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Characterization of Bacterial Pathogens Isolated from Calf Diarrhoea in Panchagarh District of Bangladesh

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ABSTRACT

The research work was conducted to isolate, identify and characterize the bacterial pathogens causing calf diarrhea in Panchagarh district of Bangladesh. The study was conducted on 114 faecal samples collected directly from the rectum of diarrhoeic calves and brought to the Microbiology laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology Universiy, Dinajpur for bacteriological examination. Isolation and identification of the microorganisms were confirmed on the basis of their morphology, staining, cultural and biochemical tests. Furthermore, the isolated bacteria were characterized by antimicrobial susceptibility testing. A total of 114 faecal samples were examined for the isolation of bacteria of which 44 (38.6%) samples were positive for *E. coli*, 25 (21.9%) samples were positive for *Salmonella spp*, 15 (13.2%) samples were positive for Staphylococcus, 18(15.8%) samples were accounted for mixed infection and 12 (10.5%) samples were negative for bacteria. The antibiogram study revealed that most of the E. coli, Salmonella spp. and Staphylococcus spp. were resistant to penicillin, ampicillin, amoxycillin and bacitracin. However, most of the *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. were susceptible to azithromycin, gentamicin and ciprofloxacin. The findings of the present study indicate that the use of azithromycin, gentamicin and ciprofloxacin may have the preference to be choice in clinical control of *Salmonella*, *E. coli*, and *Staphylococcus* causing calf diarrhoea in Panchagarh district of Bangladesh.
KEYWORDS: *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., antimicrobial resistance, calf diarrhoea

INTRODUCTION

Livestock is an integral part of the agricultural production system in Bangladesh and plays an important role in national economy as well as in socio-economic development of millions of rural household. New born farm animals suffer fairly higher mortality than their adult counterparts and it is one of major reprisal over economy in livestock Industry. Diarrhoea in farm animals, especially in neonatal calves is one of the most challenging clinical syndromes encountered by practicing large animal's Veterinary practitioners. Diarrhoea is a leading cause of economic losses to the cattle industry and major cause of calf mortality and morbidity during first few weeks of life in most countries (Radostits *et al*., 2000). The economic losses occur not only from mortality but also from treatment costs and time spent on care as well as subsequent chronic ill thrift and impaired growth performance (Bazeley, 2003). Overfeeding, overpopulation, cold temperature, bad hygiene, artificial feeding and colostrums deprivation are all predisposing factor which can be important in the complex etiology of the disease. Diarrhoea caused by different enteropathogens has been recognized as a major clinical problem for calves in Bangladesh (Debnath *et al.*, 1987). Among these organism *Escherichia coli* is the main cause for the calf diarrhea as "white" scour (Hemashenpagam *et al.*, 2009).

Calf scours is a complex disease, with many interrelated causes. Agent, host, and environmental factors collectively explain scours, and these factors interact dynamically over the course of time (David R. Smith, 2007). Discontinuation or incomplete course of treatment and continuous indiscriminate uses of antibacterial drugs against diarrhoeal infection of man and animal might have influenced to produce a new generation of virulent and resistant type of bacteria. Although routine laboratory isolation and drug sensitivity testing are expensive and impractical, the periodical check of the pattern of the drug sensitivity of organisms is more significant. It is, therefore, important that sensitivity of different bacteria isolated from diarrhoeic calves needs to be studied from time to time in order to formulate appropriate therapeutic measures (Kaura *et al.* 1988). Reports on enteropathogens associated with calf diarrhoea are very limited in Bangladesh. Therefore, an attempt

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was made to investigate the bacterial pathogens associated with calf diarrhea in Panchagarh district of Bangladesh.

2. MATERIALS AND METHODS

2.1. Selection of study area

The research work was carried out in cattle farm of different area under Panchagarh district of Bangladesh during the period of July to December 2012.

2.2. Isolation and identification of bacterial pathogens from calf diarrhoea

2.2.1. Collection of sample

A total number of 114 field samples comprising of loose feces were aseptically collected for isolation and characterization of bacterial pathogens from calf diarrhea and carried to the Microbiology laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology Universiy, Dinajpur. Isolation and identification of bacterial pathogens were performed as per procedures described by Marchant and Packer (1967), OIE (2000) and Cowan (1985).

