Global Veterinaria 3 (5): 363-368, 2009
ISSN 1992-6197

Tissue and Egg Residues and Adverse Effect of Two Oral Enrofloxacin Preparations; Baytril® and Enrotryl®

[1]H.M. Elkholy, [1]A.A. Elkomy, [2]S.K. Awidat and [2]A.A. Elmajdoub

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Al-Fateh University, Libya

Abstract: The comparative blood, egg and tissue residues as well as the adverse effects of enrofloxacin represented in Enrotryl® and Baytril® were investigated in laying hens following repeated oral administration at a dose rate 10 mg enrofloxacin base /kg b. wt., once daily for 5 consecutive days. Enrofloxacin in both products was microbiologically assayed in blood, eggs and tissues using *Bacillus cereus* as a test organism. It was completely cleared from blood, egg and all tissue tested on the 5th day after the last oral dose. The effect of enrofloxacin in the two tested preparations on some blood parameters (glucose, cholesterol, total proteins, urea, creatinine) and blood picture (Hb, PCV%, RBCs and WBCs counts) revealed insignificant changes after discontinuation of drug regimen. From this study it was concluded that the withdrawal time of enrofloxacin in Enrotryl® and Baytril® in eggs and tissues of laying hens was 5 days and that repeated administration of enrofloxacin in the form of Enrotryl® and Baytril® did not induce any adverse effect when used in a therapeutic regimen.

Key words: Quinolones · Fluoroquinolones · Enrofloxacin · Chickens

INTRODUCTION

Enrofloxacin is a abroad-spectrum antibiotic that belongs to the fluoroquinolones family of drugs, which has been developed exclusively for use in animals [1]. Enrofloxacin is bactericidal and has excellent activity against both Gram-positive and Gram-negative pathogens. This antibiotic has also been used to control certain intracellular pathogens [2, 3]. The mode of action of enrofloxacin is by inhibition of DNA gyrase enzyme. Enrofloxacin produces a comprehensive control against infections of respiratory, urogenital and digestive systems. Enrofloxacin is rapidly absorbed and distributed in all body organs, so it has a rapid bactericidal activity against Gram-positive and Gram-negative bacteria including *Staphylococci, Haemophilus, Pasteurella, Streptococci, Salmonella, E.coli*….. etc. Also, it is highly effective against chronic respiratory diseases (CRD) and canine contagious respiratory diseases (CCRD) caused by *Mycoplasma spp* in poultry and canines [4].

According to the Food and Marketing Institute Report [5], food safety has become one of the most visible and emotional issues confronting affluent societies. In a national survey, they found that the first concern of consumers pertained to residues in meat. Health-related issues, such as cholesterol and saturated fat content, are perceived by the public as less threatening than chemical residues. There is a risk that residues of hypersensitivity-inducing drugs may elicit hypersensitivity in human consumers of food of animal origin. The residual levels present in food are unlikely to be sufficient to cause initial sensitization. Levels that would illicit sensitization in human are most likely to occur by therapeutic use of these substances. However, these levels may occasionally elicit hypersensitivity in previously sensitized patients. The available data suggest that incidences of such reactions are exceedingly low, and the risk can be minimized by the careful use and observance of sufficiently long withdrawal periods of substances fed to livestock [6, 7].

The objectives of this study were to determine the egg and tissue residues and the possible adverse effects induced by enrofloxacin in Enrotryl® and Baytril® in laying hens after multiple dosage regimen of 10 mg enrofloxacin base / kg b. wt.

MATERIALS AND METHODS

Drugs: Enrotryl® (The Egyptian Co. for Chemicals & Pharmaceuticals ADWIA-Egypt) and Baytril® (Bayer Corp. Germany) are formulated as oral solution in one liter plastic bottles. Each one ml contains 100 mg enrofloxacin base.

Corresponding Author: H.M. Elkholy, Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt

Hens and Experimental Design: Forty six single comb white leghorn laying hens (18-20 months of age) were used in this study. They were kept individually in ventilated cages, room temperature (20°C) and 14 hours of day light. They received a standard commercial layer feed free from any medications and water ad libitum.

Eggs and Tissue Residues Study: Thirty six hens were used to study the egg and tissue residues of Enrotryl® and Baytril®. Hens were divided into two equal groups (n=18). The 1st group was administered Enrotryl® and 2nd group was administered Baytril® at a dose equivalent to 10 mg enrofloxacin base/ kg b. wt., once daily for 5 consecutive days regimen. A subgroup of 3 hens each was euthanized every day for six days after the end of medication. Eggs were taken daily before slaughtering the hens. Blood and tissue samples (lung, liver, kidney, muscle, ovary and oviduct) were taken from each slaughtered hen 24 hours after the last treatment and daily for 6 days for both drugs.

