3).

Seropositivity shown in this study was similar to that found by Alves et al.[20] (7.5%; 6/80), who used the AGID test and polymerase chain reaction (PCR) for amplification of DNA from *Brucella* spp. The uterus, testis, and epididymis samples were withdrawn from sheep slaughtered in an abattoir in the state of Paraíba.

Values higher than those obtained in this study were observed in the states of Rio Grande do Sul (13.4%; 220/1638)[21], Rio Grande do Norte (34.0%; 100/290)[13] and São Paulo (12.0%; 14/1033)[12] in sera from sheep analyzed by AGID. However, rates of animals seropositive to *B. ovis* lower than those obtained in this study (6.94%) were observed in the states of Alagoas (3.1%; 18/579)[14] and São Paulo (1.96%; 4/204)[22], in rural properties with history of reproductive disorders.

Among sheep, 5.13% (95% CI:1.67-8.59%) of those aged up to one year, 7.03% (95% CI:3.90-10.16%) of those aged between one and four years, and 7.61% (95% CI:4.95-10.27%) of those older than four years were seropositive in the serological test. Although the rate for seropositive adult animals was the highest, the difference observed showed no statistical significance (p>0.05) in relation to younger animals. However, Ficapal et al.[4] emphasized that adult animals are the most commonly infected because occurrence of testicular alterations and positive serology to brucellosis increase with age and sexual maturity.

In relation to gender 7.69% (95% CI:2.22-13.17%; 7/91) of males and 6.84% (95% CI:4.97-8.70%; 48/702) of females showed antibodies to *B. ovis*. Occurrence of seropositivity in both groups is comparable to what was observed by Silva et al.[8] in the state of Bahia, with 2.63% of seropositivity in males (1/38) and 3.44% (5/145) in females, without significant difference between genders. Such situation is similar to that observed in this and another study conducted in the state of Rio Grande do Norte, with sheep and lambs are equally exposed to infection with *B. ovis*[13].

Regarding race of animals, 65.45% (95% CI: 52.89-78.02%) of the Santa Inês, 5.45% (95% CI:0-11.46%) of Dorper, and 29.1% (95% CI:17.09-41.09%) of mixed breeds and UDB were seropositive, and significant differences (p>0.05) were not found between them. Clementino et al.[23] searched *B. ovis* in sheep in the semi-arid region of Paraíba (PB, Brazil) and 49.38% (199/403) of seropositive animals were of the Santa Inês breed. However, no significant difference was found between breeds.

Among the seven seropositive males (7.69%), none showed reproductive change on physical examination. However, 12% (11/91) of sampled sheep exhibited signs of unilateral or bilateral orchitis, asymmetry and/or testicular atrophy, and all of them were serologically negative. This observation could be related to other causative agents of reproductive alteration.

Table 2 - Sheep in municipalities in the micro region of Feira de Santana (Bahia, Brazil) tested in agar gel immunodiffusion for diagnosis of brucellosis by *B. ovis*. Blood samples were collected in the period Sep 2010 - Nov 2011

Municipalities	Number of sheep examined	Number of seropositive sheep	Seropositivity rate (%)
Pintadas	70	13	18.57
Serra Preta	68	8	11.76
Santa Teresinha	34	3	8.82
Rafael Jambeiro	94	7	7.45
Feira de Santana	84	6	7.14
Ipirá	286	15	5.24
Ipecaetá	48	2	4,17
Antônio Cardoso	35	1	2.86
Itatim	32	0	0.00
Santo Estévão	42	0	0.00
TOTAL VALUES	793	55	6.94

Table 3 - Rural properties with flocks of sheep in the micro region of Feira de Santana (Bahia, Brazil) with animals examined by the agar gel immunodiffusion test applied to the diagnosis of brucellosis by *B. ovis*. Blood samples were collected in the period Sep 2010 - Nov 2011

Municipalities	Number of rural properties		
	Examined	With positive sheep	%
Pintadas	4	4	100
Santa Teresinha	2	2	100
Serra Preta	4	3	75.0
Ipecaetá	3	2	66.67
Rafael Jambeiro	6	4	66.67
Ipirá	17	11	64.71
Feira de Santana	5	3	60.0
Antônio Cardoso	3	1	33.33
Itatim	2	0	0
Santo Estévão	3	0	0
TOTAL VALUES	49	30	61.22

* The flock was considered positive when at least one animal showed positive reaction in the AGID test

In the state of Santa Catarina, serological evaluation of 69 sheep revealed no seropositivity for *B. ovis*, but 18.84% of them had alterations in their reproductive organs. This highlights the importance of periodic reproductive examinations to prevent signs of subfertility and infertility in the flock[11]. In the state of Rio Grande do Sul, Gomes et al.[24] reported that epididymitis was detected by palpation in 15.1% (5/33) of animals, in which samples of *Actinobacillus seminis* but not of *B. ovis* were isolated. This suggests that serological assessment of animals with signs of orchitis and/or epididymitis be performed for final diagnosis.