2.2.2. Cultural characterization

Primary culture was performed in Nutrient agar and Nutrient broth media. Subcultures were performed in Mac Conkey agar, Blood agar, Staphyloccous Agar no.110, Eosin-Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar to obtain pure culture and to study the cultural characteristics.

2.2.3. Morphological characterization

The representative bacterial pathogens were isolated from suspected cases of fecal samples then stained with Gram's staining techniques (Marchant and Packer,1967). Motility test was performed by Hanging drop method.

2.2.4. Biochemical characterization

Isolated bacterial pathogens with specific cultural characteristics of E.coli, Salmonella and Staphylococcus on various culture media were maintained on EMB agar, SS agar and Staphylococcus agar No. 110 and were subjected to biochemical reaction such as Triple sugar iron (TSI) agar slant reaction, Methyl- Red (MR), Voges – Proskauer (VP) test, Indole test and Motility indole urease (MIU) test (Marchant and Packer,1967).

2.3. Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were performed using Kirby Bauer's disc diffusion method according to performance standards of CLSI (Clinical and Laboratory Standards Institute, 2006). The antimicrobial agents used were penicillin, ampicillin, amoxycillin, chloramphenicol, erythromycin, azithromycin, gentamicin, bacitracin and ciprofloxacin.

2.4. Maintenance of stock culture

The stock culture was maintained following the procedures of Choudhury *et al.* (1987). During the experiment it was necessary to preserve the isolated organisms for longer periods. For this purpose, pure culture of the isolated organisms were stored in sterilized 80% glycerin and used as stock culture. The equal volume of 80% glycerin and bacterial culture were mixed and sealed with paraffin wax and stored at -80°C in freezer for future use.

3. RESULTS AND DISCUSSION

The present research work was conducted to isolate, identify and characterize the bacterial pathogens causing calf diarrhea in Panchagarh district of Bangladesh. A total of 114 faecal samples were collected and examined bacteriologically for the isolation, identification, frequency distribution, and the degree of antibiotic sensitivity of bacteria isolated from diarrhoeic calves. The results of isolation and identification of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. from suspected birds by using staining, cultural and biochemical tests are summarized in Tables 1, 2 and 3. In this study, 3 different types of bacteria were isolated from a total of 114 faecal samples collected from diarrhoeic calves. Out of 114 faecal samples 44 samples were found positive for *E. coli* giving positive reaction to lactose fermentation on MacConkey agar plate, metallic green sheen colonies on EMB plates and yellowish green colonies on BGA. Twenty five samples were found positive for *Salmonella* producing negative reaction to lactose fermentation on MacConkey agar plate. The organism produce opaque, translucent and colorless colonies on S-S agar, pale pink colour colonies against a pinkish background on BGA and deep blue colour from green colour simmons citrate agar. Fifteen samples

were found positive for staphylococcous producing yellowish colonies on Staphylococcus agar no. 110, hemolysis on Blood agar. Differences in colony morphology manifested by the isolates may be due to loosing or acquiring some properties by the transfer of host or choice of host tissue as observed by Dubreuil *et al.*, (1991).

The different isolates of *E. coli*, *Staphylococcus* and *Salmonella* showed identical results in different biochemical tests including sugar fermentation, TSI, MIU, Indole, MR-VP and citrate utilization tests. *Staphylococcus* spp. produce acid but no gas by fermenting various sugars and gave positive reaction to Catalase and Methyl red test but negative reaction to Indole and Voges Proskeur test.The actual causes for which the manifestation of an identical result in biochemical tests by the three groups of known identified isolates were not clear. It is not unlikely that almost all isolates in the present study possess some common genetic materials which might be responsible for the manifestation of similar type of biochemical reaction as reported by Pandey *et al*. (1979) and Honda *et al.* (1982). It is reported that more than one predisposing factors such as environmental and management factors (housing, climate), imbalance nutrition, immune status of the calves etc. might help in the production of calf diarrhoea along with the presence of one or more than one types of bacteria (Fouquet, 1979 and Debnath *et al*., 1987).

