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DOSE OPTIMIZATION AND PHARMACOKINETIC/PHARMACODYNAMIC INTEGRATION OF AMOXICILLIN IN LOHI SHEEP (*Ovis aries*) AND BEETAL GOATS (*Capra hircus*)

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ABSTRACT

The present study aimed to investigate the interspecies variations in relation to pharmacokinetic profile and optimal dosage regimen of amoxicillin in Lohi sheep and Beetal goats. For this purpose, 16 healthy, adult animals, 8 from each species of Lohi sheep were selected. The animals were 1-2 years of age and 35-45 kg body weight. Amoxicillin was injected as a single intramuscular dose of 15 mg/kg body weight to each animal. Blood samples were collected at specific time intervals over 24 hrs. Plasma drug concentrations and time data was calculated by WinNonlin® for the estimation of pharmacokinetic parameters. Optimal dosage of amoxicillin was also calculated in both species. The elimination half-life and mean residence time were higher ($P < 0.05$) in Beetal goats than Lohi sheep indicating the slow elimination of the drug in Beetal goats. A priming dose of amoxicillin of 21.6 ± 24.4 mg/kg body weight was suggested with a maintenance dose of 15.7 ± 18.9 mg/kg likely to be repeated every 24 hours in order to provide successful treatment against bacteria with susceptibility of minimum inhibitory concentration (MIC ≤ 1.6 µg/mL) in Lohi sheep and Beetal goats respectively.

Key words: Amoxicillin, Lohi sheep, Beetal goats, Pharmacokinetics, Dosage regimen.

INTRODUCTION

Antibiotics play a significant role to combat various bacterial infections in livestock sector. Amoxicillin is one of the commonly prescribed antibiotics in humans and animals as well. It is semi-synthetic penicillin belongs to the beta-lactam group of antibiotics and have good affinity to penicillin-sensitive gram-positive and gram-negative bacteria (Plumb, 2005). The action of amoxicillin is bactericidal in nature. It interferes with the bacterial cell wall synthesis and inhibits the enzyme (DD-transpeptidases) which is responsible for the transpeptidation reaction; a vital step to maintain the integrity of cell wall (Chowdhury and Lieberman, 1999). Because of this enzyme inhibition, bacterial cell wall becomes fragile which ultimately leads to the bacterial death. Amoxicillin has great potential against respiratory as well as urinary tract pathogens such as *Streptococcus pneumonia, Pasteurella multocida*, *Escherichia coli* and *Haemophilus influenza* (File *et al*., 2004; Hernandez *et al.*, 2005).

The binding affinity of amoxicillin to plasma protein is very low. Hence, the major fraction of the drug is widely distributed in the body and penetrates to the different organs and tissues. But it fails to cross the blood brain barrier except in case of meningitis (Abad *et al*., 2003). Amoxicillin is mainly metabolized in the liver where its two metabolites (amoxicilloic acid and diketopiperazine) are formed (Elsheikh *et al*., 1999). These are the same metabolites in sheep and goats as reported by Reyns *et al*. (2008) in pigs.

The pharmacokinetic profile of a drug varies with a change in the environment and topography. A number of studies provide some evidences that the pharmacokinetic behavior and dosage regimen of investigated drug were different under local conditions when compared to the foreign documented data in the same animal species (Munawar *et al*., 2017; Manzoor *et al*., 2017; Manzoor and Iqbal, 2016; Javed *et al*., 2006, 2009). That's why pharmacokinetic study of a drug should be conducted under different environmental conditions in order to optimize proper dosing schedule in each species. In addition, the emergence of bacterial resistance is another alarming issue since the last few years which results due to indiscriminate and improper use of antibiotics or genetics (Gouvea *et al*., 2015). Hence, it is necessary to use the antibiotic drug only when it is required. Further, the proper dosage regimen of all antibiotics in each animal species should also be strictly followed during the course of treatment. The pharmacokinetic behavior of amoxicillin has been previously studied in different foreign species such as sheep (Delis *et al.,* 2009), goats (Craigmill *et al*., 1992), llamas (Kreil *et al.,* 2012), buffalo calves (Rasheed *et al*.,

2013) and pigs (Agerso *et al*., 2000). But there is a lack of pharmacokinetic data in local caprine species. Therefore, the present study is planned to evaluate the pharmacokinetic profile of amoxicillin in local breeds of sheep and goats in order to predict the proper dosage regimen of amoxicillin in these species.

