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ROLE OF *CLOSTRIDIUM PERFRINGENS* IN PATHOGENICITY OF SOME DOMESTIC ANIMALS

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ABSTRACT

Clostridium perfringens, is an anaerobic, gram-positive, pathogenic and spore-forming bacillus and broadly gave out in our territory. This bacterium has spore formation capability and creating gangrene and gastrointestinal disease, for example food poisoning and necrotic enteritis in human, whilst in other animals, gastrointestinal and enterotoxemic diseases more happening. Prevalence of necrotic enteritis, created by *C. perfringens*, has been often stated in sheep, chickens and ostrich throughout the world. The most critical problem for epidemiological investigations and vaccines improvement is accurate recognition of *C. perfringens* variants. Moreover, Small ruminants, especially native breed types, play an important role to the livelihoods of a considerable part of human population in the tropics from socio-economic aspects. Therefore, integrated attempt in terms of management and genetic improvement to enhance production is of crucial importance. Poultry provide humans with companionship, food and fiber in the form of eggs, meat and feathers. Many people love to raise and show chickens and other poultry species at fairs and other poultry shows. Others just love to raise them for backyard pets and for fresh eggs every day. In the last few years, ostrich farming has progressed dramatically and the world ostrich industry has achieved some economic stability. There is considerable scope for improvement in the areas of artificial incubation, chick nutrition, environmental requirements and selective breeding. Hence, the aim of this paper was to study role of *Clostridium perfringens* in pathogenicity of sheep, broilers and Ostrich. In conclusion, recognition of toxins producing by *C. perfringens* is very momentous because their toxin types are related to particular gastric and intestinal animal sickness and PCR has become an essential research and diagnostic tool, being a powerful technique with a vast and increasing range of applications. Hence, it is better that animal breeders identify different types of *C. perfringens* using PCR technique to prevent the damage caused by this bacterium.

Keywords: *Clostridium perfringens*, sheep, chickens, ostrich.

INTRODUCTION

Clostridium perfringens, is an anaerobic, gram-positive, pathogenic and spore-forming bacillus and broadly gave out in our territory (Ahsani et al., 2011). This pathogen very quickly grows in the soil, water, air, food, and gastroenteric of organisms (Songer et al., 1996) and is one of the most causing agents of clostridial disease in the world. Moreover, human food poisoning generally originates from domestic animals. For decreasing and removing this danger, we have to able expand some plans for identifying and avoiding infected animals to get involved in the food chain (Piatti et al., 2004). Hence, the aim of this paper was to study role of *Clostridium perfringens* in pathogenicity of sheep, broilers and Ostrich.

CLOSTRIDIUM PERFRINGENS

Clostridium perfringens (*C. perfringens*) has spore formation capability and creating gangrene and gastrointestinal disease, for example food poisoning and necrotic enteritis in human, whilst in other animals, gastrointestinal and enterotoxemic diseases more happening. This bacterium does not foray healthy cells however generates different toxins and enzymes making accountability for the related wounds and signs (Sawires and Songer, 2006). A toxin creating appertains on the *C. perfringens* strain and every kind of toxin causes a special sickness. The most critical problem for epidemiological investigations and vaccines improvement is accurate recognition of *C. perfringens* variants. Five types (A–E) of *C. perfringens* based on the synthesis of four major lethal toxins, alpha, beta, epsilon and iota identified and introduced (Gurjar et al., 2008; Ahsani et al., 2010). Types A, B, C, D and E of this bacterium produce alpha toxin; alpha, beta and epsilon toxins; alpha and beta toxins; alpha and epsilon toxins; and alpha and iota toxins, respectively (Kalender et al., 2005; Gurjar et al., 2008; Ahsani et al., 2011). Recognition of toxins producing by *C. perfringens* is very momentous because their toxin types are related to particular gastric and intestinal animal sickness (Van Asten et al., 2009; Gurjar et al., 2008; Ahsani et al., 2011).

