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Chemotherapeutic Efficacy of Oxytetracycline, Enrofloxacin and Imidocarb for the Elimination of Persistent *Anaplasma marginale* Infection in Naturally Infected Sahiwal Cattle

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Abstract.- The objective of this study was to compare the efficacy of oxytetracycline, enrofloxacin and imidocarb for the elimination of persistent *Anaplasma marginale* infection in naturally infected Sahiwal cattle. A total of sixty *Anaplasma marginale* seropositive adult Sahiwal cattle were selected having their ages between 3-4 years ranging in weight from 246-341 kg. The animals were divided in four groups designated as OXY-group-I, ENRO-group-II, IMC-group-III and control-group-IV, comprising 15 animals each. The seropositive animals received either oxytetracycline (22 mg/kg IV once in a day for five days), enrofloxacin (5 mg/kg IV once in a day for five days) or imidocarb (5 mg/kg IM twice, 7 days apart). The results of chemosterilization study indicated that oxytetracycline 13/15 (86.67%) and imidocarb dipropionate 11/15 (73.33%) eliminated *Anaplasma marginale* infection in adult naturally infected carrier cattle around the 56th post-treatment day. The carrier clearance was confirmed by cELISA followed by subinoculation of blood in seronegative splenectomized calves. It was concluded that oxytetracycline is more effective and safe in chemosterilization of persistent *Anaplasma marginale* infection in cattle.

Key words: Persistent infection, *Anaplasma marginale*, elimination.

INTRODUCTION

Anaplasmosis is one of the most important tick-borne disease (TBD) of cattle worldwide, including Pakistan (Khan *et al*., 2004; Afridi *et al*., 2005). The disease is caused by hemo-rickettsial parasite *Anaplasma marginale* (Dumler *et al*., 2001) characterized by weight loss, severe anaemia, jaundice, weakness, fever, brownish urine, pale mucous membranes, decreased milk production, abortion, hyper-excitability and mortality (Richey and Palmer, 1990). Animals recovered from clinical disease become life-long carriers (Aubry and Geale, 2011). The persistently infected animals resist subsequent clinical disease and serve as reservoirs for maintenance and further spread of infection in a herd (French *et al*., 1999). Immunosuppression in persistently infected cattle during pregnancy may lead to acute infection (Jones and Brock, 1966).

The carrier free status is required as per free trade policy for the movement of cattle between endemic and non-endemic regions of the world (Reinbold, 2009). Keeping *Anaplasma* free herds is one the important strategy for controlling bovine anaplasmosis (Aubry and Geale, 2011).

Before the development of tetracycline antimicrobials various drugs including antimalarials, antimony derivatives, dyes and arsenic compounds have been used for the treatment of acute anaplasmosis. These compounds were unable to control mortality because they had little if any therapeutic effect (Potgieter and Stoltsz, 1994). Earlier studies have shown that oxytetracycline, imidocarb and enrofloxacin are effective in the treatment of clinical and carrier states of anaplasmosis to a variable extent (Roby *et al*., 1978; Magonigle *et al*., 1975; Sharma *et al*., 1977; Kuttler and Simpson, 1978; Swift and Thomas, 1983).

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antimicrobial drug for carrier elimination in Pakistan. Therefore, a study was aimed to compare the chemotherapeutic efficacy of oxytetracycline, enrofloxacin and imidocarb for the elimination of persistent *Anaplasma marginale* infection in cattle.

MATERIALS AND METHODS

Experimental cattle

A total of sixty seropositive adult Sahiwal cattle were selected having their ages between 3-4 years ranging in weight from 246-314 kg. Before induction in the study the cattle were twice screened for TBDs at an interval of 60 days using microscopic blood smear examination or enzyme linked immunosorbent assay (ELISA). A commercially available competitive ELISA Kit, Anaplasma Antibody Test Kit cELISA (VMRD, Pullman, WA, USA) was used to detect *Anaplasma marginale* antibodies in serum and SVANOVIR® *B. bigemina*-Ab (Svanova Biotech AB, Uppsala, Sweden), diagnostic kit based on ELISA was used to rule out serum antibodies against *Babesia bigemina*. A total of 60 cattle that showed cELISA inhibition >30% and percent parasitized erythrocytes (PPE) lower than 1% for *Anaplasma marginale* were enrolled in chemosterilization study with no history, clinical, parasitological or serological evidence of babesiosis and theileriosis. The study was conducted during December to March.