MATERIALS AND METHODS

Experimental animals and drug administration: A total number of sixteen healthy, adult, non-lactating, female animals were selected, 8 from each species of local breed of sheep (Lohi) and goats (Beetal), weighting between 35-45 kg and 1-2 years of age in both species. All the animals were examined clinically for any symptoms of disease prior to the experiment. Each animal was housed in a separate pen provided with wire mesh to restrict any social contact. All the animals had free access to the drinking water at all the time and were offered seasonal fodder. Special care of each animal was taken by a veterinarian and national guidelines for animal care were strictly followed. The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC) vide notification no. IPPP/UAF/1245-14 dated 05 April 2014.

A commercial preparation of amoxicillin (Trioxyl, 15%) was procured from Univet® Pvt. Ltd. Poland. For the collection of blood samples one of the jugular vein was cannulated under aseptic conditions with plastic cannula No. 90 (Protex Ltd., England). Prior to drug administration, a control blood sample was collected from each experimental animal. Amoxicillin was then injected as a single intramuscular dose of 15mg/kg body weight to each animal (Rasheed *et al*., 2013).

1 mL of blood was drawn at 0.5, 1.0, 2.0, 3, 4, 6, 8, 12, 18 and 24 hours post drug administration in heparinized plastic tubes (Kreil *et al*., 2012). These samples were centrifuged at 4000 rpm for 15 minutes. Plasma was separated from these samples and subjected to analysis.

Pharmacokinetic parameters: The obtained data of plasma concentrations at different time intervals were subjected to a computer program WinNonlin® (Pharsight, version 4.6, USA). Data were analyzed by non-compartmental and compartmental analysis. Minimum Akaike Information Criteria was compared to determine the best fit model among these animals.

***In vitro* and *Ex vivo* bacterial killing curves of amoxicillin:** The isolate of *M. haemolytica* WOO221 was grown freshly from beads already stored at -70°C on TSA. Ten to twelve bacterial colonies were used to inoculate 9mL of MHB. The culture was allowed to grow overnight at 35°C. Multiple of MIC (0.25-8 times) were used to determine the *in vitro* time-kill curves in Lohi sheep and Beetal goats.

Serum samples collected at 1, 2, 4, 6, 8, 10, 12 and 24 hours after i.m. administration of amoxicillin were used to determine the *ex vivo* bacterial killing curves in Lohi sheep and Beetal goats according to the method previously described by Aliabadi and Lees (2002). The limit of detection was 10 cfu/mL in both experiments.

Pharmacokinetic-Pharmacodynamic integration: From the *in vivo* PK parameters and *in vitro* MIC values, the surrogate marker (AUC/MIC) was determined according to the method determined by Sidhu *et al*. (2010) AUC_{24}/MIC *ex vivo* data were modeled to the following Sigmoid E_{max} equation to quantify the three levels of antimicrobial efficacy of amoxicillin i.e. bacteriostatic level (E = 0, no change in bacterial count after 24 hour of incubation), bactericidal (AUC_{24}/MIC = -3, a 3-log or 99.9% reduction in the original inoculums bacterial count after 24 hour incubation), eradication (AUC_{24}/MIC = -4, a 4-log reduction in bacterial count of original inoculums).

$$E = E_0 + E_{max} \times C^N_e / EC_{50} + C_e$$

Where, E_0 denotes a change in log_{10} cfu/mL of sample post incubation (24 hour) in the control sample (without amoxicillin) compared with the initial inoculum log_{10} cfu/mL, E_{max} represents the maximum antibacterial effect determined as difference in log_{10} cfu/mL in samples incubated with amoxicillin between time 0 and 24 hour when the detection limit (10 cfu/mL) is reached, C_e denotes the AUC_{24}/MIC in the effect compartment (*ex vivo* site), EC_{50} is the AUC_{24}/MIC value producing a 50% reduction in bacterial count from the initial log_{10} cfu/mL, and N is the Hill-coefficient that describes the steepness of the AUC_{24}/MIC curve-effect curve.

These PD parameters were calculated using the nonlinear WinNonlin® regression program (Pharsight Corporation).

Determination of drug concentration: The concentration of amoxicillin in plasma was determined by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method previously described by Rasheed *et al.* (2013).

Dosage regimens: Based on the kinetic parameters, the optimal dosage regimen of amoxicillin was calculated in adult healthy Lohi sheep and Beetal goats. The calculation of priming and maintenance dose of amoxicillin after intramuscular administration were based on minimum inhibitory concentration (MIC) of amoxicillin in blood. The MICs of 0.1, 0.4, 0.8, 1.2, 1.6 and 2 μg/mL has been used in the current study for the calculation of dosage regimens of amoxicillin at 12, 24 and 48 hours time intervals in Lohi sheep and Beetal goats.

