Vet.Med.J.,Giza. Vol.53, No.2. (2005): 353-361.

EFFECT OF OXYTETRACYCLINE ON IMMUNOCOMPETENT DAIRY GOAT

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Received: 28.3.2005.
Accepted: 30.3.2005.

SUMMARY

Dairy goats treated with oxytetracycline revealed a significant reduction in lymphocyte transformation which occurred 3 days post treatment and lasted for 4 weeks. At the same time the chemotactic index of PMN cells showed a significant decline for 2 weeks. Decline in phagocytosis and killing percentage started on 3rd day and persisted through the 2nd and 3rd weeks. No changes have been recorded for IgM, but IgG started to decline after one week and continued for 2 weeks.

Milk parameters recorded decrease in milk protein for two weeks and elevation in milk urea for one week and decline in milk lactose for 3 days. On the other hand no significant alteration in milk, fat, T.S., SNF or SCC. has been recorded.

INTRODUCTION

Oxytetracycline are bacteriostatic antibiotics that act as inhibitors of microbial protein synthesis by chelating cation - containing enzymes at the ribosomal level Seligman et al.,(1980). They are supposed not to influence the protein synthesis in mammalian cells. In the last years it has been demonstrated repeatedly that oxytetracycline are taken up by mammalian cells and even that they are concentrated intra-cellularly. Being chelating agent, it is reasonable to expect tetracyclenes to have effects also on the mammalian cells Somlen et al., (1983). It has been shown in several reports that oxytetracycline do affect a number of cellular functions i.e, those of granulocytes, lymphocytes (Hassan, 1994).

Stemmed from the previous notion the present study was designed to gain more explicit information on the possible effect of oxytetracycline

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on the systemic immunity in dairy goats and its possible profile in their milk yield, since oxytetracycline are frequently used for the treatment of many infections.

MATERIAL AND METHODS

1- **Drug :-** panterramycine long acting (oxytetracycline- phyzer).

2- **Animals** :- 6 dairy goats were devided into two equal groups. The first group was injected with oxytetracycline. Meanwhile, the second served as acontrol.

3- **Blood samples (Heparinized blood and serum):-** Jugular vein puncture was used to collect blood samples at one hour prior to oxytetracyline injection, after 3 days post treatment then weekly up to the 6th week for determination of the cellular and humoral immune performances .

4-**Milk samples :-**

Four milk samples were collected aseptically one hour before oxytetracyline treatment and post treatment for the evaluation of milk constituents.

5- Tissue culture media RPMI 1640 (Sigma) was prepared with addition of 10% foetal calf serum (Sigma).

- **Determination of phagocytic killing and chemotaxis activities of PMN :-**

Polymorphonuclear cells were isolated from blood by the method described by Rouse et al., (1980). The mixture of PMN and bacteria (S- aureus) was incubated at 37°C for 2 hours with regular stirring and then the mixture was centrifuged at 200xg for 5 min at 4°C. The supernatant was used to estimate the percentage of bacteria phagocytosed, Woldehiwet and Rowan, (1990). The mixture of bacteria and PMN was treated with one cycle of freezing and thawing and the percentage of bacteria killed was estimated according to the formula described by Woldehiwet and Rowan, (1990). The chemotactic index of PMN were calculated using the chemotaxis under agarose technique based upon migration patterns to chemotactic factor. (*E. coli* filtrate) (Nelson and Imons 1975).

- **Determination of immunoglobulin levels in serum :-**

Commercial radial immunediffusion plates were used to quantitate the immunoglobulin (IgG and IgM) in serum according to the instructions supplied with each kit (the binding site, U.K.) (Mancini et al., 1965).

Lymphocyte transformation assay :-

The lymphocyte transformation using MTT lymphocyte blastogensis micro assay was carried out as described by (Denizot and Lang 1991) with some modifications (Malsak and Reynolds 1995). Lymphocyte proliferation was measured by the blastogenesis assay using 3- (4,5 dimethyl thiazol 1-2) - 2,5 diphenyl tetrazolium bromide MTT (Sigma USA) as a tetrazolium dye. MTT is reduced to blue for mason compound by succinate

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dehydrogenase (Mitochondrial enzyme produced by living cells). Two hundred µl culture medium containing mitogen phytohaemagglutinine (PHA) at 25 µg/ml were dispensed into each of 96-well flatbottom tissue culture plate. Ten µl/cell suspensions containing 10^5 lymphocyte were added to each well. Plates were incubated at 37°C for 72 hours in humid Co_2 incubator (5-10% Co_2). Two hours before the termination of incubation 20 µl MTT (10mg/ml) were added to each well. At the end of incubation period 10% SDS in phosphate buffer saline was used to lyse the cells. Four percent 1N HCL in isopropanol was added to dissolve the formazone crystals. Plates were centrifuged at 300g for 10minutes at 27°C One hundred mL of supernatent from each well was transferred to wells of new flatebottom 96well plates before reading. The absorbance was detected with automated microtitre plate reader at a wave length of 550 nm.