Housing and husbandry

The selected cattle were divided in four groups designated as OXY-group-I, ENRO-group-II, IMC-group-III and control-group-IV, comprising 15 animals each. Whereas, group-IV acted as infected non-treated control (Fig. 1). The animals were housed in a group of 15 animals at Livestock Experiment Station, Sargodha. The cattle were offered green fodder and water ad libitum on a daily basis during the study. No drugs were given by feed or through drinking water during the entire study period.

Treatment

The following treatments were given to each group except infected non-treated control.

OXY-group-I: Oxytetracycline L.A (Tridox L.A., Farvet, Prix Pharmaceutica) at 22 mg/kg intravenous (IV) once a day for five days.

ENRO-group-II: Enrofloxacin (Enrotil Injection 10%, Selmore Agencies Pvt. Ltd.) at 12.5 mg/kg IV once a day for five days.

IMC-group-III: Imidocarb dipropionate (Imizole, Azco Nobal, ICI) at 5 mg/kg intramuscular (IM) twice, 7 days apart.

Control-group-IV: Infected, non-treated.

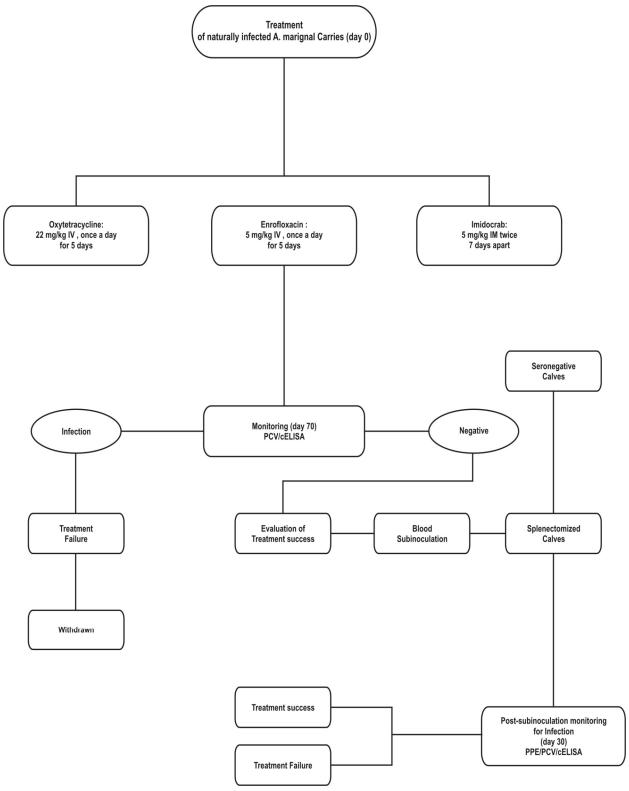

Fig. 1. Flow diagram of chemosterilization trial.

Theoretical dosage was calculated for each animal according to body weight based on the active ingredient in each formulation. Oxytetracycline (Tridox L.A., Farvet, Prix Pharmaceutica) and enrofloxacin (Enrotil Injection 10%, Selmore Agencies Pvt. Ltd.) were administered by slow intravenous injection in jugular vein. Whereas, imidocarb dipropionate (Imizole, Azco Nobal, ICI) was administered by deep intramuscular injection in a neck.

Post-treatment monitoring

Blood samples were collected weekly for the determination of packed cell volume (PCV) and cELISA up to 70 days to evaluate the success of chemosterilization. All the cattle that remained seropositive for *Anaplasma marginale* infection up to 70 days after first treatment were designated as treatment failure and removed from the study. After 70 days the success of chemosterilization in treated cattle was evaluated by further subinoculation of 50ml heparinized blood into splenectomized seronegative calves (cELISA inhibition <30%). The splenectomy was performed as mentioned in "Regional and Clinical Surgery and Lameness MANUAL" (2007). The splenectomized calves were monitored for 30 days to evaluate the final success of chemosterilization.