The priming and maintenance doses of amoxicillin were determined by the following equations as described by Baggot (1997).

$$\text{Maintenance dose } (D') = C^{\circ}P_{(min)}.\ V_d\ (e^{\beta t-1})$$

Where, $C^{\circ}P$ (min) = Minimum effective concentration, V_d = Volume of distribution, e =exponantional term, β = over all elimination rate constant, t = Time at which dose was calculated.

The priming dose was obtained by omitting -1 from the above equation:

$$\text{Priming dose } (D) = C^{\circ}P_{(min)}.\ V_d\ (e^{\beta t})$$

Statistical analysis: Mean with standard deviation values for each parameter was calculated by Microsoft Excel version 2007. Two tailed Student's T test was used to compare the pharmacokinetic parameters in Lohi sheep and Beetal goats. $P \leq 0.05$ was considered as significant.

RESULTS

Pharmacokinetic: Data were best fitted by one compartmental model in each animal of both species. The (mean±SD) plasma values of amoxicillin at different time intervals in both species are presented in Figure 1.

The pharmacokinetic parameters of amoxicillin following single intramuscular administration at dose of 15 mg/kg in Lohi sheep and Beetal goats are depicted in Table 1.

After intramuscular administration, drug was absorbed from the site of administration to the systemic circulation with $T_{1/2abs}$ values of 0.77 and 0.69 hours in Lohi sheep and Beetal goats respectively. The difference between the absorption half-life was insignificant among both species. Drug reached its maximum concentration as 3.13±0.24 and 3.43±0.12 µg/mL at T_{max} of 4.24±0.10 and 4.15±0.53 hours in Lohi sheep and Beetal goats respectively. There was an insignificant difference for these values in both species. After achieving the maximum concentration in plasma, amoxicillin was decreased gradually with a constant rate of K_{el} = 0.14±0.04 hour in Lohi sheep and K_{el} = 0.10±0.06 hours in Beetal goats. The elimination half-life was observed to be 4.73±0.93 hour in Lohi sheep and 6.91±1.24 hour in Beetal goats. Amoxicillin was slowly eliminated ($P < 0.05$) in Beetal goats and remained in the body for a longer period of time as indicated by their respective MRT values when compared to Lohi sheep. The total body clearance was 0.37±0.12 L/h/kg in Lohi sheep and 0.29±0.14 L/h/kg in Beetal goats.

***In vitro* bacterial killing of amoxicillin:** The *in vitro* antibacterial activity of amoxicillin is shown in Lohi sheep (Figure 2) and in Beetal goats (Figure 3). A concentration dependent activity of amoxicillin was observed in both species. In Lohi sheep, the initial bacterial count ranged from 6.1-6.3 cfu/mL. At MIC of 2 mg/mL the bacterial count reduced to 5.8 cfu/mL after 2 hours of incubation time. At MIC of 8 mg/mL, the maximum antibacterial activity was observed and bacterial count reduced to 2 cfu/mL after 12 hour incubation period. A similar *in vitro* antibacterial pattern of amoxicillin was observed in Beetal goats and there was no statistical difference between these data.

***Ex vivo* antibacterial activity of amoxicillin:** The *ex vivo* bacterial killing of amoxicillin in serum against *M. haemolytica* WOO221 are shown in Lohi sheep (Figure 4) and in Beetal goats (Figure 5). Samples collected at 6 hours post drug administration decreased bacterial growth to < 10 cfu/mL after 6 hour incubation. The antibacterial activity of amoxicillin was almost similar in both species and not any significant difference was observed in these species. The 2 and 4 hour samples were bactericidal whereas 10 and 12 hour samples were bacteriostatic in both Lohi sheep and Beetal goats. There was no bacterial growth between 6 to 24 hour incubation times.

PK/PD integration: The sigmoid E_{max} equation was used to estimate the three antibacterial levels of amoxicillin in Lohi sheep and Beetal goats. The high ratios of AUC_{24}/MIC of 86 and 92 are suggested for complete bacterial eradication in Lohi sheep and Beetal goats respectively. The AUC_{24}/MIC value of 71 and 79 are proposed for bactericidal action of amoxicillin whereas AUC_{24}/MIC of 37 and 41 are likely to have bacteriostatic action in Lohi sheep and Beetal goats respectively.