DETECTING METHODS OF CLOSTRIDIUM PERFRINGENS

Prevalence of necrotic enteritis, created by *C. perfringens*, has been often stated in sheep, chickens and ostrich throughout the world. Detecting different types of *C. Perfringens* applying biochemical tests is inconceivable (MacFaddin et al., 2000). Defining and distinguishing of bacterial toxins was done using serum neutralization test in mice and guinea pigs in several investigation centers. This tiresome, prolonged, costly and monovalent method is inappropriate and immoral for applying at the expense of laboratory animals (Ahsani et al., 2010b). Instead of the serum neutralization test, ELISA was used to quantify the antitoxin ε in sheep blood serum and showed that this technique is more economical, faster and more specific (Timoney et al., 1988). In ELISA method polyclonal antibodies are used for recognition of *C. perfringens* toxins (Baron et al., 1990). But, created antibodies operate versus toxins, because there is an interaction between them. Hence recognition of toxin types will be laborious. Furthermore ELISA cannot identify β2 toxins and for recognition toxins from spore-forming bacteria must be activated using particular culture (Baums et al., 2004). Biochemical tests are also incapable of distinguishing different types of *C. perfringens* (MacFaddin et al., 2000). The newest applied distinguishing technology for infectious diseases is polymerase chain reaction (PCR). In comparison with classical methods, PCR is faster and more valid (Mohammadabadi et al., 2004; Miyashiro et al., 2007; Ahsani et al., 2010a; Mohammadabadi et al., 2011). Therewith, quick and direct recognition of bacterium in clinical samples is possible using PCR (Ahsani et al., 2010b). Genotyping using DNA is reliable and promising, because DNA is a stable marker, is independent from gene expression and less dependent on phenotype. Identifying potency among genomes is momentous

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(Baums et al., 2004). The most important characteristics of genotyping method are the typing capability, reproducibility, discriminatory power and also the easiness and price of performing the analysis. Several PCR methods have since been established for typing isolates of *C. perfringens* (Ahsani et al., 2010b; Ahsani et al., 2011; Zandi et al., 2014; Shahdadnejad et al., 2016). Ahsani et al. (2010b) identified the several types of *C. perfringens* using multiplex PCR in sheep. They concluded that molecular methods provide a new insight into bacterial classification. They offer essential genetic information about the organism of interest, which comprises one of the most practical and helpful aspects of PCR. As PCR employs specific primers, an individual species or strain may be traced among different species or types of organisms. Compared to other identification techniques that are based on large amounts of samples, PCR is safer for researchers; since sample preparation for PCR starts with cell lysis and DNA extraction. It is unnecessary to say that the cell cannot survive this stage and loses its pathogenic property. In conclusion, PCR has become an essential research and diagnostic tool, being a powerful technique with a vast and increasing range of applications.

SHEEP

Small ruminants, especially native breed types, play an important role to the livelihoods of a considerable part of human population in the tropics from socio-economic aspects. Therefore, integrated attempt in terms of management and genetic improvement to enhance production is of crucial importance (Mohammadabadi and Sattayimokhtari, 2013). Economical and biological efficiency of sheep production enterprises generally improves by increasing productivity and reproductive performance of ewes (Soufy et al., 2009). There are more than 50 million heads of sheep in Iran, of 27 breeds and ecotypes (Khodabakhshzadeh et al., 2016) that have not defined well as distinct breeds. However, they are considered as geographically defined populations.

One of the most important breeds of Iranian sheep is Kermani sheep (Mohammadabadi et al., 2017). This local breed lives in the south-eastern of Iran and is a fat-tail breed and well adapted to a wide range of harsh environmental conditions in Kerman province. The ability to adapt to different environmental circumstances is a desirable characteristic of this breed. This breed is one of the best Iranian wool sheep breeds which fleece is white and the wool is coarse and curly. The average weight for the rams is 48-50 kg and for the ewes, 45-48 kg (Khodabakhshzadeh et al., 2016). Hence rapid identification of pathogens especially *C. perfringens* in this sheep breed is very important.

Detecting different types of *C. perfringens* in an area is important for the improvement of the most appropriate vaccines (Kalender et al., 2005). In addition, the effects of the developed vaccines on prevention of the disease need to be investigated. De la Rosa et al. (1997) compared vaccination schedules for ewes and their lambs to raise antibody concentrations against epsilon toxin of *C. perfringens*, the causative agent of enterotoxaemia. They observed that vaccination of lambs did not increase sera antibody concentration but prepartum vaccination of the dams increased antibody concentrations in the lambs when compared with the lambs reared by unvaccinated ewes. El Idrissi et al. (1992) reported that C. sordellii alone or with *C. perfringens* may be an important pathogen in sudden mortality in sheep (El Idrissi et al., 1992). They found that as there was no significant difference between vaccinated and unvaccinated sheep with regard to clostridial infections, vaccines or vaccination programs need to be improved (El Idrissi et al., 1992). Ahsani et al. (2010b) identified the different types of *C. perfringens* using multiplex PCR in sheep. Based on their results, out of 23 *C. perfringens* types, isolated by biochemical tests, four (17.39%) were type A, five (21.74%) were type B, eight (34.78%) were type C and six (26.09%) were type D, with type C as the dominant one. Therefore, they recommended that vaccination against enterotoxemia in Kerman province should provide adequate immunity, especially against *C. perfringens* types A and C. Furthermore, enterotoxemia in sheep is asymptomatic; thus, molecular detection of *C. perfringens* in farms may be useful for prophylaxis.