Measurment of milk parameters :-

This was performed by using infrared milk analyzer-150, Bently, USA - Hungria.

RESULTS

Dairy goats treated with oxytetracycline revealed a significant reduction in lymphocyte transformation, that occurred 3 days post treatment and returned to control values 4 weeks post injection. At the same time, chemotaxis index of polymorphnuclear cells isolated from blood showed a significant decline, that continued for 2 weeks and subsided to control levels after 3 weeks (Table, 1).

Table (2) demonstrates that oxytetracycline injection induced a significant decrease in phagocytosis and killing percentages that appeared on the 3rd day post treatment. The previous decline persisted through the 2nd and 3rd weeks respectively following treatment.

Table (3) illustrates a significant reduction in serum IgG levels after oxytetracycline treatment. The previous decline originated after one week. This decrease lasted for 2 weeks. Meanwhile, IgM level demonstrated no significant effect due to oxytetracycline injection.

- Dairy cattle exposed to oxytetracycline treatment revealed a significant reduction of milk protein. The previous reduction continued for two weeks. On the other hand, milk fat didn't show any significant alterations (Table, 4).

Table (5) demonstrates a significant elevation of milk urea for one week, while, milk lactose showed a significant decline for 3 days. No significant alterations, throughout the experiment were,

Table (1): The effect of oxytetracycline on lymphocyte transformation and chemotactic activity of PMN cells.

Control			Treated	
Days	lymph	Chem	lymph	Chem
0 day	1.67±0.13	2.51±0.19	1.61±0.26	2.35±0.29
3 days	1.74±0.18	2.35±0.21	1.51±0.12*	1.97±0.08*
1 week	1.89±0.03	2.26±0.15	1.63±0.05*	1.81±0.16*
2 weeks	1.81±0.11	2.31±0.20	1.53±0.07*	1.72±0.13*
3 weeks	1.82±0.23	2.41±0.51	1.64±0.12*	1.97±0.32
4 weeks	1.96±0.45	2.52±0.85	1.73±0.26	2.31±0.34
6 weeks	1.62±0.42	2.36±0.63	1.76±0.28	2.55±0.27*

Lymph : lymphocyte transformation index.
Chem. : chemotactic index.

Table (2): The effect of oxytetracycline on phagocytosis and killing percentage of bacteriainfected animals

Control			Treated	
Days	Phag	Kill%	Phag	Kill
0 day	90.4±8.61	86.6±4.54	87.3±6.73	85.8±8.81
3 days	87.9±5.63	81.3±2.05	71.6±2.19*	69.3±4.26
1 week	89.3±5.97	83.4±2.63	73.5±3.08*	71.9±5.19
2 weeks	84.6±4.81	80.3±5.19	72.9±3.46*	68.4±2.74
3 weeks	86.5±3.95	81.5±8.63	75.8±5.21	72.5±6.06
4 weeks	88.6±8.73	83.7±4.81	81.4±6.17	80.5±3.57
6 weeks	87.3±8.15	84.2±6.93	83.9±8.89	81.7±5.86

* Significant at P <0.05
Phag : phagocitosis %.
Kill : killing percentage

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Table (3): The effect of oxytetracycline on IgM and IgG levels in treated animals.

	Control		Treated	
	IgG	IgM	IgG	IgM
0 day	1631.36±17597	493.63±83.81	1586.19±81.52	461.63±46.48
3 days	1451.21±13032	469.72±75.35	1235.83±75.47	413.75±59.95
1 week	1503.83±736	443.88±48.66	1189.56±42.95*	395.47±85.33
2 weeks	1681.75±835	456.91±37.05	1057.18±97.97*	406.84±69.48
3 weeks	1593.97±1865	396.76±69.76	1408.23±113.61	385.71±73.75
4 weeks	1405.8±1636	419.45±57.92	1397.85±147.19	387.09±59.08
6 weeks	1643.3±2539	435.63±61.78	1508.94±156.93	439.34±91.29

* Significant at P <0.05

Table (4): Measurements of fat and protein amounts in oxytetracycline treated and control animals

	Control		Treated	
	Fat	Protein	Fat	Protein
0 day	3.65±0.58	4.66±0.77	3.58±0.76	4.85±0.83
3 days	4.78±0.36	5.96±0.26	4.19±0.81	3.56±0.14*
1 week	3.95±0.63	4.81±0.36	4.35±0.59	3.21±0.26*
2 weeks	4.05±0.82	5.06±0.47	4.67±0.85	3.45±0.19*
3 weeks	3.18±0.56	4.93±0.31	4.18±0.69	3.14±0.30
4 weeks	4.79±0.71	3.96±0.47	3.41±0.53	4.46±0.25
6 weeks	3.67±0.72	3.79±0.56	3.67±0.91	4.59±0.17

* Significant at P <0.05

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Table (5): Evaluation of milk urea and lactose levels in oxytetracycline treated and control animals

