EFFICACY OF CIPROFLOXACIN AND ENROFLOXACIN IN THE TREATMENT OF A RESPIRATORY PASTEURELLOSIS OUTBREAK IN NEW ZEALAND RABBITS

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ABSTRACT

Pasteurelosis is an important economic disease in rabbit production. The objective of this study was to determine the individual therapeutic efficacy of fluoroquinolones such as ciprofloxacin and enrofloxacin on *Pasteurella multocida* in one outbreak of respiratory pasteurellosis in a New Zealand rabbit farm in central Mexico. The drugs were administrated subcutaneously during three days in animal groups: A ciprofloxacin 20/mg/kg b. wt.; B enrofloxacin 10/mg/k b. wt.; C receiving a placebo 1 mL/k b. wt.; D untreated animals. The efficacy was evaluated as disease recovery and mortality reduction after treatment. *Pasteurella multocida* A3 was isolated from clinical cases and postmortem examination from acute or chronic lesions. The rabbit survival in groups A and C was higher with low mortality compared with untreated animals in which the clinical sings were persistent. Pathological findings related to the infection involved abscedative pneumonia and pleuritis and extrapulmonary lesions such as otitis, meningoencephalitis and subcutaneous abscesses. Our results indicate that ciprofloxacin and enrofloxacin are efficient as medical treatment of acute respiratory pasteurellosis in young rabbits, reducing the clinical cases and lesion severity and increasing survival in animals.

Key words: rabbits, pasteurellosis, ciprofloxacin , enrofloxacin, pathological findings.

INTRODUCTION

Pasteurella multocida is an important bacterial pathogen in rabbit production. Outbreaks are caused by environmental changes, stress or it is associated to other illnesses. *Bordetella bronchiseptica, Staphylococcus aureus* and *Streptococci* are commonly associated to *P. multocida* infection (GOLDSTEIN *et al.,* 1997).

The adherence to respiratory epithelium and the ability to resist phagocytosis as well as intracellular killing by neutrophils may be present in serotypes including 12:A, 3:D, and 3:A. The degeneration of the nasal turbinates has been associated with toxin-production in rabbits infected with capsular types D and A (DIGIACOMO *et al.,*1989; DEEB *et al.,*1990).

Pasteurelosis is a persistent disease in most rabbit hutches. The clinical cases in animal population occurs in acute and chronic presentations associated to septicaemia, pneumonia, meningoencephalitis, otitis, reproductive failures and located abscesses (DIGIACOMO *et al.,*1989; KUNSTYR and NAUMANN, 1985; SUCKOW *et al.,* 1991;RICHARDSON *et al.,* 1997).

Antibiotics treatment in affected animals reduce the number of clinical cases as well as the economic impact (LION *et al.,* 1996). Antibacterial activity of fluoroquinolones have shown a broad spectrum of activity against gramnegative and grampositive bacteria. The post-antibiotic effect is considered as an additional therapeutic benefit (SPRENG *et al.,* 1995). *In vitro* sensitivity tests indicate the activity of ciprofloxacin and enrofloxacin on *Pasteurella multocida* (GOLDSTEIN *et al.,* 1997 ; HANAN *et al.*, 2000).

The objective of this study was to determine the individual therapeutic effect of two fluoroquinolones, ciprofloxacin and enrofloxacin on *Pasteurella multocida* in one outbreak of Pasteurellosis occurred in a New Zealand rabbit farm in central Mexico.

MATERIAL AND METHODS

Animal farm

During the dry season in winter time (November 2002), in the Northern region of the state of Mexico, a commercial breeder New Zealand rabbit hutch located 19º47´27´´ North latitude was highly affected by *Pasteurella multocida* infection in an acute respiratory form, with a morbidity rate of 85% estimated from a 3700 total animal population.

200 rabbits were randomly selected in the same proportion of females and males aging ten to twelve weeks. They were not medicated animals which suffered the clinical disease characterized by fever, eyes redness, conjunctivae secretion with predominant respiratory signs; sneezing, coughing, nasal discharge as well as acute pneumonia manifestations.

Experimental groups

The experimental clinically affected animals, were distributed in four groups of 50 animals each from the husbandry and maintained in isolated conditions out of the farm. The treatment was given with two fluoroquinolones subcutaneously administrated for three days with the following treatments: group A ciprofloxacin* (CPX) 20/mg/kg b. wt (**Primecin, Lapisa, México)*., group B enrofloxacin**(ERF) 10/mg/k b.wt.(** *Piroflox, Pisa Agropecuaria, México)* , group C receiving a placebo physiologic saline solution (PSS) 1 /mL/k b. wt., and the fourth group D was affected but not treated during the experimental period. After the drug medication period (seven days later) ten rabbits of each group were euthanized and sampled for bacteriology and pathological routinary procedures. The rest of the animals were observed for twenty one days until the remission of clinical signs.