Adverse Effect Study: Ten hens were used to investigate the effect of the tested drugs on some blood picture, constituents and some enzymatic activities. Hens were divided into two equal groups (G3 and G4, 5 hens each). The third group (G3) and fourth group (G4) were administered Enrotryl® or Baytril® at a dose equivalent to 10 mg enrofloxacin base /kg b. wt. once daily for 5 days.

Analytical Methods: Enrofloxacin was assayed in the blood and tissue samples by a microbiological method using *Bacillus cereus* as a test organism. The preparation of microbial suspension of *Bacillus cereus* was carried out according to Arret *et al.* [8]. For tissue extraction of enrofloxacin, 2 grams of tissue were crushed in a porcelain dish with 2 milliliters of distilled water, samples were centrifuged at 3000 r.p.m. for 5 minutes. Serum and tissue supernatant were directly microbiologically assayed as follows: three plates were used for each sample. Three wells on each plate were filled with the reference concentration. The other three wells filled with the sample (serum or tissue supernatant). The plates were incubated at 37°C for18 hours. The diameters of the inhibition zones were then measured by triplicate manner. Average reading of the tested concentration and the reference concentration were calculated. Average of the whole readings of the reference concentration was considered the correlation point to correct the average value obtained from each tested concentration. The increase in values of the average reference determinations in set of three plates than the whole reference determinations were added to the average determination of the tested concentration. On the other hand, the decrease in values of average reference determination in set of three plates than the average of the whole reference determinations were subtracted from the average determinations of the tested concentration. From the standard curve, the concentrations corresponding to the corrected values of the zone diameter were obtained.

Haematological Examination and Biochemical Analysis: Two blood samples were collected from each hen (G3 and G4) before and on days 1, 5, 10, and 15 after the last treatment. One blood sample was collected in tubes containing EDTA as anticoagulant for determination of RBCs and WBCs counts [9], haemoglobin concentration [10] and packed cell volume; PCV% [11]. The other blood sample was collected in a clean tube and left to clot, centrifuged and the obtained clear serum analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities [12], alkaline phosphatase (ALP) [13], glucose [14], cholesterol [15], total proteins [16], urea [17] and creatinine [18].

The results are represented as mean ±S.E. Statistical significance was determined by Student's (t) test for paired observations [19].

RESULTS

Blood and tissue residues following oral administration of Enrotryl® and Baytril ®at a dose equivalent to 10 mg enrofloxacin base /kg b. wt. of laying hens, once daily for 5 consecutive days are recorded in Tables 1 and 2. Enrofloxacin could be detected in the lung, liver and kidneys till 4 days after the last dose. The higher drug residue levels were detected in the liver and kidney.

The effect of repeated oral administrations of Enrotryl® and Baytril® on some enzymatic activities (ALT, AST and ALP) and some blood constituents (glucose, cholesterol, total protein , urea and ctreatinine) are tabulated (Tables 3 and 4) .

The reported data revealed that Enrotryl® and Baytril® produced non-significant alterations in blood picture (Hb, PCV, RBCs and WBCs counts) of laying hens following repeated oral administrations (Tables 5 and 6).

Table 1: Blood (µg ml^{-1}), egg and tissue (µg g^{-1}) residues of enrofloxacin in Enrotryl® in laying hens following repeated oral administration of 10 mg enrofloxacin base /kg b. wt. once daily for 5 consecutive days (n=3 at each slaughter)

Blood, egg and tissue concentration	Time of slaughter after the last dose (X±S.E.)					
	1st day	2nd day	3rd day	4th day	5th day	6th day
Blood	5.00±0.29	2.60±0.09	0.85±0.01	0.00	0.00	0.00
Lung	17.50±1.11	11.30±1.96	4.50±0.73	1.09±0.02	0.00	0.00
Liver	42.80±2.32	20.00±2.22	6.50±0.89	2.00±0.04	0.00	0.00
Kidney	35.60±2.40	16.30±2.01	4.40±0.56	0.90±0.03	0.00	0.00
Muscle	16.70±1.96	4.90±0.98	1.05±0.01	0.00	0.00	0.00
Ovary	10.80±1.23	3.60±0.31	0.70±0.01	0.00	0.00	0.00
Oviduct	11.90±1.79	5.30±0.99	1.20±0.06	0.00	0.00	0.00
Egg albumin	13.0±2.11	4.20±0.08	0.80±0.01	0.00	0.00	0.00
Egg yolk	8.50±1.01	2.50±0.69	0.25±0.00	0.00	0.00	0.00