	Control		Treated	
	Lactose	Urea	Lactose	Urea
0 day	3.57±0.75	23.15±1.45	4.11±0.68	22.16±1.39
3 days	4.05±0.25	20.27±1.93	3.33±0.09*	27.25±1.56*
1 week	3.95±0.23	19.25±1.43	3.45±0.15	26.58±2.81*
2 weeks	4.16±0.18	21.33±0.98	386±0.33	22.68±3.36
3 weeks	4.38±0.17	23.51±2.85	4.06±0.49	26.83±2.85
4 weeks	3.81±0.18	18.79±3.89	4.15±0.58	20.15±2.13
6 weeks	3.97±0.35	20.82±2.45	3.83±0.51	23.43±2.79

* Significant at P <0.05

Table (6): Measurements of T.S., SNF in oxytetracycline treated and control animals.

	Control		Treated	
	T.S	SNF	T.S	SNF
0 day	14.68±2.56	7.19±1.89	15.61±1.59	8.21±1.36
3 days	13.01±3.17	8.43±1.57	13.65±2.39	6.89±1.82
1 week	17.26±1.98	7.59±0.97	14.18±2.79	7.11±0.82
2 weeks	15.38 ±2.05	6.97±1.08	14.85±2.58	7.05±0.78
3 weeks	14.33±1.98	8.01 ±0.75	13.93±1.71	7.85±0.81
4 weeks	12.86±1.58	7.81±1.17	14.81±1.93	8.15±1.51
6 weeks	13.52±2.05	8.96±0.93	13.48±1.58	8.36±1.31

T.S. : Total solids
SNF. : Solid non - fat

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Table (7): Measurements of S.C.C. in oxytetracycline treated and control animals.

Days	Control	treated
0 day	358.19±49.56	405.68±58.42
3 days	341.58 ± 59.73	357.88± 42.33
1 week	289.95± 39.71	395.71± 67. 58
2 weeks	368.59± 41.82	248.54± 58.83
3 weeks	281.49± 33.33	295.61± 61.07
4 weeks	294.59± 58.78	311.29±29.85
6 weeks	315.93± 41.05	357.63± 72.48

S.C.C. : Total somatic cell count

recorded in T.S, SNF and SCC (Table,5, 6 and 7).

DICUSSION

Nowadays, antibiotics are widely utilized for the control of diseases in human and veterinary fields with concentration enough to evoke a significant immunosuppressive effects (Kapry, 1977).

In the present study, it has been shown that in dairy goats, the usage of oxytetracycline injection evoked a significant reduction in both the cellular components of immunity represented largely by lymphocyte blastogenesis, chemotaxis, phagocytosis and bacterial killing and the humoral arm portrayed as immunoglobulin G. These findings have been demonestrated in both blood and milk samples.

As revealed from table (1), the significant reduction in lymphocyte transformation and the decline in the chemotactic index of the polymorphnuclear neutrophils (PMN) both have been achived 3 days post treatment with oxytetracycline and lasted for 4 weeks and 3 weeks respectively before they turned back to normal control levels. This had a serious impact on the immune system leading to a state that could be described as " transient immune suppression, " during which the animal becomes liable to be infected with the opportunitic microorganisms which are frequently present as inhabitants either in the animal body or in its environment.

Since lymphocytes are one of the main components of both the cell mediated and humoral immune responses, as well as the inflammatory process, so these functions are actually affected.

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(Tizard, 2003).

On the other hand, and as depicted in table (2) the significant decrease in phagocytosis and killing percentages that occurred 3 days post oxytetracycline and which has continued throughout the third and second weeks respectively, aid greatly the state of transient immunosuppression directly through hindering the effect of the type of cellular response and its activity, and indirectly through obstacling the antigen presentation to the immune system with its impact on the immune response, not only during the treatment with the drug but throughout several weeks after. This indicates that the activity of the drug residues in the body of the animals is of serious hazardous significance not only for the animal itself but also for the human beings who consume its meat and milk or their products.

The fade response of the immune system, whose components have been injuried through the attack of the drug and its residues would certainly interfere with successful vaccination (Tizard, 2003). The data illusterated in table (3) substantiate this statement as a significant reduction was evident in serum IgG levels one week after treatment with the drug and the decline in the level remained throughout the next two weeks. On the other hand, the non-significant change of IgM level was expected because of its production shortly after infection and before the effect of the drug on the pathogens become evident. On the other hand the situation with the IgG is different; as it is formed after the appearance of the IgM and its level is affected primarily by the size of the pathogen inoculum, virulence and specificity which has been actually influenced with the direct effect of the drug on the pathogen during the first week of treatment.

The return to the normal levels after that is due to several factors, one of which is the activity of the residual amount of the pathogen, since the drug is bacteriostatic and its effect is compeleted by the body defenses (inflammatory process, pharmacologically active products,etc.).

The idea becomes clear when we go through table (4) which illusterates the milk protein levels, that could be also considered as a mirror for the protein pool of the body during the period of the treatment with the durg.

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