The percent parasitized erythrocytes (PPE) were calculated using formula (Coetzee and Apley, 2006).

$$PPE = \frac{\text{No. of infected cells}}{\text{Total no. of cells counted}} \times 100$$

Statistical analysis

Repeated measure analysis of variance was applied for chemosterilization studies using SPSS (2004). The interaction between the days and animals was evaluated by the Wilk's lamda, which was calculated by the software along with repeated measure analysis of variance. Tukey's Honestly significant difference test was used to discriminate differences between treatments as well as to discriminate between days. The *P*-value <0.05 was considered statistically significant.

RESULTS

Oxytetracycline administration caused mild localized pain at injection site in 6 out of 15 animals while imidocarb caused cholinergic reaction with mild pain, serous nasal discharge and lacrimation in 13/15 animals. None of the animal from enrofloxacin showed any local or systemic reactions. Before the start of chemosterilization study the data regarding PCV, cELISA, PPE and the body weights of the selected animals were found non-significant (>0.05).

Pre-treatment PCV ranged from 30.16±0.74% (OXY-group-I) to 32.78±0.58% (IMC-group-III). Twenty eight days after treatment PCV ranged from 32.13±0.85% (ENRO- group-II) to 33.38±0.79% (IMD-group-III). At 49th post treatment day the mean PCV of OXY-group-I, ENRO-group-II and IMC-group-III were 36.03±0.63%, 25.47±0.57% and 36.45±0.58, respectively. At 56th post treatment day the mean PCV of OXY-group-I, ENRO-group-II and IMC-group-III were 38.45±0.73%, 23.56±0.94% and 37.38±0.80, respectively. Packed cell volume of 2 animals (cattle # 19 and 26) from ENRO-group-II declined to 23%.

At the end of 70 day evaluation period the mean PCV for OXY-group-I, ENRO-group-II and IMC-group-III was recorded as 36.42±0.93, 22.24±0.89, and 35.65±0.74, respectively. No significant change in PCV was observed between pre and post treatment.

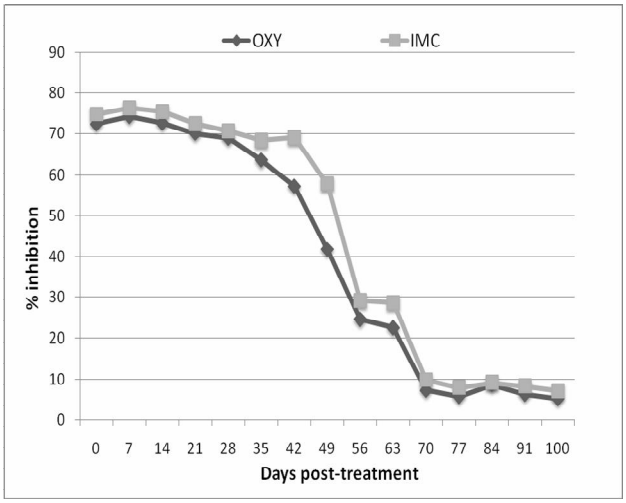

Fig. 2. Mean competitive inhibition ELISA in naturally infected *Anaplasma marginale* carrier cattle treated with oxytetracycline or imidocarb with successful chemosterilization.
Wilk's lamda, *P*<0.001; OXY, oxytetracycline; IMC, imidocarb.

