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Immunological responses of enrofloxacin in goats

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Abstract
Antimicrobial therapy constitutes a major component of modern medical and veterinary practices. Several studies indicate that antimicrobial agents have role which affect the host immunity and thereby alter course of diseases. The present study was conducted to assess the immunological effect of enrofloxacin in goats administered @ 5 mg/kg body weight by multiple intramuscular administration. Three groups of goats comprising of five animals in each group were selected for the study. Group I consisted of saline control, group II contained animals with antigen (Ag) control [2 ml of 7% sheep red blood cell (SRBC) Ag given on 1st day as sensitizing dose and on 10th day as challenging dose], group III consisted of animals exposed to enrofloxacin and SRBC Ag (enrofloxacin @ 5 mg/kg I.M. daily for 7 days during sensitizing and challenging period and SRBC as given in group II). Blood samples were collected on 1, 7, 10, 14, 21, 28, 35 and 42 days of the experiment. The immunological parameters evaluated were haemagglutination (HA) test for humoral immune response (HIR) whereas absolute lymphocyte count (ALC) and delayed type hypersensitivity (DTH) for evaluating cell mediated immune response (CMIR). DTH was assessed by injecting 1-chloro-2, 4-dinitrobenzene (DNCB), phytohemagglutinin-P (PHA-P) and purified protein derivative (PPD) in the skin of the neck region. The agglutinating antibody titers recorded higher titre in enrofloxacin treated group as compared to antigen treated group. Three mitogens used in the study revealed very least immunomodulatory effect when the drugs were given alone. The ALC revealed an apparent decrease of lymphocyte count when enrofloxacin was given alone. It is concluded that these drugs when given in in multiple doses exert immunomodulatory effect on humoral immunity while only very little effect was evident on the CMIR.

Keywords: Enrofloxacin, Immunomodulation, goat, HIR, CMIR, PPD, PHA-P, DTH

Introduction
The knowledge of possible influence of antimicrobials on the immune response (immunomodulation) seems to be of great importance for the clinical approach to the process of therapy [1-3]. Enrofloxacin is an antibacterial agent which belongs to fluoroquinolones [4]. The fluoroquinolones are popular class of antibiotics for use in a variety of infections in humans and animals. They are also known to have direct effects on the immune system [1, 5]. The production and secretion of various cytokines and chemokine's in vitro (i.e., IL-I, IL-3, 1L-6, 1L-8, TNF-α, and IFN-γ) was affected by various fluoroquinolones [1, 2, 6]. The immunomodulatory effects of fluoroquinolones are probably due to their effects on intracellular cyclic AMP and phosphodiesterase, on transcription factors, such as NF-Kappa B and activator protein 1 [5]. Enrofloxacin is very popular is veterinary medicine because of its pharmacokinetic properties, low toxicity and a broad spectrum of activity [4, 8-9]. Enrofloxacin is metabolized into pharmacologically active metabolite, ciprofloxacin, which is also known to have modulatory effect on the immune system [1, 5, 10, 11]. Ciprofloxacin has been shown to modulate phagocytic and killing capacity of neutrophils and macrophages as well as affects the expression of tall – like receptors in monocytes [12-14]. There is lack of immunological studies of enrofloxacin in small ruminants, particularity in goats. Hence, the present study was undertaken to assess the immunological effect of enrofloxacin in goats.

Materials and Methods
Experimental Animals: In the present study, 15 clinically healthy Black Bengal female goats (*Capara hircus*) between 20 to 24 months of age weighing 18-22 kg body weight were used.
Experimental Design: For conducting immunological study, clinically healthy goats were divided into three groups consisting of five animals in each group. Details of treatment given to different groups are summarized as given below.

Group I: Saline Control Group.
Group II: Antigen Control Group. 2 ml of 7 % sheep red blood cell (SRBC) suspension intravenously (I.V.) given in each goat on the first day of experiment (sensitizing dose) and on the 10th day of experiment (challenging dose).
Group III: Enrofloxacin + Antigen – Apart from antigen (SRBC) given as in Group II, enrofloxacin was administered @ 5mg/kg. I.M. daily for 7 days during sensitizing and challenge period.