Laboratory procedures and clinical findings

The postmortem examination was conducted by evaluation of the severity lesions as well as the lungs distribution and other lesions in organs. The pathologic category in lungs were considered as follows: C0 none macroscopic findings of pneumonia lesions; C1 light lesions occurring in < 10% of the lung with a non progressive process; C2 15 to 25 % affected lung surface; C3 > 30% lung area including other thoracic lesions and/or extra pulmonary affections. Bacteriological diagnosis of the necropsy cases were made from samples in affected organs by inoculating blood agar slops and culturing at 37ºC for 18 to 24 h.

The isolates of *Pasteurella multocida* were identified by Gram staining, biochemical tests, motility and haemolytic activity. The clinical signs and mortality were registered from all the groups.

Statistical analysis

The data were analyzed using the estimation proportions test, considering the observations derived from the survival of the animal groups, clinical signs and pathological findings as limit value ($P<0.05$) (DANIEL, 1984).

RESULTS AND DISCUSSION

Table 1 shows the animal recovery after ciprofloxacin and enrofloxacin treatment. The survival in both groups was higher compared with the untreated animals, in which mortality was proportionally increased ($P<0.05$). It is possible that the clinical results obtained using ciprofloxacin are related to high drug concentrations in tissues reported in kidneys, lung, spleen, liver, and muscle during the trial (HANAN *et al.,* 2000), MIC at which 90% of isolates are inhibited [MIC90] marbofloxacin and ciprofloxacin is 0.5 µg/mL in *Pasteurella* species and other bacteria of canine-feline isolates (SPRENG *et al.,*1995).

Table 1. ***Ciprofloxacin And Enrofloxacin Efficacy Treatment In Animal Recovery***

Clinical condition	*Ciprofloxacin*	*Enrofloxacin*	*Placebo*	*Non Medicated*
% Affected	*100*	*100*	*100*	*100*
% Recovery	*95*	*95*	*32.5*	*27.5*
% Deaths	*5*	*5*	*67.5*	*72.5*

Similar reduction of clinical signs were observed 72 h after parenteral treatment formulation of enrofloxacin in naturally occurring *Pasteurella multocida* (BROOME and BROOKS, 1991). The experimental treatment for enrofloxacin during the periparturient period in rabbit mothers limited the nasal carriage stage of *Pasteurella multocida* ($P<0.05$) (SUCKOW *et al.,* 1996). Low dosage administration of enrofloxacin (5 mg/kg subcutaneously) failed to eliminate the infection from nasal cavities, turbinates, trachea, middle ear and outer ear in naturally and experimentally infected rabbits (MAHLER *et al.,* 1995). In this clinical study fewer cases of subcutaneous abscesses were observed in enrofloxacin treated animals.

The macrolide antibiotics is an alternative in emergency cases in which bacterial antibiotic resistance occurs in the upper and lower respiratory tract diseases, as well as in the same infection in man following animal bites (LION *et al.,* 1996; GOLDSTEIN *et al.,* 1997). Experimental *Pasteurella multocida* infection studies on drug treatment in rabbits may be conducted in an adapted animal model (PAUSEN *et al.,*1992).
Clinical signs of the treated and untreated diseased rabbits are shown in Table 2. Dyspnea, seromucous nasal discharge and cough were the predominant clinical manifestations. This signs are commonly observed in acute pasteurelosis outbreaks.
Other progressive neurological and torticollis may be present associated to otitis, acute meningoencephalomyelitis in chronically infected animals with *Pasteurella multocida* (KUNSTYR and NAUMANN, 1985; KUNSTYR and NAUMANN, 1985) some of these cases could be related with pasteurellosis and encephalitozoonosis (MURRAY *et al.,* 1985).

Table 2. *Predominant Clinical Signs In Diseased Animals Groups*

Signs	***%***
Red eyes	*25*
Sneeze	*40*
Cough	*15*
Nasal discharges/ mucous	*50*
Dispnea	*75*
Cianosis	*8*
Torticolis	*1*
Neurological	*5*
Orquitis	*1*
Nasal purulent discharge	*35*
Subcutaneous abscess	*9*

Pathological findings are shown in Table 3.and 4. All treated and untreated animal groups, number of deaths, conjunctivitis, rhinitis, bronchopneumoniae were common alterations present in the acute form of the disease. C1 and C2 pathology categories were observed (DEEB *et al., 1990*). Chronical lesions in affected rabbits were mostly observed in non medicated animals as a fibrinopurulent pneumonia, otitis, meninghoencephalitis and subcutaneouos abcesesses as a C3 category in the postmortem study (KUNSTYR and NAUMANN, 1985; MURRAY *et al.,* 1985).
About 30 % had concurrent infection of the paranasal sinuses, 52% had infection of the bronchi and lungs. All isolated strains were biochemically and serologically identified as *Pasteurella multocida type 3A* considered as a widely distributed infection in rabbit population (DABO *et al.,*1999; HANAN *et al.,* 2000).

CONCLUSIONS

In conclusion, our results indicate that the therapeutic effect of fluoroquinolones such as ciprofloxacin and enrofloxacin for the treatment of *Pasteurella multocida* acute infection, is effective in reducing the clinical cases of pasteurellosis in young rabbits and prevents

the chronic evolution of the disease related to respiratory, torticolis and neurological cases.