Statistical significance was not observed in the risk factors (lack of veterinary care, poor sanitary management, lack of primary care for newborns, lack of requirement for health documents to purchase animals or non segregation of animals by age group, and not performing periodic soundness examination in the breeding animals) for the sheep flocks analyzed in this study (Table 4).

Conclusion

Occurrence of seropositivity for ovine brucellosis widely distributed in the micro region of Feira de Santana, a distinct region in sheep production, highlights the need for a more comprehensive investigation in the state of Bahia (Brazil). Significant effect of age group or gender on the prevalence of brucellosis was not observed.

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Table 4 - Risk factors for brucellosis by *B. ovis*, values for odds ratio (OR), and probability of random occurrence of brucellosis (*p*) in sheep flocks in the micro region of Feira de Santana (Bahia, Brazil) in 2011

Factors	***OR***	**95% CI**	***p* values**
Lack technical monitoring	0.48	0.14-1.57	0.22
Does not treat the navel with iodine	0.64	0.20-2.03	0.44
Does not require sanitary documentation	1.36	0.35-5.36	0.66
Origin of the flock			
Location: another municipality	0.22	0.02-2.25	0.18
Location: another state	1	0.19-5.16	1
Other municipality / other state	4.5	0.34-60.15	0.26
Participates in exhibitions	0.2	0.03-1.16	0.73
Does not perform soundness examination	0.2	0.03-1.16	0.07
Does not separate the animals	0.27	0.04-1.65	0.15
Creation system			
Extensive / semi-extensive	1.2	0.32 -4.47	0.79
Semi-extensive / semi-intensive	1	0.14-6.91	1
Extensive / semi-intensive	1.2	0.15-9.77	0.86
Mode of reproduction			
Natural/controlled mount	2.63	0.40-17.41	0.32

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Referências

1. ROBLES, C. A.; UZAL, F. A.; OLAECHEA, F. V.; LOW, C. Epidemiological observations in a Corriedale flock affected by *Brucella ovis*. **Veterinary Research Communications**, v. 22, n. 7, p. 435-443, 1998.

2. BAIGÚN, R.; CONIGLIARO, A. S.; LUNA, F. Aislamiento de *Brucella ovis* y control de reaccionantes serológicos en epididimitis ovina. **Veterinaria Argentina**, v. 7, n. 162, p.103-107, 2000.

3. MARINHO, M.; MATHIAS, L. A. Pesquisa de anticorpos contra *Brucella ovis* em ovinos do estado de São Paulo. **Pesquisa Veterinária Brasileira**, v. 16, n. 2/3, p. 45-48, 1996.

4. FICAPAL, A.; JORDANA, J.; BLASCO, J. M.; MORIYÓN, I. Diagnosis and epidemiology of *Brucella ovis* infection in rams. **Small Ruminant Research**, v.29, n. 1, p.13-19, 1998.

5. MANTEROLA, L.; TEJERO-GARCÉS, A.; FICAPAL, A.; SHOPAYEVA, G.; BLASCO, J. M.; MARIN, C. M.; LÓPEZ-GOÑI, I. Evaluation of a PCR test for diagnosis of *Brucella ovis* infection in semen samples from rams. **Veterinary Microbiology**, v. 92, n. 1/2, p. 65-72, 2003.

6. LÓPEZ, G.; ESCOBAR, G. I.; AYALA, S. M.; LUCERO, N. E. Detection of antibodies to *Brucella ovis* in sheep milk using *B. ovis* and *B. canis* antigen. **Veterinary Microbiology**, v. 116, n. 1/3, p. 232-238, 2006.

7. ROBLES, C. A. Epididimitis contagiosa de los carneros por *Brucella ovis*. **Microbiologia y Enfermedades Infecciosas**, v.79, n.1, p. 67-71, 1998.

8. SILVA, N. S.; BARROS, I. N.; DASSO, M. G.; ALMEIDA, M. G. A. R.; LABORDA, S. S.; ANUNCIAÇÃO, A. V. M.; MOREIRA, E. L. T.; LIMA-SILVA, A. E.; OLIVEIRA, E. M. D. Detecção de anticorpos anti-*Brucella ovis* em ovinos do estado da Bahia. **Revista Brasileira de Saúde e Produção Animal**, v. 10, n. 4, p. 852-859, 2009.

9. SOUZA, T. S.; COSTA, J. N.; MARTINEZ, P. M.; LIMA, C. C. V.; ARAÚJO, B. R.; NETO, A. O. C.; ANUNCIAÇÃO, A. V. M.; ALMEIDA, M. G. A. R.; PINHEIRO, R. R. Inquérito soro-epidemiológico de *Brucella ovis* em rebanhos ovinos no semiárido baiano. **Arquivos do Instituto Biológico**, v. 79, n. 2, p. 277-281, 2012.