The results of frequency distribution of bacterial isolates are presented in Table 4. Out of 114 faecal samples, 44 (38.6%) samples were positive for *E. coli*, 25 (21.9%) samples were positive for *Salmonella* spp., 15 (13.2%) samples were positive for *Staphylococcus* spp., 18 (15.8%) samples were accounted for mixed infection and 12 (10.5%) samples were negative for bacteria. The frequency distributions of different species of bacterial isolates in different faecal samples were found variable. Results of the present study indicated that all the three different types of bacteria were not present in the same faecal sample collected from diarrhoeic calves. The incidence of different types of bacteria isolated from calf diarrhoea, correlate with the findings of Raseswari--shomel *et al.* (1996). Haque and samad (1996) isolated 9.61% *Salmonella* from calves, Joon and Kaura (1993) isolated 23(23%) *E. coli* and 5 (5%) *Salmonella* from 100 fecal samples.The results are dissimilar with the result of Hemashenpagam *et al.* (2009) who isolated 75% *E. coli* (12 positive samples from 16 samples), Oporto *et al*. (2008) stated that the prevalence of *E. coli* in bovine herds was 35.9%, Kim-ChulMin *et al*, (2000) isolated 30 *E. coli* from 56 calves (53.37%), Valdivia-Andy *et al*., (2000) found verotoxin producing *E. coli* in 63.7% of the samples tested, Bendali et al. (1999) isolated 20.3% *E. coli* from fecal samples of diarrhoeic calves, and Khan and Khan (1997) isolated enteropathogenic *E. coli* (54-58%), *Staphylococcus* (7-10%) and *Salmonella (*13-14%). In addition to the bacteria isolated during the present study the *Campylobacter jejuni* was isolated by Barrandeguy *et al*.(1988); *Campylobacter coli* by Waltner Toews et al.(1986d), *Yersinia enterocolitoca* by Cygan and Buezek (1993), *Streptoccus* spp. by Rajeswari-Shorne *et al.* (1996), *Klebsiella* spp. by Dodson ket *et al*.(2005). and *Shigella spp*. by Rajeswari-Shome *et al*. (1996) from the diarrhoeic calves, they also isolated different types of viruses and protozoa.

The results of antimicrobial susceptibility of the isolated *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. are summarized in Tables 5, 6 and 7. The in-vitro antibiotic sensitivity test of three different types of bacterial isolates to 9 different antibiotics such as penicillin, ampicillin, amoxycillin, chloramphenicol, erythromycin, azithromycin, gentamicin, bacitracin and ciprofloxacin were studied. A slight variation was noticed in the results of sensitivity of isolates against 9 different antibiotics used. The antibiogram study revealed that most of the *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. were resistant to penicillin, ampicillin, amoxycillin and bacitracin. However, most of the *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. were susceptible to azithromycin, gentamicin and ciprofloxacin. These findings satisfy the result of Nazir (2007), Ahmed *et al.* (1986) and Genovese *et al.* (2006) who stated that calf isolates were highly sensitive to ciprofloxacin, levofloxacin and resistant to ampicillin, erythromycin, gentamicin and amoxycillin. These results were slightly dissimilar with the findings of Tripathi and Soni (1982), Joshi *et al*. (1986), Panower *et al* (1990) and Joon and Kaura (1993) who reported that most of the bacteria isolated from calf diarrhoea were highly sensitive to tetracycline, chloramphenical, streptomycin moderately sensitive to ampicillin, amoxicillin, less sensitive to penicillin gentamycin and kanamycin. The variation in the sensitivity of antibiotics of the faecal isolates may be due to the out come of choice and also the indiscriminate use of antibiotic in different disease stage to various species of animals.

Table 1. Characterization of isolated bacterial pathogens from diarrhoeic calves by Gram's staining technique

Gram's Staining			Identification
shape	Arrangement	Gram's staining reaction(+/-)	
Short plump rods	Single, paired or in short chain	Gram negative	*E.coli*
Very short plump rods	Single	Gram negative	*Salmonella* spp.
Cocci arranged	grape- like clusters	Gram-positive	*Staphlococcus* spp.