Dosage regimen: The intramuscular priming and maintenance doses of amoxicillin in mg/kg body weight for 12, 24 and 48 hours intervals in Lohi sheep and Beetal goats have been presented in Table 2.

At MIC of 1.6 µg/mL, the priming dose of amoxicillin in Lohi sheep was suggested to be 11.3, 21.6, 79.1 mg/kg and in Beetal goats as 14.01, 24.49, 83.67 mg/kg at 12, 24 and 48 hours time interval respectively. The corresponding maintenance doses in Lohi sheep were 5.4, 15.7, 73.1 and in Beetal goats these values were 9.01, 18.93, 77.22 mg/kg to be repeated at 12, 24, and 48 hours time intervals respectively.

Table 1. Pharmacokinetic parameters of amoxicillin after single intramuscular administration at a dose of 15mg/kg body weight in Lohi sheep and Beetal goats (n = 8).

Parameters	Unit	Lohi sheep	Beetal goats
C_{max}	(µg/mL)	3.13±0.24^{NS}	3.43±0.12^{NS}
T_{max}	(h)	4.24±0.10 ^{NS}	4.15±0.53 ^{NS}
K_{abs}	(h^{-1})	0.35±0.03 ^{NS}	0.48±0.06 ^{NS}
$T_{1/2abs}$	(h)	0.77±0.02^{NS}	0.69±0.04^{NS}
V_c	(L/kg)	2.56±0.21^{NS}	2.90±0.14^{NS}
K_{el}	(h^{-1})	0.14±0.04^{NS}	0.10±0.06^{NS}
$T_{1/2\ el}$	(h)	4.73±0.93*	6.91±1.24*
Cl_B	(L/h/kg)	0.37±0.12^{NS}	0.29±0.14^{NS}
AUC	(µg.h/mL)	19.9±1.47^{NS}	21.47±1.97^{NS}
MRT	(h)	6.83±1.07*	9.89±0.86*

*Means are significant (P < 0.05) to each other in a row, ^{NS}Non-significant difference, C_{max} = Maximum plasma concentration, T_{max} = Time to reach maximum concentration, K_{abs} = Absorption rate constant, $T_{1/2abs}$ = Absorption half-life, V_c = Volume of central compartment, K_{el} = Elimination rate constant, $T_{1/2el}$ = Elimination half-life, Cl_B = Total body clearance, AUC = Area under plasma concentration-time curve, MRT = Mean residence time.

Table 2. Dosage regimen of amoxicillin at 12, 24 and 48 hours in Lohi sheep and Beetal goats.

Dosing interval (hours)		12						24						48					
C^op (min) µg/mL		0.1	0.5	1.0	1.3	1.6	2	0.1	0.5	1.0	1.3	1.6	2	0.1	0.5	1.0	1.3	1.6	2
D	Sheep	0.71	2.83	5.66	8.49	11.3	14.2	1.35	5.41	10.8	16.2	21.6	27.1	4.94	19.8	39.5	59.3	79.1	98.8
D´	Sheep	0.34	1.35	2.74	4.18	5.42	6.85	0.98	3.93	7.9	11.8	15.7	19.6	4.6	18.2	36.6	54.8	73.1	91.4
D	Goats	1.57	3.63	7.41	9.79	14.01	17.45	2.71	6.98	13.43	18.56	24.49	29.89	6.65	22.44	43.41	62.99	83.67	99.13
D´	Goats	0.82	3.23	5.34	6.88	9.01	10.92	2.22	6.54	9.76	14.95	18.93	23.42	5.78	19.42	38.86	56.88	77.22	95.88

Table 3. PK/PD integration of *ex vivo* serum data (mean±SEM, n = 8) after intramuscular administration of amoxicillin at a dose of 15 mg/kg body weight in Lohi sheep and Beetal goats.

Parameter	Lohi Sheep		Beetal Goats	
	Mean	SEM	Mean	SEM
Log E_0 (cfu/mL)	-5.04	0.65	-4.95	0.45
Log E_{max} (cfu/mL)	1.55	0.28	1.78	0.33
Log E_{max} - Log E_0 (cfu/mL)	6.59	1.02	6.73	0.81
EI_{50}	9.48	1.18	8.78	1.08
AUC_{24h}/MIC for bacteriostatic action (h)	37	8	41	9
AUC_{24h}/MIC for bactericidal action (h)	71	13	79	12
AUC_{24h}/MIC for bacterial elimination (h)	86	11	92	10
Slope *(n)*	6.31	0.98	6.47	0.77

E_o = Difference in number of bacteria (cfu/mL) in control sample in absence of drugs between time 0 and 24 hour. E_{max} = Difference in number of bacteria (cfu/mL) in sample incubated with amoxicillin between time 0 and 24 hour. AUC_{24}h/MIC values for bacteriostatic, bactericidal and eradication responses derived from the sigmoid E_{max} curve. N = Slope of AUC_{24} h/MIC-response curve.