Table 2: Blood (µg ml^{-1}), egg and tissue (µg g^{-1}) residues of enrofloxacin in Baytril® in laying hens following repeated oral administration of 10 mg enrofloxacin base /kg b. wt. once daily for 5 consecutive days (n=3 at each slaughter)

Blood, egg And tissue concentration	Time of slaughter after the last dose (X±S.E.)					
	1st day	2nd day	3rd day	4th day	5th day	6th day
Blood	5.70±2.09	1.90±	0.20±0.00	0.00	0.00	0.00
Lung	20.00±3.84	9.40±1.78	2.50±0.09	0.95±0.03	0.00	0.00
Liver	37.30±2.99	14.30±1.99	3.20±0.92	1.05±0.03	0.00	0.00
Kidney	3.90±0.10	12.20±3.00	4.40±0.99	0.85±0.01	0.00	0.00
Muscle	15.30±1.99	6.30±1.90	2.40±0.36	0.60±0.00	0.00	0.00
Ovary	9.50±2.11	2.40±0.71	0.50±0.01	0.00	0.00	0.00
Oviduct	10.00±1.78	3.00±0.62	0.70±0.02	0.00	0.00	0.00
Egg albumin	14.60±2.39	6.50±1.04	1.20±0.09	0.00	0.00	0.00
Egg yolk	9.40±2.00	2.60±0.08	0.50±0.02	0.00	0.00	0.00

Table 3: Effect of enrofloxacin in Enrotryl® on some enzyme activities and blood constituents of laying hens after repeated oral administration of 10 mg enrofloxacin base /kg b. wt. once daily for 5 consecutive days (n=5)

Parameters (Unit)	Before administration	Time after the last dose (X±S.E.)			
		1st day	5th day	10th day	15th day
ALT (u/ml)	80.60±5.65	77.50±6.59	82.30±6.32	79.30±5.96	81.40±3.22
AST (u/ml)	165.00±7.90	166.00±13.04	165.50±10.66	159.20±9.88	164.30±8.49
ALP (mu/ml)	8.65±1.98	10.00±2.03	9.60±1.79	8.70±2.01	8.50±1.49
Glucose (mg/100 ml)	155.50±8.00	157.00±9.88	157.50±9.87	159.20±11.11	156.50±11.05
Cholesterol (mg/100 ml)	157.80±11.12	160.30±12.17	159.40±10.90	156.00±14.01	155.50±9.56
Total protein (g/100 ml)	17.30±3.12	19.50±2.08	18.30±3.28	17.80±3.21	19.30±1.80
Urea (g/L)	30.40±5.90	29.70±2.19	29.20±2.98	32.00±4.12	30.50±4.11
Creatinine (mg/L)	1.05±0.07	0.89±0.01	0.95±0.17	1.10±0.27	0.95±0.21

Table 4: Effect of enrofloxacin in Baytril® on some enzyme activities and blood constituents of laying hens after repeated oral administration of 10 mg enrofloxacin base /kg b. wt. once daily for 5 consecutive days (n=5)

Parameters (Unit)	Before administration	Time after the last dose (X±S.E.)			
		1st day	5th day	10th day	15th day
ALT (u/ml)	76.70±4.09	77.30±4.34	75.30±5.15	75.90±6.00	78.70±4.19
AST (u/ml)	155.50±16.19	157.90±12.17	15.50±2.01	156.20±9.69	155.30±8.19
ALP (mu/ml)	10.40±2.98	9.80±1.98	11.30±1.65	11.20±1.60	9.90±1.90
Glucose (mg/100 ml)	149.50±12.56	150.50±9.9.8414	153.20±10.45	148.30±9.98	150.50±8.89
Cholesterol (mg/100 ml)	155.00±11.32	156.20±10.90	155.70±12.23	153.60±10.00	155.80±10.03
Total protein (g/100 ml)	18.60±2.67	20.30±2.12	19.50±2.99	19.30±3.12	18.20±2.93
Urea (g/L)	27.30±3.33	28.00±2.64	27.60±2.92	26.90±3.10	25.30±2.89
Creatinine (mg/L)	0.90±0.03	1.05±0.02	0.95±0.03	0.89±0.03	1.07±0.06

