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Short Communication

Effect of Vitamin E Supplementation on Humoral Immunity Following the Administration of Enterotoxaemia Vaccine in Goats

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Authors' Contributions
MAK, AZD, SBK and SA designed the study. MAK, IA, IH, KP, AU, MS, SZ, AIA, GR and NUK executed the experiment and analyzed the samples. IUK, MA, NU, IK and MS helped in data analysis and article drafting.

Key words
Humoral immunity, *Clostridium perfringens*, Epsilon toxin, Vaccine, Haemagglutination.

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ABSTRACT

This study was conducted to evaluate and compare the effects of vitamin E supplementation on humoral immunity against *Clostridium perfringens* type D epsilon toxin in goats to sort out better immunogenic approach. This study was conducted on total of 36 healthy animal of equal number of rabbits and goats. Each species was divided randomly into three equal (n=6) groups. Group A was supplemented vitamin E and group B was without vitamin E supplementation in both species. Animals of both groups were vaccinated with enterotoxaemia vaccine. Group C was kept as negative control in both rabbits and goats. The immune titer against *C. perfringens* type D was evaluated on 0, 7, 14, 28th and 60th day of vaccination with indirect haemagglutination test (IHA). The results showed significantly higher (P< 0.05) immune titer in vitamin E supplemented animals groups against *C. perfringens* type D epsilon toxin.

Goats are the main part of livestock population in Pakistan, enumerating 70.34 millions animals where 6.48 millions goats are present in Khyber Pakhtunkhwa (KPK) province (Livestock census. 2010). Enteric *Clostridium perfringens* (*C. perfringens*) infections known as enterotoxemia is an important bacterial disease of goats and sheep causes huge losses to small ruminants industry (Niilo, 1980; Kriek *et al*., 1994). The disease has very short course and provide less or no time to control in goats (Veschi *et al*., 2008). The pathogen produce four important types of exotoxins (α, β, ε and ί), on the ability of producing various type of toxins are classified into five toxinotypes *i.e.* A-E (Uzal and Songer, 2008). Among all these five toxinotypes, *C. perfringens* type D is the mainly involved in caprine enterotoxaemia (Smith and Sherman, 2011). The *C. perfringens* type D is (19.53%) prevalent in enterotoxemia suspected goats in northern Khyber Pakhtunkhwa province in Pakistan (Khan *et al.,* 2017). Humoral immunity plays key role in protecting animals from enterotoxaemia but the antibody titer remains lower and shorter duration in goats after enterotoxaemia vaccination in comparison to the sheep (Green *et al.,* 1987). The reason is still not known that why enterotoxaemia vaccine responses differently to goats and sheep (Uzal and Kelly, 1998). Enhancing humoral immunity to *Clostridium* type D toxins, therefore, may lead to increased protection against enterotoxaemia. Vitamin E significantly enhanced resistance to infectious diseases in chickens (Tengerdy

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et al., 1978), mice (Hcinzerling *et al.,* 1974) and sheep (Stephens *et al.*, 1979; Tengerdy *et al.,* 1983).

The study was planned to investigate the immuno-enhancing effect of vitamin E when supplement to goats vaccinated against *Clostridium perfringens* type D.

Materials and methods

Thirty six apparently healthy and not vaccinated against *Clostridium perfringens*, animals (eighteen goats and rabbits each) of four to six months of age, were purchased from local markets of district Mardan and were kept at experimental farm in Animal Health Department, the University of Agriculture Peshawar, Pakistan. The selected animals were confirmed healthy since their feces gave no growth on tryptose sulfite cycloserine agar (TCA) medium. All the animals were kept on ad-libtum green grass for two weeks. Each species was divided in 3 groups (A, B and C) of each 6 animals. Group A's diet was supplement with vitamin E at 30 mg [dl] tocopheryl acetate (Hoffman LaRoche, 50% dry vitamin E) per kg live body weight. Animals in groups A and B were twice vaccinated with enterotoxemia-cum-lamb dysentery vaccine 1 mL subcutaneously (Veterinary Research Institute, Peshawar) with interval of two weeks. This vaccine is *C. perfringens* types B and D bacterin toxoid inactivated with formalin and adjuvated with alum hydroxide.

Isolates of *C. perfringens* type D was obtained from vaccine section of Veterinary Research Institute Peshawar, Khyber Pakhtunkhwa Pakistan. The isolate sample was identified through Gram staining and then grown on Robertson's Cooked Meat medium in CO_2 incubator at 37°C for 36 h. Biochemical characteristics were tested through tests kit (remel Rapid ANA II test kit Lenexa, USA). PCR was performed to confirm type D by using their genomic DNA through alpha and epsilon specific primers F- 5-TGC TAA TGTTAC TGC CGT TGA TAG-3; R- 5-TGC TAA TGTTAC TGC CGT TGA TAG-3 & F-5-ATT AAA ATC ACA ATC ATT CAC TTG-3; R-5-CTT GTG AAG GGA CAT TAT GAG TAA-3 (Khan *et al,*. 2017).