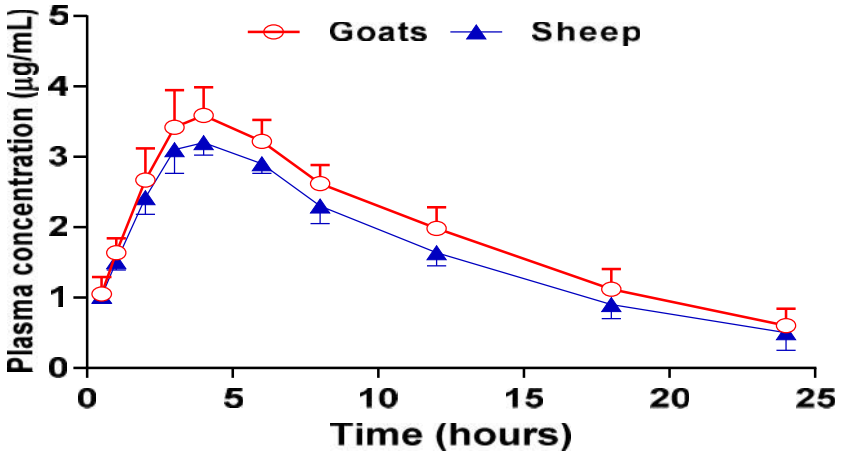

Fig. 1. Mean ± S.D. plasma concentration of amoxicillin (15mg/kg) after a single intramuscular administration in Lohi sheep and Beetal goats (n = 8).

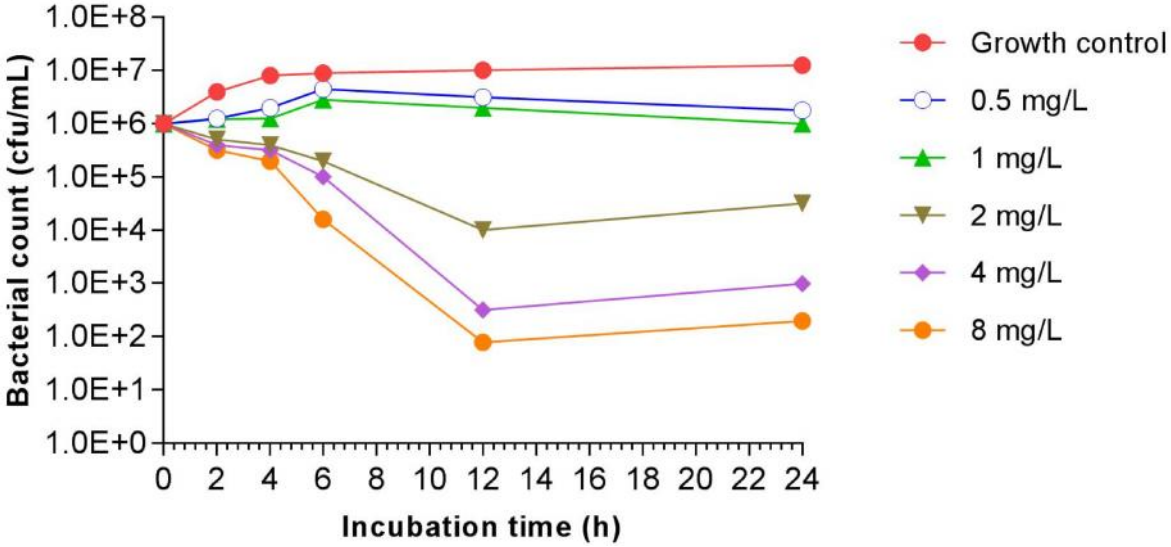

Fig. 2. *In vitro* antibacterial activity of amoxicillin is shown in Lohi sheep.