Pre-treatment competitive ELISA ranged from 71.03±0.82% (ENRO-group-II) to 74.71±0.97% inhibition (IMC-group-III). The percentage inhibition of cELISA after 28 days of first treatment ranged from 68.83±0.60 (OXY group) to 71.67±0.76 (ENRO group). All the cattle remained

seropositive on 28 days after treatment (Fig. 2). On 49th day post treatment the cELISA inhibition from OXY, ENRO and IMC groups remained above 55%. The cELISA inhibition ranged from 41.78±0.86 (OXY-group-I) to 61.59±0.72 (ENRO-group-II), respectively. At 56th post treatment day the mean ELISA for oxytetracycline (OXY), enrofloxacin (ENRO) and imidocarb (IMC) treated groups were 24.53±0.85%, 58.51±0.60% and 29.13±0.72, respectively. The cELISA of 27 treated cattle (14 OXY and 13 IMC) fell below 30%. The major decline in cELISA inhibition was noticed between 42-56 days (Fig. 2).

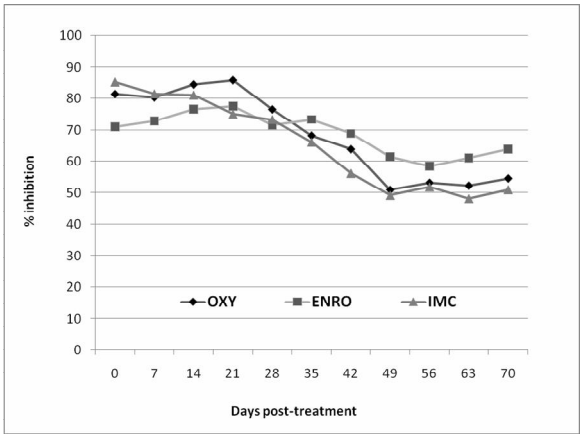

Fig. 3. Mean competitive inhibition ELISA in naturally infected *Anaplasma marginale* carrier cattle treated with oxytetracycline, enrofloxacin or imidocarb with chemosterilization failure.
OXY, oxytetracycline; ENRO, enrofloxacin; IMC, imidocarb.

At the end of 70 day chemosterilization trial period (first phase) the mean cELISA inhibition of OXY-group-I and IMC-group-III was 7.29±0.57 and 9.81±0.65, respectively, indicated carrier clearance based on cELISA. While the mean cELISA of ENRO-group-II was 63.92±0.72, indicated treatment failure (Fig. 3). Fourteen cattle from OXY-group-I and 13 cattle from IMC-group-II showed cELISA inhibition less than 30% assessed on individual animal basis. One animal from OXY-group-I, two from IMC-group-III and all animals from ENRO-group-II including infected non-treated control remained carriers after 70 days of treatment (Fig. 3). All animals that demonstrated a considerable decrease in PCV and cELISA values >30% were considered as treatment failure and withdrawn from study.

Post-treatment subinoculation of blood into splenectomized calves

All the cattle that showed cELISA inhibition <30% after 70 days of treatment, their blood were further subinoculated into 20 splenectomized Sahiwal calves (5-6 months of age). Prior to subinoculation, PPE values were not detectable in any of the treated cattle except control. Five infected non treated carrier cattle were used as source of pooled control blood. Twenty splenectomized calves were used for subinoculation of blood from treated carrier and non-treated control cattle. A batch of 4-5 animal's blood from OXY and IMC treated groups were pooled to subinoculate into splenectomized calves according to their respective groups. One batch each from OXY and IMC treated group showed either parasitological or serological evidence of *Anaplasma marginale*; their blood were further subinoculated into individual splenectomized calves to discriminate chemosterilization on individual basis. Whereas, each control animal blood was subinoculated into each splenectomized calf. The summary of the clinical parameters before and after subinoculation are given in Table I. Three calves exposed to blood from infected non treated control cattle were died between 16-20 days after subinoculation. The mean PCV of these calves ranged from 12-17%.

One calf out of fourteen from OXY treated cattle had seroconverted on day 21 after subinoculation while two calves out of thirteen exposed to blood from IMC treated cattle had seroconverted on 14th day after subinoculation. After 30 days of post inoculation monitoring one animal from OXY-group-I and two cattle from IMC-group-III failed to clear infection upon subinoculation (Table I). Clearance of persistent *Anaplasma marginale* infection was achieved in 13/15 (86.67%) cattle from OXY group-I whereas, IMC group-III was able to clear 11/15 (73.33%) cattle. The repeated measure analysis of variance revealed significant difference (P<0.001) between 42-56 days as well as between OXY and IMC treatments (P<0.001).