Table 2. Characterization of isolated bacterial pathogens by cultural properties

Name of Culture media used	Observation		
	E. Coli	***Salmonella* spp.**	**Staphyloccous spp.**
Nutrient agar	Smooth, circular, white to grayish colony with peculiar fetid odour	Small, round and smooth colony	growth of circular, small smooth, convex, and golden yellowish colonies
Blood agar	Produce haemolysis	Produce haemolysis	Produce haemolysis
Staphyloccous Agar no.10	No growth (-)	No growth (-)	Yellowish color colony
Mac Conkey agar	Rose pink lactose fermenter colony.	Colourless, pale, translucent colony.	No growth (-)
Eosin-Methylene Blue (EMB) agar	Moist circular colonies with dark centers yellow green metallic sheen	No growth (-)	No growth (-)
Salmonella- Shigella (SS) agar	Pink colour colony	Translucent colourless smooth colony	No growth (-)

Table 3. Characterization by biochemical reactions of *E. coli, Salmonella* spp. and *Staphylococcus* spp.

Isolated organism	Indole production test	Methyl-red test	Voges-Poskauer reaction	Citrate utilization test	MIU test	TSI Test	Hydrogen sulphide
E.coli	+	+	–	–	All +	Butt-Y Slant-Y	–
Salmonella spp.	–	+	–	–	+	Butt-Y Slant-R	+
Staphylococcus spp.	–	+	–	–	–	Butt-Y Slant-Y	+

Table 4. Frequency of distribution of positive *E. coli, Salmonella* spp. and *Staphylococcous* spp.

Total number of samples examined	Name of isolated bacteria	Total number of positive samples	Frequency of distribution (%)
114	*E. coli*	44	38.6%
	Salmonella spp.	25	21.9%
	Staphylococcus	15	13.2%
	Mixed infection involved	18	15.8.%
	Negative for bacteria	12	10.5%

Table 5. Results of antimicrobial susceptibility test of the isolated E. coli (n = 44).

Antimicrobial agent	No (%) of *E. coli*		
	Susceptible	Intermediate	Resistant
Penicillin	0 (0)	0 (0)	44 (100)
Ampicillin	0 (0)	0 (0)	44 (100)
Amoxycillin	0 (0)	0 (0)	44 (100)
Chloramphenicol	18 (40.90)	0 (0)	26 (59.10)
Erythromycin	25 (56.82)	0 (0)	19 (43.18)
Azithromycin	40 (90.90)	0 (0)	4 (9.10)
Gentamicin	38 (86.36)	0 (0)	6 (13.64)
Bacitracin	0 (0)	0 (0)	44 (100)
Ciprofloxacin	36 (81.82)	0 (0)	8 (18.18)

Table 6. Results of antimicrobial susceptibility test of the isolated *Salmonella* spp. (n = 25).

Antimicrobial agent	No (%) of *Salmonella* spp.		
	Susceptible	Intermediate	Resistant
Penicillin	0 (0)	0 (0)	25 (100)
Ampicillin	0 (0)	0 (0)	25 (100)
Amoxycillin	0 (0)	0 (0)	25 (100)
Chloramphenicol	6 (24)	0 (0)	19 (76)
Erythromycin	18 (72)	0 (0)	7 (28)
Azithromycin	21 (84)	2 (8)	2 (8)
Gentamicin	20 (80)	0 (0)	5 (20)
Bacitracin	0 (0)	0 (0)	25 (100)
Ciprofloxacin	19 (76)	1 (4)	5 (20)

Table 7. Results of antimicrobial susceptibility test of the isolated *Staphylococcus* spp. (n = 15).