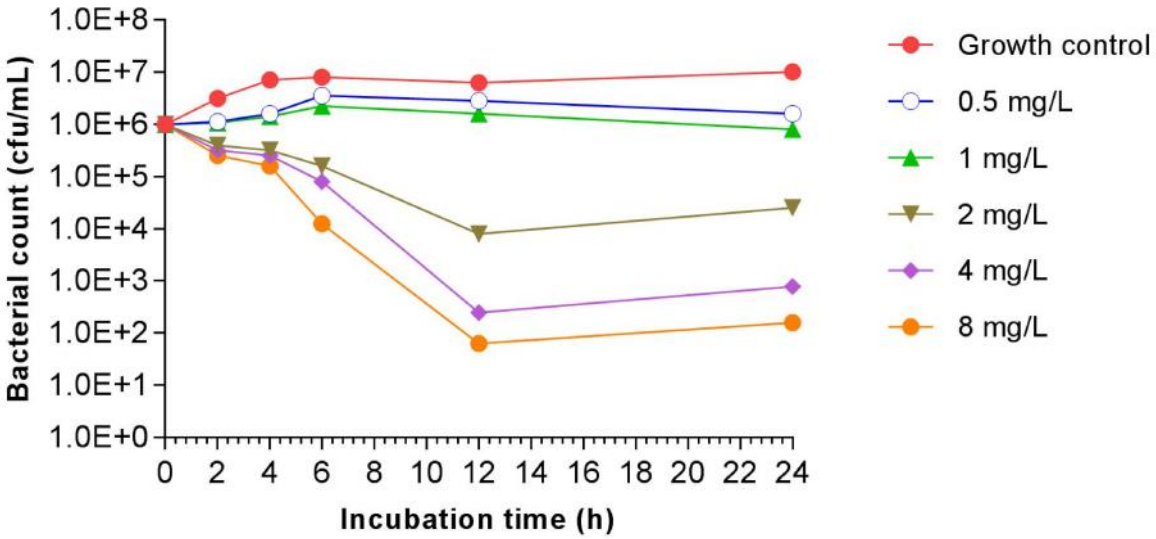

Fig. 3. *In vitro* antibacterial activity of amoxicillin is shown in Beetal goats.