Administration of Drugs
Enrocin®, an injectable commercial preparation containing Enrofloxacin (10%) in concentration of 100 mg. ml^{-1} marketed by Ranbaxy Laboratories Limited, India was used in the present experiment. Enrofloxacin (5mg/kg) was administered I.M. for 7 days in healthy goats during period of pre and post challenge.

Collection of biological fluids and their timings
Blood samples were collected on day 1, 7, 10, 14, 21, 28, 35 and 42 days of the experiment without anticoagulant for serum separation. For Haemagglutination test (HA) and absolute lymphocyte count also, blood samples were collected on same days as mentioned above with anticoagulant.

Analysis of Immunological parameters
Preparation of buffers: Phosphate buffer saline (PBS) was prepared using NaCl, KCl, $Na_2HPO_4.2H_20$, KH_2P04 and distilled water, pH 7.2 to 7.4 as per a prescribed method [15]. PBS was used for reconstitution and preparation of sheep red blood cells (SRBC). Alsever's solution was prepared using dextrose, sodium citrate, sodium chloride, citric acid and distilled water. Equal volume of Alsever's solution and anticoagulant was used for collection of sheep blood.

Mitogen used for cutaneous basophilic hypersensitivity reactions (delayed type hypersensitivity)
1% DNCB (1-Chloro-2, 4-dinitrobenzene) solution was prepared in acetone (10 mg/ml). PHA-P (Phytohaemagglutinin-P) was used as mitogen for cutaneous basophilic hypersensitivity reaction. A concentration of 1 mg/ml of PHA-P in PBS solution was prepared. PPD (Tuberculin) @ 10 IU/0.1 ml was used.

Assessment of immune response after administration of different drugs
Humoral immune response (HIR) was assessed by haemagglutination (HA) test. HA test was performed in the sera of test and control goats. The anti-SRBC antibody titres were measured using micro-titration technique [16]. The HA pattern was read with the aid of reading mirror and result of HA titre was recorded reciprocal of the highest dilution showing 100% HA and expressed a log_2 HA titre/0.5 ml of goat's serum.
Cell-medicated immune response (CMIR) was assessed by Delayed type hypersensitivity (DTH) reactions which included DNCB, PHA-P and PPD Skin sensitivity tests. DNCB test was done as per a previously described [17]. 0.25 ml of DNCB (10mg/ml) in acetone vehicle was applied on right side. On left side, 0.25 ml of acetone was applied which served as control. DNCB was applied on 5th day and challenged on 15th day of experiment by applying 0.25 ml of DNCB in acetone on right side and 0.25 ml acetone on the left side at the same site of first application. The skin thickness was measured with the help of slide caliper at 4, 8, 12, 24, 48, 72 and 96 h during pre- and post-challenge. The CMI response was calculated by subtracting the thickness of right side from left side.
PHA-P skin sensitivity test was done as per a previously described method [18]. 0.1ml of PHA-P (1mg/ml) in 0.1ml of PBS was injected intradermally on the right side of neck. The left side received 0.1ml of sterile PBS and served as control. The PHA-P stimulation index was calculated as the difference in swelling on PHA-P injected and PBS injected site with the help of slide caliper on 4, 8, 12, 24, 48, 72 and 96 h. Purified protein derivative (PPD) Skin Sensitivity test was done as per a previous method [19]. The results were expressed as the difference of swelling on PPD injected site and PBS injected site at 4, 8, 12, 24, 48, 72 and 96 h post injection during pre- and post-challenge periods.
Cell-medicated immune response was also assessed by absolute lymphocyte count as the method described in Schalm's veterinary Haematology:-
Absolute lymphocyte count = (Total no. of lymphocyte ÷ 100) × Total no. of leukocyte.

Statistical analysis
The effects of drug on immune response at different time intervals and on various days of post treatment in groups was done by random design (CRD).

Results and Discussion
Humoral Immune Response (HIR)
Effects of enrofloxacin on humoral immune response in goats were recorded using sheep red blood cell (SRBC) as an indicator of humoral immunity. Table 1 showed the humoral immune response of enrofloxacin against – SRBC antigen (CRD mean ± S.E.) to HA antibody titre (log 2 Value) in goats. The study revealed that there is no significant immunomodulatory effect of enrofloxacin on humoral immunity, however, the HA antibody titre recorded by Enrofloxacin (1.65565 ± 0.34735) was non-significantly higher as compared to antigen treated group (0.88050 ± 0.19901).