Table 5: Effect of enrofloxacin in Enrotryl® on blood picture of laying hens after repeated oral administration of 10 mg enrofloxacin base /kg b. wt. once daily for 5 consecutive days (n=5)

		Time after the last dose (X±S.E.)			
Parameters (unit)	Before administration	1st day	5th day	10th day	15th day
Hb (g%)	15.60±1.06	16.20±1.79	16.10±1.70	16.00±2.10	15.70±1.91
PCV%	30.30±2.08	29.40±2.90	29.00±2.50	31.00±4.01	30.80±3.90
RBCs (x10^6)	2.60±0.12	2.75±0.18	2.50±0.20	2.65±0.18	2.62±0.19
WBCs (x10^6)	21.00±1.95	20.50±2.22	21.40±1.90	20.50±2.12	19.60±1.69

Table 6: Effect of enrofloxacin in Baytril® on blood picture of laying hens after repeated oral administration of 10 mg. enrofloxacin base /kg b. wt. once daily for 5 consecutive days (n=5)

		Time after the last dose (X±S.E.)			
Parameters (unit)	Before administration	1st day	5th day	10th day	15th day
Hb (g%)	16.00±2.05	17.20±2.12	16.75±1.99	15.90±1.96	16.30±1.33
PCV%	28.80±2.24	30.00±2.98	27.60±3.33	28.20±3.54	28.00±3.03
RBCs (x10^6)	3.00±0.12	2.60±0.09	2.70±0.08	3.20±0.13	3.20±0.19
WBCs (x10^6)	20.50±3.06	19.70±1.99	22.10±3.00	21.30±2.02	22.00±3.00

DISCUSSION

Enrofloxacine is an antibacterial that is widely used as a veterinary drug or as a feed additive to promote growth. Some studies have introduced pharmacokinetic data for eggs or tissues in laying hens [20-22]. Following repeated oral administration of enrofloxacin in the form of Enrotryl® and Baytril® to laying hens the drug could be detected in some body tissues till 4 days after the last dose. The higher drug residues were detected in the liver and kidney like those recorded for other antibacterials [23, 24].

Mycoplasma spp is of considerable importance in veterinary practice. For poultry, the predominant mycoplasmal pathogens are *M. gallisepticum, M. synoviae M. iawa.* and *M. gallisepticum* infection commonly induce chronic respiratory disease in chickens, CRD [25]. The clinical signs of CRD include nasal discharge, coughing, sneezing, tracheal rales and mild conjunctivitis. Turkeys are more susceptible than chickens and often develop moderate sinusitis. *M. synoviae* most frequently occurs as a subclinical upper respiratory infection but may result in air sacculitis and synovitis in chickens and turkeys [26]. *M. iawae* infection is associated with reduced hatchability in turkeys, and has been shown to induce experimentally mild to moderate air sacculitis and leg lesions in chickens and turkeys [27].

The control of *Mycoplasma* infections by vaccination is limited because only a few vaccines are available. Control of these infections by chemotherapy is sometimes necessary in complement of bio-security measures to minimize economic losses and lateral and vertical transmission. Many antimicrobial agents, such as macrolides, pleuromutilins, tetracyclines and fluoroquinolones have been shown to possess *in vitro* activity against various veterinary *Mycoplasmas* [28, 29] however, increasing resistance of the *Mycoplasmas* against tetracyclines [29, 30], macrolides [28-32] and quinolones [33, 34] has been reported in animal species and human.

In the present study, blood, lung and ovary enrofloxacin levels exceeded the MIC against most Mycoplasma spp. affecting poultry (MIC = 0.06 μg ml^{-1}) as recorded by Kleven [26].

The eggs and tissue residue disposition profiles for the antibacterials are influenced by structural and physiochemical conditions of the animal in use [23, 35, 36]. It has been proposed that the degree of lipid solubility and extent of ionization of enrofloxacin were distinguishing factor in describing their pharmacokinetic and disposition characteristics [3], that explains its high disposition characteristics of enrofloxacin in the ovary and oviduct due to its high distribution level in the egg yolk. The study indicated that 5 days for withdrawal of enrofloxain are adequate to get the tissues lower than the maximum residue limits of 100, 200 and 300 μg kg^{-1} for muscle, liver and kidney respectively [37].