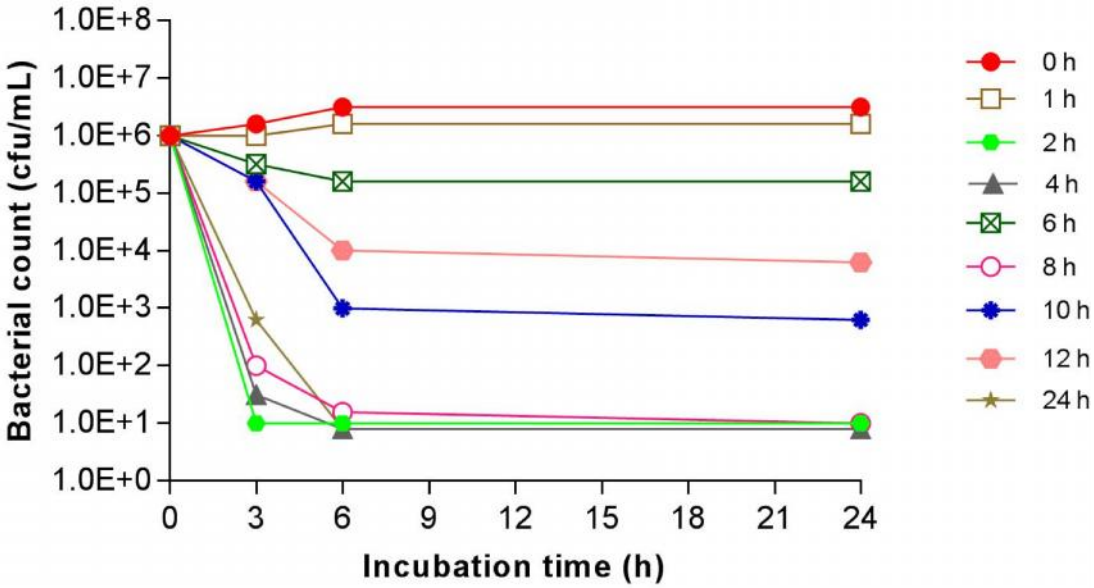

Fig.4. *Ex vivo* antibacterial activity of amoxicillin in Lohi sheep

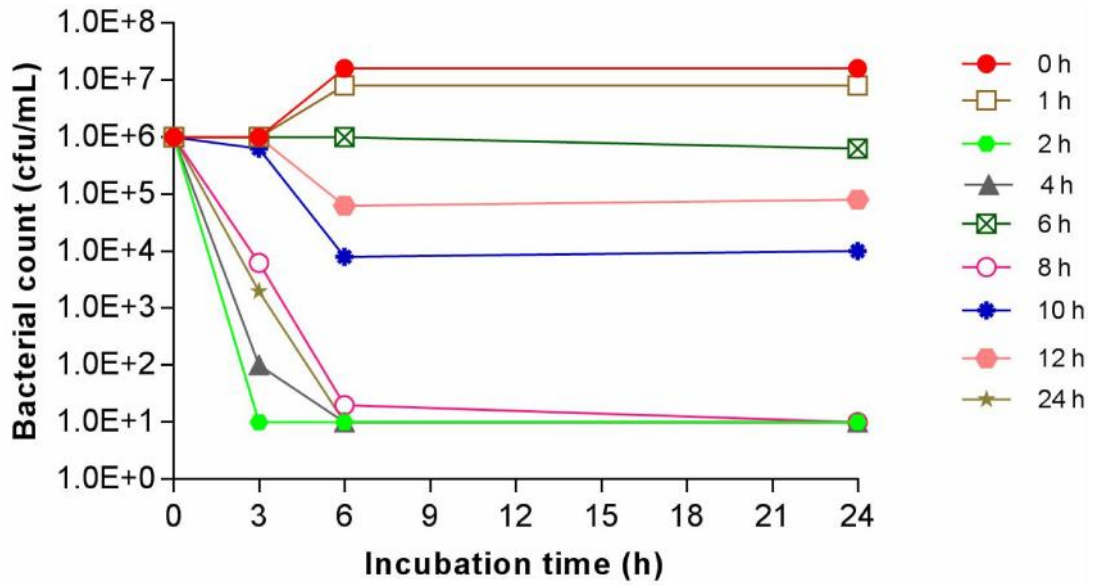

Fig. 5. *Ex vivo* antibacterial activity of amoxicillin in Beetal goats

DISCUSSION

In the current study, the pharmacokinetic profile of amoxicillin following intramuscular administration was in perfect accordance with one compartment disposition model in Lohi sheep and Beetal goats. Contrary to our findings, Anadon *et al*. (1996) described two compartmental open model for amoxicillin in broiler chickens. Similarly, Spyker *et al*. (1977) also used two compartmental model for the pharmacokinetic behavior of amoxicillin in human subjects.

In the current study, amoxicillin achieved its maximum plasma concentration as 3.13±0.24 µg/mL and 3.43±0.12 µg/mL at T_{max} of 4.24±0.10 hour and 4.15±0.53 hour in Lohi sheep and Beetal goats respectively. In contrast to our results, Raheed *et al*. (2013) reported lower value of amoxicillin (C_{max} = 1.82 µg/mL) at T_{max} of 2.73 hour in buffalo calves. Carceles *et al*. (1995), however, determined higher values of C_{max} of 10.71mg/mL after oral administration of amoxicillin in sheep.

A higher value of volume of distribution suggests the rapid distribution of the drug in both species. Rasheed *et al*. (2013) reported almost parallel values of volume of distribution (4.36 ± 1.64 L/kg) in buffalo calves this study. However, Kreil *et al*. (2012) determined higher values of volume of distribution (7.42 ± 2.3 L kg^{-1}) in llamas than the current study. In conflict

with our findings, Khargharia *et al*. (2012) and Delis *et al*. (2009) found some lower values of V_d in sheep (0.79 ± 0.16 L⁄kg) and in goats (0.31 ± 0.01 L/kg).

The shorter value of elimination half-life ($T_{1/2el}$) suggests the rapid elimination of the drug in Lohi sheep as compared to Beetal goats. These findings are consistent with the results described by Delis *et al*. (2009) in sheep and Carceles *et al*. (1995) in goats. Similarly, Montesissa *et al*. (1998) also determined the shorter half-life (1.42 h) of amoxicillin in horses. On the other hand, some higher values of elimination half-life than the current study were also reported by Kreil *et al*. (2012) and Agerso *et al*. (2000) in llamas and pigs respectively.

The pharmacokinetic interpretation of serum amoxicillin data revealed that there was an insignificant difference regarding the total body clearance of amoxicillin in Lohi sheep and Beetal goats. The drug clearance was rapid in both species. Similar to our findings rapid clearance of amoxicillin was also determined by Carceles *et al*. (1995) in goats, Carter *et al*. (1986) in foals and Montesissa *et al*. (1988) in horses. Kreil *et al*. (2012) reported very rapid clearance of amoxicillin of 8.62 ± 0.01 L/h/kg in llamas. In conflict with our findings, Khargharia *et al*. (2012) determined much slower clearance (Cl = 22.76 ± 3.41 mL/h/kg) of amoxicillin after intravenous administration goats. In the current study, total body clearance of amoxicillin was higher than the average value (0.13 L⁄h⁄kg) of glomerular filtration rate (GFR) suggesting the involvement of both GFR and active tubular secretion processes in both species of Lohi sheep and Beetal goats. However, further detailed studies at molecular level are required to predict the exact excretory mechanisms in these species.