Table I.- Summary of mean PPE, PCV and cELISA following subinoculation of blood into splenectomized calves from treated and control carrier cattle.

Days	Parameters	OXY		IMC		Control (n=5)
		Successful chemosterilization (n=6)	Chemosterilization failure (n=1)	Successful chemosterilization (n=6)	Chemosterilization failure (n=2)	
	No. of donors	13	1	11	2	5
0	PPE	0.00	0.00	0.00	0.00	0.08
	PCV	28.44±2.62	32.00	26.22±2.65	30.5±1.75	34.18±0.93
	cELISA	6.34±0.79	8.13	4.28±0.82	7.51±0.51	48.32±0.49
7th	PPE	0.00	0.53	0.00	0.46±0.10	0.92±0.13
	PCV	27.03±1.92	33.00	26.33±1.89	3.15±0.84	31.24±0.81
	cELISA	5.48±0.45	6.72	4.34±5.6	8.38±0.47	53.54±0.68
14th	PPE	0.00	2.83	0.00	3.51±0.23	5.57±1.1
	PCV	26.51±0.66	34.00	24.97±0.74	32.25±0.73	17.31±0.18
	cELISA	10.84±0.26	18.42	7.08±0.25	39.56±0.21	78.47±0.75
21st	PPE	0.00	8.58	0.00	7.14±0.58	14.36±1.5
	PCV	28.23±0.86	34.00	26.33±0.78	26.00±0.76	16.63±0.75
	cELISA	4.21±0.88	62.98	4.13±0.85	69.39±0.52	81.38±0.37
30th	PPE	0.00	4.56	0.00	3.19±0.85	8.36±1.6
	PCV	31.98±0.98	19.00	31.36±0.9	24.75±0.73	14.36±0.67
	cELISA	3.83±0.85	-	2.75±0.91	80.56±0.89	74.13±8.7
Outcome		Negative	Positive	Negative	Positive	Positive

OXY, oxytetracycline; PPE, percent parasitized erythrocytes; PCV: packed cell volume; cELISA: competitive enzyme linked immunosorbent assay; Enro, enrofloxacini; 3, IMC, imidocarb dipropionate; 4, oxytetracyclin; 5, PCV, packed cell volume; 6, percent parasitized erythrocytes

DISCUSSION

Successful elimination of the naturally infected carriers of *Anaplasma marginale* has been achieved previously using oxytetracycline at the dose rate of 22 mg/kg intravenous daily for five days (Magonigle *et al*., 1975). This dosage earlier formed the basis of OIE recommendation for the clearance of *Anaplasma marginale* in persistently infected cattle (Coetzee *et al*., 2005). Chemosterilization in naturally infected carrier cattle was also achieved by Swift and Thomas (1983) using three to four treatments of long acting oxytetracycline administered intramuscularly at the dose rate of 20 mg/kg 3 days apart. The carrier elimination was confirmed by complement fixation and rapid card agglutination tests 120 days after treatment. Another study reported *Anaplasma marginale* carrier clearance using 2-4 intramuscular injections of oxytetracycline at the dose rate of 20 mg/kg with the interval of 3-7 days (Roby *et al*., 1978; Kuttler, 1983; Rogers and Dunster, 1984). Carrier elimination was also achieved in adult persistently infected cows by feeding 1.1 mg/kg of chlortetracycline (Richey *et al*., 1977). Reinbold and associates chemosterilized persistently infected cattle by daily feeding chlortetracycline hydrochloride in experimentally infected splenectomized calves (Reinbold *et al*., 2010).

Results of the present chemosterilization study showed elimination of *Anaplasma marginale* from the majority of carrier cattle on 56th day after the initiation of treatment using oxytetracycline and imidocarb while enrofloxacin failed to clear

persistent infection in adult naturally infected cattle.