In the present study, it has been shown that administration of Enrotryl® and Baytril® at a dose equivalent to 10 mg enrofloxacin /kg b. wt daily for five successive days, into hens induced insignificant changes of serum ALT, AST and ALP activities, and serum glucose, cholesterol and creatinine levels.

Measurement of serum creatinine levels yields the same diagnostic information concerning renal function as that obtained by the measurement of urea.

The effect of Enrotryl® and Baytril® on blood picture (Hb, PCV%, RBCs, and WBCs) of laying hens following repeated oral administration at a dose equivalent to 10 mg enrofloxacin base/ kg b. wt. for 5 days caused insignificant changes.

Conclusively, the obtained results suggests that the withdrawal times for Enrotryl® and Baytril® in eggs and tissues were 5 days . Thus chicken must not be slaughtered for human consumption for a period of at least 5 days after the last treatment with Enrotryl® and Baytril®. The repeated oral administrations of Enrotryl® and Baytril® in a dose level of 10 mg enrofloxacin base / kg b. wt. once daily for 5 days not affect liver or kidney functions and had no effect on blood constituents and blood pictures.Blood and tissue levels exceeded the MIC against common *Mycoplasma spp* affecting chickens and turkeys; a factor resulting in, Enrofloxacin is the drug of choice for treatment of avian Mycoplasmosis (CRD-Air sacculitis).

REFERENCES

1. Mitchel, M.A., 2006. Enrofloxacin. Journal of Exotic Pet Medicine, 15: 66-69.
2. Hooper, D. and J. Wolfson, 1985. The fluoroquinolones: structures, mechanisms of action and resistance and spectra of activity *in vitro*. Antimicrobial Agents Chemotherapy, 28: 581-586.
3. Vancutsem, P.M., J.G. Babish and W.S. Schwark, 1990. The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. The Cornell Veterinarian, 80: 173-186.
4. Wagman, A.S. and M.P. Wentland, 2007. Quinolone antibacterial agents. Comprehensive Medicinal Chemistry II, Chapter 7, pp: 567-596 .
5. Food Marketing Institute, 1988. Food Marketing Institute Report: Pesticide residues in food: Technologies for detection. Consumer attitudes and supermarket updates. Food Marketing Institute, Washington, DC.
6. Takabatake, E., 1981. Feed additives and drugs for animal use. Journal of Hygienic Chemistry (Eisei Kagaku), 27: 127-143.
7. Woodward, K.N., 1991. Hypersensitivity in humans and exposure to veterinary drugs. Veterinary and Human Toxicology, 33: 168-172.
8. Arret, B., E.P. Johnson and A. Kirshbaum, 1971. Outline of details for microbiological assay of antibacterial, second revision. Journal of Pharmacological Sciences, 60: 1690-1694.
9. Nutt, M.P. and C.A. Herrick, 1952. A new blood diluent for counting of erythrocytes and leucocytes of the chicken. Poultry Science, 34: 360-368.
10. Crosby, W.H., J.I. Munn and F.W. Fruth, 1954. Standardizing a method for clinical hemoglobinmetry. United States Armed Forces Medical Journal, 5: 693-703.
11. Schalm, O.W., N.C. Jain and E.J. Carroll, 1975. Veterinary Haematology, 3rd Ed. Lea and Febiger, Philadelphia, USA., pp: 42.
12. Reitmann, S. and S. Frankel, 1957. Colorimetric method for the determination of serum Gultamic and pyruvic transaminases activity. American Journal of Clinical Pathology, 28: 56-58.
13. Kilching, H. and B. Freiburg, 1951. Inorganic phosphorus and alkaline phosphatase in serum. International Clinical Photometry 3rd Ed. Wiss Verl. Ges. mbh Stutgart.
14. Siest, G., J. Henny and F. Schiele, 1980. Interpretation des examens de laboratorie, Karger ed., pp: 206-223.
15. Watson, D., 1960. A simple method for the determination of serum cholesterol. Clinica Chimica Acta, 5: 637-643.
16. King, E.J. and D.P. Wooton, 1959. Micromethods in Medical Biochemistry. 3rd Ed. Churchill, London.
17. Patton, C.J. and S.R. Crouch, 1977. Determination of blood urea. Analytical Chemistry, 49: 464-469.
18. Husdan, H. and A. Rapoport, 1968. Estimation of creatinine by the Jaffe reaction, A comparison of three methods. Clinical Chemistry, 14: 222-238.
19. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed. The Iowa State University Press, Ames. Iowa, USA., pp: 30-60.
20. Roudaut, B., J.P. Moretain and J. Biosseau, 1987. Extraction of Baytril in eggs after medication of laying hens. Food Additive Contaminants, 4: 297-307.
21. Yoshida, M., D. Kubota, S. Yonezawa, H. Nakamura, H. Azechi and N. Terakado, 1971. Transfer of dietary spiramycin into the eggs and its residue in the liver of laying hens. Japanese Poultry Science, 8: 103-110.
22. Omija, B., E.S. Mitema and T.E. Maito, 1994. Baytril residue levels in chicken eggs after oral administration of medicated drinking water to laying hens. Journal of Poultry Sciences, 8: 103-110.
23. Ziv, G., M. Shem-Tov, A. Gilckman, M. Winkler and A. Saran, 1995. Tilmicosin antibacterial activity and pharmacokinetics in cows. Journal of Veterinary Pharmacology and Therapeutics, 18: 340-345.
24. Furusawa, N., 2001. *In vitro* hepatobiotransformation of sulphadimethoxine in laying hens. Journal of Veterinary Medical Series A, 48: 147-152.