Table 3. ***Pathological Findings In Dead Animals***

LESIONS	*%*
Non supurative Conjunctivitis	*38.3*
Supurative Conjunctivitis	*61.6*
Serous Rhinitis	*61.6*
Supurative Rhinitis	*38.3*
Bronchopneumoniae	*33.3*
Pleuritis	*30*
Fibrinopurulent pneumoníae	*63.3*
Oedema	*11.6*
Hemorrage	*5*
Pyothorax	*3.3*
Meningoencephalitis	*3.3*
Supurative otitis	*3.3*
Subcutaneous Abscess	*31.6*
Lung Abscess	*53.3*

Table.4 ***Lung Gross Pathology In Dead Animals (n = 50)***

Categories	*%*
C0	*11.7*
C1	*10.0*
C2	*23.3*
C3	*55.0*

REFERENCES

BROOME RL., BROOKS DL., 1991.Efficacy of enrofloxacin in the treatment of respiratory pasteurellosis in rabbits. *Lab Anim Sci.,41(6):572-576.*

CHU DT., 1999. Recent progress in novel macrolides, quinolones, and 2-pyridones to overcome bacterial resistance. *Med Res Rev. 19(6):497-520.*

DABO SM., CONFER AW., MONTELONGO M., LU YS., 1999.Characterization of rabbit Pasteurella multocida isolates by use of whole-cell, outer-membrane, and polymerase chain reaction typing. *Lab-Anim-Sci. 49(5): 551-559*

DANIEL, WW. 1984. Bioestadística. Limusa. México.

DEEB BJ., DIGIACOMO RF., BERNARD, BL; SILBERNAGEL, SM.1990. January

Pasteurella multocida and Bordetella bronchiseptica infections in rabbits *J Clin Microbiol. 28(1):70-75*

DIGIACOMO R.F., DEEB BJ., GIDDENS WE JR., BERNARD BL., CHENGAPPA MM., 1989. Atrophic rhinitis in New Zealand rabbits infected with Pasteurella multocida. Am *J veterinarian Res. 50:1460-1465.*

GOLDSTEIN EJ., CITRON DM., HUNT GERARDO S., HUDSPETH M., MERRIAM CV., 1997. Comparative in vitro activities of DU-6859a, levofloxacin, ofloxacin, sparfloxacin,

and ciprofloxacin against 387 aerobic and anaerobic bite wound isolates. *Antimicrob Agents Chemother. 41(5):1193-1195.*

HANAN MS., RIAD EM., EL-KHOULY NA., 2000. Antibacterial efficacy and pharmacokinetic studies of ciprofloxacin on Pasteurella multocida infected rabbits. *Dtsch Tierarztl Wochenschr. 107(4):151-155.*

KUNSTYR I., NAUMANN S., 1985. Head tilt in rabbits caused by pasteurellosis and encephalitozoonosis. *Lab Anim 19:208-213.*

LION C., LOZNIEWSKI A., CONROY MC., WEBER M,,1996. In vitro susceptibility of Pasteurella and related bacteria to five orally administered antibiotics. *Pathol Biol (Paris). 44(5):341-346.*

MURRAY KA.; HOBBS, BA; GRIFFITH, JW. 1985. Acute meningoencephalomyelitis in a rabbit infected with Pasteurella multocida*. Lab Anim Sci 35 (2):169-171.*

MAHLER M., STUNKEL S., ZIEGOWSKI C., KUNSTYR I., 1995. Inefficacy of enrofloxacin in the elimination of Pasteurella multocida in rabbits. *Lab Anim. 29(2):192-199.*

PAULSEN DB., CORSTVET RE., MCCLURE JR., ENRIGHT FM., MCBRIDE JW., MCDONOUGH KC.,1992. Use of an indwelling bronchial catheter model of bovine pneumonic pasteurellosis for evaluation of therapeutic efficacy of various compounds. *Am J Vet Res.53(5):679-83.*

RICHARDSON M., DE RESKE M., DELANEY K., FLETCH A., WILCOX LH., KINLOUGH-RATHBONE RL., 1997. Respiratory infection in lipid-fed rabbits enhances sudanophilia and the expression of VCAM-1. *Am J Pathol. 151(4):1009-1017.*

SUCKOW M.A., CHRISP C.E., FOGED NT., 1991. Heat-labile toxin-producing isolates of Pasteurella multocida from rabbits. *Lab Anim Sci. 41:151-156.*

SUCKOW MA., MARTIN BJ., BOWERSOCK TL., DOUGLAS FA., 1996. Derivation of Pasteurella multocida-free rabbit litters by enrofloxacin treatment.*Vet Microbiol. 51(1-2):161-168.*

SPRENG M., DELEFORGE J., THOMAS V., BOISRAME B., DRUGEON H., 1995. Antibacterial activity of marbofloxacin. A new fluoroquinolone for veterinary use against canine and feline isolates. J Vet Pharmacol Ther. 18(4):284-289.