Ahsani et al. (2011) genotyped *C. perfringens* isolates from vaccinated and unvaccinated sheep and evaluated enterotoxaemia vaccine effects on reduction of *C. perfringens* isolations and prevention of enterotoxaemia in Kermani sheep. Their genotyping of 2 strains isolated from the vaccinated sheep indicated that these strains were type D, while the strains isolated from the unvaccinated sheep were types A, B, C and D; 14.8% (4 out of 27), 22.2% (6 out of 27), 40.7% (11 out of 27) and 22.2% (6 out of 27), respectively. However, no isolate containing the iota gene (type E) was detected. Vaccination against enterotoxaemia had a significant effect ($P< 0.01$) on reducing *C. perfringens* isolates. Occurrence of the disease in the vaccinated and unvaccinated groups was 3.3% and 64.0% ($P < 0.01$), respectively.

The immune response to a vaccine depends on the type of the vaccine used and the ability of the animal to respond to the vaccine administered. Some vaccines can protect up to 98% of the vaccinated animals while some produce lower protection rates. A vaccine may induce a weaker immunoprotective response if the animal has a weak immune system (Leite-Browning, 2007). Vaccines such as enterotoxaemia vaccine require that two doses be given (Leite-Browning, 2007). It has been recommended that a booster dose of enterotoxaemia must be used 4–6 weeks after the first vaccination (De la Rosa et al., 1997; Uzal and Kelly, 1998). Veschi et al. (2006) concluded that the first enterotoxaemia vaccination followed by a booster dose after 40 days triggered satisfactory antibody levels (Veschi et al., 2006). In this research, a booster dose vaccine was applied 1 month after the first vaccination and the results showed that the prophylaxis of enterotoxaemia in sheep could be achieved by this vaccination program. The enterotoxaemia vaccine used in the present study was 96.7% efficient indicating that this vac cine has adequate protective immunity. However, as only *C. perfringens* type D was isolated from the vaccinated group, it is recommended that the vaccine be more protective against this type of *C. perfringens*. Enterotoxaemia is one of the most frequently occurring diseases in sheep and goats worldwide that has been ranked as the third one amongst the importance diseases causing death (Tooloei and Masodie, 2008). Reports from different countries around the world indicated that prevalence rates of enterotoxaemia vary between 10% and 100% (Miyashiro et al., 2007). It has been also reported that *C. perfringens* D is the dominant type causing enterotoxaemia in sheep in Iran (Tooloei and Masodie, 2008). In present study, type D was also found as the dominant type in the vaccinated group though type C was the dominant type (40.7%) in the unvaccinated sheep. It should be noted that some animals having high levels of enterotoxins could move around without showing the signs of illness until found dead or

exhibit the acute form of the disease (Vaikosen and Ikhatua, 2005). In addition, some strains of *C. perfringens* may not be able to produce toxin under laboratory conditions and this complicates classical typing methods (Kalender et al., 2005).