Antimicrobial agent	No (%) of *Staphylococcus* spp.		
	Susceptible	Intermediate	Resistant
Penicillin	0 (0)	0 (0)	15 (100)
Ampicillin	0 (0)	0 (0)	15 (100)
Amoxycillin	0 (0)	0 (0)	15 (100)
Chloramphenicol	5 (33.33)	0 (0)	10 (66.67)
Erythromycin	11 (73.33)	0 (0)	4 (26.67)
Azithromycin	12 (80)	0 (0)	3 (20)
Gentamicin	14 (93.33)	0 (0)	1 (6.67)
Bacitracin	0 (0)	0 (0)	15 (100)
Ciprofloxacin	10 (66.67)	0 (0)	5 (33.33)

4. CONCLUSIONS

Cattle farming are emerging as a traditional small and large scale agro-based industry according to the demand of time. Due to the shortage of land and dense population, domestic cattle have the chance to become the carrier of *Salmonella*, *Staphylococcus* and *E. coli*, the common food borne pathogens for human beings. The findings of the present study indicate that the use of azithromycin, gentamicin and ciprofloxacin may have the preference to be choice in clinical control of *Salmonella, E. coli,* and *Staphylococcus* causing calf diarrhoea in Panchagarh district of Bangladesh.

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REFERENCES

Ahmad, R.; M. Amin and S.E. Kazmi (1986). Studied on the bacterial causes of calf mortality. Pak. Vet. J., 6:116-118.

Barrandeguy, M.E.; E.M. Cornaglia; M. Gottschalk; N. Fijtman; M.I. Pasim; A. Gomez-Yafa; J.R. Parraud and A.A. Schudel (1988). Rotavirus, enterotoxigenic *Escherichia coil* and other agents in the faeces of dairy calves with and without diarrhoea. Revisla Lalinoamericana-de' Microhiologia, 30: 239-245.

Bazeley, K. (2003). Investigation of diarrhoea in the neonatal calf. In Practice, 25: 152-159.

Bendali, F.; H. Bichet; F. Schelcher and M. Sanaa (1999). Pattern of diarrhoea in new born calves in South west France. Vet. Res., 30: 61-74.

Choudhury, K.A.; M.M. Amin; A.J. Sarker; M. Ali and A.R. Ahmad (1987). Immunization of chickens against fowl cholera with oil adjuvanated broth culture vaccine. Bangladesh Vet. J., 21: 63-73.

Cowan, S.T. (1985).Cowan and Steels manual for identification of medical bacteria, 2nd edition.

Clinical and Laboratory Standards Institute (2006). Performance standard for antimicrobial disk susceptibility testing. 16th Informational Supplement. M100-S16. Wayne, Pa.

Cygan, Z. and J. Buczek (1993). Non enterotoxigenic *Clostridium perfringens-A* as a cause of calf diarrhoea. Medycyna Weterynaryina, 49: 469-471.

David, R. and Smith (2007). Basic principles used in the "Sand hills calving system" & how they apply to other production environments. Proceedings, the range of beef cow symposium XX. Fort collins, colorado.

Debnath, N.C.; M.I. Huq and A. Rahman (1987). A microbial investigation of neonatal calf diarrhoea in Bangladesh. Indian J. Anim. Sci., 57 : 1035-1038.

Dubreuil, M.A. ; S.E. Ives; M.J. Engler and M.L. Looper (1991). *Escherichia coli* O157:H7 in cattle feces using a polymerase chain reaction-based fluorogenic 5' nuclease (TaqMan) detection assay after secondary enrichment. American J. Vet. Diagn. Invest., 6: 543 - 552.

Fouquet, G. (1979). Diarrhoea of newborn Charolais calves. Aetiology, pathogenesis, prophylaxis, treatment. Bulletin Mensuel-de-la-Sociele Veterinaire Pratiquede-France, 63: 589-607.

Genovese, K.J.; K.M. Bischoff ; J.L. McReynolds; R.C. Anderson and D.J. Nisbet (2004). Variation in the fecal shedding of *Salmonella* and *E. coli* 0157:H7 in lactating dairy cattle and examination of salmonella genotypes using pulsed-field gel electrophoresis. Lett. Appl. Microbiol., 38: 366-72.