Dose optimization of antibiotics is a very serious concern as the emergence of bacterial resistance arises when an inappropriate dosing of antibiotic is used in animals. To minimize the bacterial resistance, it is necessary to use an accurate dose of an antibiotic which is sufficient to kill the mutant resistant bacteria in order to achieve successful outcome of the therapy. Inter and intra species variations are common in animals. Hence, antibiotic dose should be optimized in each animal species. Amoxicillin has a great potential against a variety of microorganisms *in vitro*. Various pharmacokinetic/pharmacodynamic (PK/PD) indices have been proposed to predict the effectiveness of an antibiotic (Aliabadi and Lees, 2000). Three PK/PD indices are commonly used. These include T > MIC when the antibiotic is time dependent, AUIC (AUC/MIC) and C_{max}/MIC when antibiotic is concentration dependent. Recently, Nielsen and Friberg (2013) proposed that the surrogate marker (AUC/MIC) is the better predictor of antibacterial activity of β-lactams antibiotics. White *et al*. (2004) determined the MIC_{50} of amoxicillin of 0.125 μg/mL for the majority of the bacterial pathogens. It is reported that the value of AUC/MIC ≥125 hour provide optimal efficacy against most pathogens (Brown, 1996). To calculate AUIC in the current study, the MIC_{50} of 0.125 μg/mL has been used for amoxicillin against most of the pathogenic bacteria. The obtained AUIC values were 160 and 172 hour in Lohi sheep and Beetal goats respectively. So, it is speculated that the current dose (15 mg/kg) of amoxicillin is sufficient to kill most of the bacteria in sheep and goats. But the optimal dosage is that amount of drug which rapidly kills the microorganisms and prevents the regrowth of bacteria without any support of the defensive system of the body (Toutain *et al*., 2002), So, MIC_{90} should be used in order to calculate the optimal dosage regimen of an antibiotic. Veloo *et al*. (2012) suggested the MIC_{90} of amoxicillin to be 1.5 μg/mL for majority of the bacterial pathogens. By incorporating (MIC_{90} = 1.5 μg/mL) of amoxicillin, the determined values of AUIC were 13.2 and 14.3 hours in Lohi sheep and Beetal goats respectively. The values of the surrogate marker obtained by PK-PD integration in the present study fall below of suggestive values (AUIC = 125 hours). Hence, it is supposed that the current dose of 15 mg/kg will promote the resistant mutants in Lohi sheep and Beetal goats. The priming dose of amoxicillin by means of pharmacokinetic parameters obtained in this study for the treatment of diseases caused by susceptible bacteria with MIC_{90} of ≤1.6 μg/mL computed after intramuscular administration at 24 hour interval is 21.6 and 24.4 mg/kg body weight with a maintenance dose of 15.7 and 18.9 mg/kg body weight in Lohi sheep and Beetal goats. It is speculated that these dosage regimens might achieve the desired value of AUIC = 125 hours; predictor of antibacterial activity. Further, the plasma concentrations will remain in mutant prevention window as a minimum for 75% of dosage time interval which is essential β-lactams to ensure the bacteriological eradication and emergence of resistance explained by Drlica (2003). However, further detailed pharmacodynamic studies of amoxicillin in Lohi sheep and Beetal goats are required to identify an actual as opposed to an assumed MIC_{90} for confirming the dose by generating sufficient data from field isolates in future studies before issuing final recommendations.

Based on the findings of the present study it was concluded that the current administered dose of 15 mg/kg body weight by the intramuscular administration seemed to be effective against most of the respiratory pathogens in Lohi sheep and Beetal goats. However, in the light of PK/PD integration, the optimal doses were suggested to be 21.6 and 24.4 mg/kg/24 hours as priming dose and 15.7 and 18.9 mg/kg/24 hours as maintenance dose which might be successful for therapeutic outcome and prevention of mutant selection pressure in Lohi sheep and Beetal goats respectively. It is prudent to conduct further detailed pharmacodynamic studies of amoxicillin against enteric disease causing organisms in Lohi sheep and

Beetal goats in order to establish PK-PD inter-relationships.

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