BROILER

Poultry provide humans with companionship, food and fiber in the form of eggs, meat and feathers. Many people love to raise and show chickens and other poultry species at fairs and other poultry shows. Others just love to raise them for backyard pets and for fresh eggs every day. There is a large commercial chicken industry that provides us with eggs and meat. Breeding of household poultry in Persia (Iran) and its dispersion all over this country has an ancient history. Persia was a tremendous empire from the 5th century BC to almost the 7th century AD, and prolonged from India (Delhi) to the Black and the Mediterranean seas. At those periods and later, in the middle Ages, Persia was placed at the crossroads of main roads for redeploying commodities, containing the household poultry, from the East to the West, both by ground and waterways. Many wars in the zone of Persia and neighbor countries in those times could also simplify the distributing of the chicken populations. Archaeological excavations affirmed the presence of the household poultry in the zone of Iran at the old periods (Mohammadabadi *et al*., 2010b). Results of investigations have demonstrated that Persian chickens from the Gilan Province play a role in the source of the Russian Orloff breed (Mohammadabadi *et al*., 2010b). Since 1981, twelve chicken breeding stations were established for multiplying native chicken vaudevilles, and entirely retained about 8000 birds. At this time, there are eight breeding stations in Fars, West Azarbaijan, Isfahan, Mazandaran, Khorasan, Yazd, Zanjan and Khuzestan provinces (Mohammadabadi *et al*., 2010b). Some investigations have performed on Iranian native chicken populations and the information on their genetic diversity of various genes have been reproduced (Mohammadabadi *et al*., 2010b; Mohammadifar *et al*., 2013; Moazeni *et al*., 2016a and b). Afshari et al. (2015) typed *C. perfringens* in broilers meat collected from retail meat shops in Mashhad city of Iran using multiplex PCR and indicated that *C. perfringens* type C is the most common type in broiler chicken carcasses. Shahdadnejad et al. (2016) determined the incidence and toxin typing of *C. perfringens* in intestinal tract of broilers collected from Kerman province in south east of Iran. They isolated only *C. perfringens* type A from broilers chicken samples in Kerman province, hence, they recommended that inoculation versus enterotoxemia in this province must cater enough inviolability, particularly versus *C. perfringens* types A and moreover, enterotoxemia in poultry is asymptomatic; thus, molecular primers employed in this research were excellently specific for detection of *C. perfringens* encoding toxin gene. All of the isolates from intestinal tract of broilers chicken in Kerman province were confirmed as *C. perfringens* type A by the detection of cpa gene using multiplex PCR. It is not surprising that, in this study, all *C. perfringens* isolates were found to be positive according to multiplex PCR results and that all of them were determined as type A. It can be explained by: (i) the fact that cpa toxin genes are common genes for all *C. perfringens* types, (ii) it is common among *C. perfringens* types worldwide, (iii) *C. perfringens* type A is dominant in almost all of the research concerning poultry Guran et al., 2013; Nowell et al., 2010).

OSTRICH

The ostrich is undoubtedly the world's largest living bird. Adult males stand 2.4 m tall and can weigh well over 100 kg; the hen is slightly smaller. Ostriches are flightless birds, with their great body size and reduced wing size rendering them incapable of flying. They have a long neck, long bare legs and two toes. Their strong legs allow them to run up to 70 km per hour when necessary, with strides of up to 8 m. Neck and thigh muscles are well developed and unfeathered. Since ancient times, ostriches have aroused people's interest. Apart from being hunted for their flesh and plumes, ostriches were kept in captivity, tamed (Siegfried, 1984). The species name *Struthio camelus* comes from Latin. The word *camelus* is based on the similarities ostriches have with camels, such as their prominent eyes and eyelashes, their large size and their remarkable tolerance to the desert habitat. The first commercial ostrich farm was established in South Africa in about 1860 solely for harvesting the feathers every six to eight months (Vyver, 1992). In the last few years, ostrich farming has progressed dramatically and the world ostrich industry has achieved some economic stability. There is considerable scope for improvement in the areas of artificial incubation, chick nutrition, environmental requirements and selective breeding. Zandi et al (12) typed toxigenic isolates of *C. perfringens* by multiplex PCR in Ostrich. Out of 30 *C. perfringens* types, isolated by biochemical tests, all (100%) were type A, 0 (0%) were type B, 0 (0%) were type C and 0 (0%) were type D. They demonstrated that *C. perfringens* isolated types were divided as 100% type A and showed that PCR is the reliable technique for detection of *C. perfringens* isolated types. Their findings disagreed with those from different countries (Albini *et al*., 2008; Kalender *et al*., 2005; Wojdat *et al*., 2006; Yoo *et al*., 1997). The difference comprises the fact that the existence and emergence of some bacterium types is closely related to geographic features, consequently several regions may totally lack one or more types of a certain bacterium. For instance, *C. perfringens* type A has been reported as most prevalent in North America, while types C, D and E are uncommon. Moreover, type A constitutes 97% of isolates in Belgium (Juneja *et al*., 2008). All researchers concluded that PCR has become an essential research and diagnostic tool, being a powerful technique with a vast and increasing range of applications. Prevention of clostridial diseases in ostriches is important in public health and food poisoning in humans. We can produce specific *clostridium* vaccine and preventing from clostridial disease in this animal by identifying the important pathogen varieties in ostrich.

CONCLUSION

Recognition of toxins producing by *C. perfringens* is very momentous because their toxin types are related to particular gastric and intestinal animal sickness and PCR has become an essential research and diagnostic tool, being a powerful technique with a vast and increasing range of applications. Hence, it is better that animal breeders identify different types of *C. perfringens* using PCR technique to prevent the damage caused by this bacterium.

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