The culture was centrifuged at 2000 rpm at 4°C for half an hour and washed in phosphate buffer (pH 7.2, 0.15 molar). The suspension then sonicated at 20 kHz and 105W for 5 min (Ultrasonic Homogenizer (MODEL 300VT USA). Human RBCs taken from blood group O was sensitized and glutaraldehyde (1%) was mixed for IHA test with sensitized human RBCs of blood group O (Tahir *et al.,* 2013).

For humoral Immunity serum collected from the blood samples of all animals included in this trial on 0, 7th, 14th, 28th and 45th day of the vaccination. All the serum samples were heated for 30 min at 56ºC for inactivation and then stored on -20°C for further analysis. The titers were measured through (IHA) indirect haemagglutination test (Khan *et al*., 2018).

A direct intravenous lethal challenge of ten times the dose of potent LD_{50} epsilon toxin D was used to determine protection percentage. Protection percentage was calculated from number of rabbits survived after lethal challenge. Antibody titers were compared among various groups of animals by one way ANOVA at the level of 95% confidence interval (P<0.05).

Results

Vitamin E supplementation significantly increased humoral antibody titers against epsilon toxin of *C. perfringens* type D in 60 days trail (Table I). Significant difference (*P<0.05*) among groups were observed on 14th, 21th, 28th and 60th after vaccination. The significantly higher geometric mean titer (GMT) 465.67 was produced by group A on 28th post vaccination. The rabbits were challenged on 45th day post vaccination to find out protection percentage. The rabbits in group A exhibited 100%, when challenged on 45th day post vaccination (Table I).

Antibody titers were similarly analyzed and compared in various groups of goats on 0, 7th, 14th, 21th, 28th and 60th day of enterotoxaemia vaccine inoculation. Significant differences (*P<0.05*) among groups were observed on 14th,

Table I.- Comparative geometric mean (Mean±SD) titer against epsilon toxin D in rabbits and goats.

Days	Rabbits			Goats		
	Vitamin E supplemented enterotoxaemia vaccinated	Enterotoxaemia vaccinated	Control	Vitamin E supplemented enterotoxaemia vaccinated	Enterotoxaemia vaccinated	Control
0	1.67±3.2^{a}	2.00±3.9^{a}	1.67±3.2^{a}	1.67±3.2^{a}	1.67±3.2^{a}	2.00±3.9^{a}
7	59.67±6.6^{a}	54.33±6.6^{b}	2.00±3.9^{b}	92.0±7.2^{a}	72.5±6.4^{b}	1.67±3.2^{c}
14	190.83±11.76^{a}	78.0±9.8^{b}	2.00±3.9^{c}	205.17±47.2^{a}	109.50±9.9^{b}	2.00±3.9^{c}
21	311.83±50.7^{a}	166.0±12.1^{b}	1.67±3.2^{c}	373.33±53.3^{a}	184.67±15.2^{b}	2.00±3.9^{c}
28	465.67±69.7 ^{a}	232.83±34.8 ^{b}	2.00±3.9 ^{c}	658.0±22.1 ^{a}	326.5±66.1 ^{b}	1.67±3.2 ^{c}
60	361.16±64.3 ^{a}	218.0±25.8 ^{b}	1.67±3.2 ^{c}	361.16±64.2 ^{a}	182.37±32.3 ^{b}	2.67±5.2 ^{c}

Difference in superscripts represent significant (*P<0.05*) difference.

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28th and 45th after vaccination. The highest geometric mean titer (GMT) 658.0 was produced by group A on 28th post vaccination while group B produced 326.5 and group C indicated baseline antibody titer (Table I).

Vaccination against *C. perfringens* type D is the main prophylactic measure in controlling the losses from this disease but there is no specific caprine enterotoxaemia vaccine. Ovine vaccine are used for the purpose (Blackwell *et al*., 1983). The antibody titre produced from these vaccines in goats were low and for shorter duration (Green *et al*., 1987). The reason for the differences between ovine and caprine are unknown (Uzal and Kelly, 1998). Monika *et al*. (2011) suggested that flaws in the present immunization strategies against enterotoxaemia needs further study in this field.

For this purpose the present project was designed to study the effect of vitamin E supplementation on immune titer, duration and protection against *C. perfringens* type D infection. The results indicated that vitamin E supplementation greatly enhances the immune response of goats against epsilon toxin D of *C. perfringens*. The results indicated that vitamin E supplementation greatly enhances the immune response of goats against epsilon toxin D of *C. perfringens*. The findings are in agreement with the previous studies (Prince *et al*., 2017; Tengerdy *et al.,* 1983) conducted in various animal species against different viral and bacterial diseases. The study indicated improved humoral immunity response with vitamin E supplementations. Our study trial proved impressive humoral immunity response with vitamin E supplementation, although a better relationship was found between increased humoral immunity and protection percentage against *C. perfringens* type D toxins.

This beneficial effect of vitamin E was studied for the 1st time in goats which protected goats from the lethal challenge of C. perfringens type D toxins. Immune titers were calculated through Indirect haemagglutination test in both animal species similarly determined by Tahir *et al*. (2013).

The results of our indicated the synergistic role of vitamin E along with enterotoxaemia vaccine in the induction of better and long immune response against *C. perfringens* type D infection.

Statement of conflict of interest

All authors declare no conflict of interest.

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