Hemashenpagam, N.; B. Kiruthiga; T. Selvaraj and A. Panneerselvam, (2009). Isolation, Identification and Characterization of Bacterial pathogens causing Calf Diarrhea with special reference to *Escherichia coli*. The Internet Journal of Microbiology, 7(2), DOI: 10.5580/9c7

Hodna,T., M. Arita; Y.Taklea and T. Miwatani, (1982). Further evaluation of the Nimon test (Modified E leck test) for deletion of enterotoxigenic *E. coli* producing heat-labile enterotoxin and application of the test to sampling of heat-stable enterotoxin. J. Clin. Microbiol., 16: 60-62.

Hoque, M.S. and M.A. Samad (1996). Prevalence of clinical diseases in dairy Cross-bred cows and calves in the urban areas in Dhaka. Bangladesh Vet. J., 30: 118-129.

Joon, D.S. and Y. K. Kaura (1993). Isolation and characterization of same of the enterobacteria from diarrhoeic and non-diarrhoeic calves. Indian J. Anim. Sci., 63: 373-383.

Joshi, B.P.; J.Z. Pociecha and Y.A. Yousif (1986). Drug sensitivity pattern of organisms isolated from calf colibacillosis in Mosul (Iraq). Indian Vet. J., 63: 783-784.

Kaura, Y.K.; D.N. Bhargava; A.K. Pruth and S. Prasad (1988). Pathology and isolation of multiple antibiotic resistant strains of *E. coli* from an outbreak of colibacillosis in turkey poultry. Indian J. Poult. Sci., 23: 9-13.

Khan, A. and M.Z. Khan (1997). Bacteria isolated from natural cases of buffalo and bovine neonatal calf diarrhoea, pneumonia and pneumoenteritis. Vetermarski Arhiv, 67: 161-167.

Merchant, I. A. and R. A. Packer (1967). *Veterinary Bacteriology and Virology. Seventh edi.* The Iowa University Press, Ames, Iowa, USA, pp. 286-306.

Nazmul Hossain Nazir, K.H.M. (2007). Plasmid profile and antibiogram pattern of *E. coli* isolates of calves feces and diarrhegenic stool of infants. Journal of Bangladesh Society for Agricultural Science and Technology, 4:149-152.

OIE (Office International Des Epizooties) (2000). Mannual of standards for diagnostics test and vaccines. OIE Guide-2.

Oporto, B.; J.I. Esteban; G. Aduriz; R.A. Juste and A. Hurtado (2008). *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses Public Health, 52: 411- 550.

Pandey, P.N.; D.C. Thaphyal and S.N. Sharma (1979). Enterotoxigenicity of some *Escherichia coli* isolates. Indian J. Anim. Research, 13: 1-4.

Panwar, B.S.; N.S. Dhanesar and K.N.P. Rao (1990). Serotypes and antibiogram of phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* O111 isolates. The Journal of Antimicrobial Chemotherapy, 57:1210-4.

Radostits, O.M.; D.C. Blood and G.C. Gay (2000). Veterinary Medicine: *A Text Book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. 8th Edn. W.B. Saunders Company, Philadelphia, London.

Rajeswari-Shome, R. R. Shome and R. Shome (1996). Mannose sensitive and mannose resistant haemagglutination of *E. coli* isolated from calf diarrhoea cases in Andamans. Indian Vet. J., 73: 998-1000.

Tripathi, R.D. and J.L. Soni (1982). Antibiotic sentivity test with *E. coli* isolates from cases of neonatal calf diarrhoea (NCD). Indian Vet. J., 59:413-416.

Valdivia- Ad, G.; Cervantes- Rosales; D.M. Soriano-becerrili; F. Alba-Hurtado; J.A. Montaraz-Crespo and J.L. Tortora- Prez (2000). Interaction of *E. coli* verocytotoxin strains and rota virus in out break of calf's diarrhoea. Veterinaria Mexico, 31: 293- 300.

Zaman, S.; U.K. Chattopadhyay; D. Hattacharya; S.C. Das and A.A. Sikdar (2000). A study on bacterial calf diarrhoea in an organised farm. Journal of Interacademicia, 4:141-144.