10. SALABERRY, S. R. S.; PAULIN, L. M.; SANTANA, R. L.; CASTRO, J. R.; LIMA-RIBEIRO, A. M. C. Pesquisa de anticorpos anti-*Brucella abortus* e anti-*Brucella ovis* em ovinos no município de Uberlândia, MG. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 63, n. 4, p. 1022-1024, 2011.

11. SCHÄFER, I.; VAZ, A.; RAMELLA, J.; COUTINHO, G. Prevalência de carneiros reagentes à prova de imunodifusão em gel para *Brucella ovis* no município de Lages-SC. **A Hora Veterinária**, v. 17, n. 99, p. 60-61, 1997.

12. NOZAKI, C. N.; MEGID, J.; LIMA, K. C.; SILVA JUNIOR, F. F.; VELOSO, C. S. Comparação das técnicas de imunodifusão em gel de ágar e ELISA no diagnóstico de brucelose ovina em Cabanhas da região centro-oeste do estado de São Paulo. **Arquivos do Instituto Biológico**, v. 71, n. 1, p. 1-5, 2004.

13. SILVA, J. B. A.; FEIJO, F. M. C.; TEIXEIRA, M. F. S.; SILVA, J. S. Prevalência de brucelose ovina causada por *Brucella ovis* em rebanhos do estado do Rio Grande do Norte, Brasil. **Ciência Animal**, v. 13, n. 1, p. 51-54, 2003.

14. PINHEIRO JUNIOR, J. W.; OLIVEIRA, A. A. F.; MOTA, R. A.; AGOTTANI, J. V.; JESUS, E. M.; ASSIS, S. T.; OLIVEIRA, C. Z. Ocorrência de ovinos sororreatores para *Brucella ovis* no estado de Alagoas, Brasil. **Veterinária e Zootecnia**, v. 16, n. 3, p. 500-508, 2009.

15. INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). **Pecuária 2010**: rebanho ovino. Available at: <http://www.ibge.gov.br> Accessed on: 26 out. 2011.

16. THRUSFIELD, M. V. Inquéritos. In:_____ **Epidemiologia veterinária**. 2. ed. São Paulo: Roca. 2004. p. 223-247.

17. RADOSTITS, O. M.; MAYHEW, I. G. J.; HOUSTON, D. M. **Exame clínico e diagnóstico em veterinária**. Rio de Janeiro: Guanabara Koogan. 2002. p. 554-565.

18. ARANGO, H. G. **Bioestatística**: teórica e computacional. 2nd ed. Rio de Janeiro: Guanabara Koogan, 2005. 423 p.

19. HAMMER, O.; HARPER, D. A. T.; RYAN, P. D. PAST: Paleontological statistics software package for education and data analysis. **Palaeontologia Electronica** v. 4, n. 1, p. 9, 2001. Available at: <http://palaeo-electronica.org/2001_1/past/issue1_01.htm>. Accessed on: 07 mar. 2008.

20. ALVES, C. J.; FIGUEIREDO, S. M.; AZEVEDO, S. S.; CLEMENTINO, I. J.; KEID, L. B.; VASCONCELLOS, S. A.; BATISTA, C. S. A.; ROCHA, V. C. M.; HIGINO, S. S. Detection of *Brucella ovis* in ovine from Paraíba State, in the Northeast Region of Brazil. **Brazilian Journal of Microbiology**, v. 41, n. 2, p. 365-367, 2010.

21. MAGALHÃES NETO, A.; GIL-TURNES, C. Brucelose ovina no Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 16, n. 2/3, p. 75-79, 1996.

22. RIZZO, H.; GREGORY, L.; PINHEIRO, E. S.; CARVALHO, A. F.; SANTANA, R. L.; SILVA, L. M. P. Incidência de *Brucella ovis* em ovinos com histórico de distúrbios reprodutivos no estado de São Paulo, Brasil. **Ciência Animal Brasileira**, 2009. Suplemento, 1. Trabalho apresentado no VIII Congresso Brasileiro de Buiatria, realizado em Belo Horizonte MG em outubro de 2009.

23. CLEMENTINO, I. J.; ALVES, C. J.; AZEVEDO, S. S.; PAULIN, L. M.; MEDEIROS, K. A. Inquérito soro-epidemiológico e fatores de risco associados à infecção por *Brucella ovis* em carneiros deslanados do semi-árido da Paraíba. **Pesquisa Veterinária Brasileira**, v. 27, n. 4, p. 137-143, 2007.

24. GOMES, M. J. P.; DRIEMEIER, D.; BONETTI, A. L.; EIDT, M.; AZAMBUJA, D. R. Epididimite ovina: isolamento de *Actinobacillus seminis*, no RS - Brasil. **Arquivos da Faculdade de Veterinária da UFRGS**, v. 29, n. 1, p. 55-58, 2001.