Table 1: Humoral immune response (HIR) of drug against SRBC antigen to antibody titre (Log 2value) in goats

Group	CRD mean ± SE
1. Saline Control	$0.30103^{a} \pm 0$
2. Antigen	$0.88050^{ab} \pm 0.19901$
3. Enrofloxacin + Antigen	$1.65565^{b} \pm 0.34735$

Means with different superscript within column differ significantly ($p < 0.05$)

The above findings are not in agreement with the result of a previous study [20] in which it was observed that the simultaneous administration of pefloxacin and diclofenac produced a marginal suppression of humoral immune response, which was not statistically significant in rabbits. Pefloxacin and ciprofloxacin were reported to alter the humoral immune response of mice against sheep red blood cells [21]. The antibodies formation was observed on 7th days of antigen exposure and highest HA titre was observed on 21st day followed by declining trend thereafter up to 42nd days. The above findings are also in agreement with finding of a previous study [22].

Cell-medicated immune response (CMIR)

The result of absolute lymphocyte count revealed that administration of enrofloxacin (4457 ± 36.310) caused significant decrease of lymphocyte count with antigen control (5400 ± 150.80).

Table 2: Cell-medicated immune response (CMIR) of drugs to absolute lymphocyte count (per cubic millimetre)

Group	CRD mean ± S.E (Per cubic millimetre)
1. Saline Control	4516^{a} ± 15.890
2. Antigen Control	5400^{b} ± 150.800
3. Enrofloxacin + Antigen	4457^{a} ± 36.310

Means with different superscript within column differ significantly ($p < 0.05$)

The DNCB and PHA-P Skin sensitivity tests do not suggest significant immunomodulatory effect on cell medicated immune response by the Enrofloxacin. Similar result was reported by a previous study [23]. But, in another study [24], it was noticed that ciprofloxacin neither diminished nor enhanced mononuclear cell proliferation. Simultaneous administration of pefloxacin and diclofenac did not affect the natural host defines response [20]. In present experiment, administration of enrofloxacin caused an apparent decrease of absolute lymphocyte count. Pefloxacin, ciprofloxacin and ofloxacin have been reported to inhibit mononuclear leukocyte proliferation in response to mitogen phytohemagglutinin [23]. The result revealed that very least immunomodulatory effect was shown by enrofloxacin.

Table 3: Cell medicated immune response (CMIR) of drugs to DNCB, PHA-P and PPD/tuberculin mitogens in goats

Pre-challenge (5^{th} day)

Group	CRD mean ± S. E		
	DNCB	PHA-P	PPD/Tuberculin
1. Saline Control	1.0440^{a} ± 0.0733	0.7988^{a} ± 0.0724	0.5734^{a} ± 0.0331
2. Antigen Control	1.0442^{a} ± 0.0945	1.0222^{a} ± 0.0724	0.6947^{ab} ± 0.0876
3. Enrofloxacin + Antigen	0.7631^{a} ± 0.0529	0.8768^{a} ± 0.0628	0.6100^{b} ± 0.0686

Means with different superscript within column differ significantly ($p < 0.05$)

Post-challenge (15^{th} day)

Group	CRD mean ± S. E		
	DNCB	PHA-P	PPD/Tuberculin
1. Saline Control	1.2308^{a} ± 0.0995	1.0485^{a} ± 0.0798	0.6628^{a} ± 0.0517
2. Antigen Control	1.2537^{a} ± 0.1140	1.774^{a} ± 0.1044	0.9337^{bc} ± 0.0641
3. Enrofloxacin + Antigen	0.8008^{b} ± 0.0515	0.9505^{a} ± 0.0699	0.7577^{ab} ± 0.0834

Means with different superscript within column differ significantly ($p < 0.05$)

The knowledge on interactions of antibiotics with the immune system is of great importance. The presented results should prompt further studies on the practical significance of recent observation in terms of clinical implications.

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