25. Ley, D.H. and H.W. Yoder, 1997. *Mycoplasma gallisepticum* infection. In : Calnek, B.W. (Ed). Diseases of poultry. Iowa State University Press, Ames, Iowa, USA, pp: 194-207.
26. Kleven, S.H., 1997. *Mycoplasma synoviae* infection. In Calnek, B.W. (Ed.), Diseases of Poultry, Iowa State University Press, Ames, Iowa, USA., pp: 220-228.
27. Kleven, S.H. and C. Baxter-Jones, 1997. *Mycoplasma iawaw* infection. In : Clanek, B.W. (Ed.). Diseases of poultry. Iowa State University Press, Ames, Iowa, USA., pp: 228-232.
28. Bradbury, J.M., C.A. Yavari and C.J. Giles, 1994. *In vitro* evaluation of various antimicrobials against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by the micro-broth method, and comparison with a commercially prepared test system. Avian Pathology, 23: 105-115.
29. Hannan, P.C., G.D. Windsor, A. de Jong, N. Schmeer and M. Stegemann, 1997. Comparative susceptibilities of various animal pathogenic mycoplasmas to fluoroquinolones. Antimicrobial Agents Chemotherapy, 41: 2037-2040.
30. Ter Laak, E.A., J.H. Noordergraaf and M.H. Verschure, 1993. Susceptibilities of *Mycoplasma bovis, Mycoplasma dispar* and *Ureaplasma diversum* strains to antimicrobial agents *in vitro*. Antimicrobial Agents Chemotherapy, 37: 317-321.
31. Levisohn, S., 1981. Antibiotic sensitivity patterns in field isolates of *Mycoplasma gallispticum* as a guide to chemotherapy. Israel Journal of Medical Sciences, 17: 661-665.
32. Kempf, I., C. Ollivier, R. L'Hospitaller, M. Guittet and G. Bennejean, 1989. Efficacy of spiramycin and tylosin in preventing mycoplasmosis in chicks experimentally infected with mycoplasma gallisepticum. Pathologie - Biologie, 37: 560-564.
33. Bebear, C.M., J. Renaudin, A. Charron, H. Renaudin, B. De Barbeyrac, T. Schaeverbeke and C. Bebear, 1999. Mutaions in the gyrA parC and parE genes associated with fluoroquinolones resistance in clinical isolates of *Mycoplasma hominis*. Antimicrobial Agents Chemotherapy, 43: 954-956.
34. Wu, C.C., T.R. Shryock, T L. Lin, M. Faderan and M.F. Veenhuizen, 2000. Antimicrobial susceptibility of *Mycoplasma hyorhinis.* Veterinary Microbiology, 76: 25-30.
35. Baggot, J. and D. Gingerich, 1976. Pharmacokinetic interpretation of erythromycin and tylosin activity in serum after intravenous administration of a single dose in cows. Research in Veterinary Sciences, 21: 318-323.
36. Carlier, M.B., A. Zenebergh, and P.M. Tulkens, 1987. Cellular uptake and subcellular distribution or roxithromycin and erythromycin in phagocytic cells. Journal of Antimicrobial Chemotherapy, 20(Suppl. B): 47-56.
37. USDA, 1998. National Residue Program Plan, Food Safety Inspection Services. Office of Public Health and Science, Washington, DC.

(Received: 30/04/2009; Accepted: 10/06/2009)