The findings of present study are in partial agreement with the results of Coetzee and associates. They reported partial clearance of carrier infection. These calves achieved up to 2.05% PPE on 38th day after infection and dosages of drugs were the same as those used in the current work. The parasitological and serological responses were more pronounced in experimental animals as compared to our study. Carrier elimination was noticed in 25% animals belonging to oxytetracycline and imidocab treated groups. They used specific *Anaplasma marginale* isolates (Oklahoma, Virginia and St. Maries) for experimental infection in Holstein calves and only four calves were enrolled in each group. Five milliliter heparinized blood was used to subinoculate in splenectomized calves (Coetzee *et al*., 2006). Failure of chemosterilization in majority of these treated calves may be due to specific *Anaplasma marginale* isolates which might be resistant to specific drugs. The Brayton and associates has also indicated the existence of drug resistant pumps in the *Anaplasma marginale* genome (Brayton *et al*., 2005).

A few studies have reported failure of carrier elimination in cattle (Kuttler *et al*., 1980; Coetzee *et al*., 2005, 2006). Kuttler and associates used two intramuscular injection of long acting oxytetracycline at the dose rate of 20 mg/kg with unsuccessful chemosterilization (Kuttler *et al*., 1980). The dosage used in this study was not comparable with the current work.

In the present study the carrier elimination was achieved on 56th post-treatment day based on cELISA. Coetzee and associates reported carrier clearance on 49th day using oxytetracycline and imidocarb drugs in Holstein calves after experimental infection with Oklahoma, St. Maries and Virginia isolates of *Anaplasma marginale*. In a separate study, chemosterilization was achieved after feeding chlortetracycline ranging from 4.4-22 mg/kg/day for 80 days. The clearance of persistent infection was noticed on 49th day after treatment using real time polymerase chain reaction (RT-PCR). Chemosterilization was recorded within 49-88 days using cELISA (Reinbold *et al*., 2010).

The carrier free status was achieved between 4-12 months after intravenous administration of oxytetracycline hydrochloride at the dose rate of 22 mg/kg daily for five days using complement fixation test (Magonigle *et al*., 1975). Magonigle and coworkers used complement fixation test to detect carrier clearance in naturally infected cattle. Lack of sensitive test and the adult naturally infected intact cattle (non splenectomized) may be the cause for reporting longer time in carrier elimination. The longer duration of treatment and success of chemosterilization is unknown (Reinbold, 2009).

The unreliable clearance might be due to specific isolates. There are reports of drug resistant pumps in *Anaplasma marginale* genome (Brayton *et al*., 2005) that support the hypothesis regarding the difference in the susceptibility of different isolates.

The imidocarb at the dose rate of 3 mg/kg is reported to be effective in the treatment of anaplasmosis. Successful chemosterilization has been reported using imidocarb at the dose rate of 5 mg/kg for 14 days apart (Roby and Mazzola, 1972). Akhter and associates evaluated the efficacy of imidocarb dipropionate against TBDs as well as against natural *Anaplasma* infected buffalo calves. The *Anaplasma* infected animals were treated with single injection and carrier status was successfully eliminated with two intramuscular injections of imidocarb dipropionate at the dose rate of 3 mg/kg, respectively (Akhter *et al*., 2010). The imidocarb had the potential to cause systemic cholinergic reaction. Serous nasal discharge and lacrimation was observed in the imidocarb treated experimental animals. Toxicity signs of imidocarb dipropionate has been reported including dyspnea, diarrhea, excessive salivation, serous nasal discharge (Adams and Corrier, 1980).

The unsuccessful immune response, lack of pharmacokinetic and pharmacodynamic association with plasma drug levels (Reinbold, 2009) as well as existence of drug resistant *Anaplasma marginale* isolates, insufficient drug duration, inadequate drug concentration at infection site and local environmental conditions might be associated with failure in carrier clearance in study animals (Coetzee, 2005). Thus, it was concluded that oxytetracycline is more effective and safe in chemosterilization of persistent *Anaplasma marginale* infection in cattle.

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