Detection of a Newly Described Bacteriocin, Perfrin, Among Clostridium perfringens Isolates from Healthy and Diseased Ostriches and Broiler Chickens in Iran

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Research Note—

Detection of a Newly Described Bacteriocin, Perfrin, Among Clostridium perfringens Isolates from Healthy and Diseased Ostriches and Broiler Chickens in Iran

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SUMMARY. Necrotic enteritis due to Clostridium perfringens strains harboring the netB gene is a well-known disorder in poultry. The aim of this study was to investigate the association of a novel bacteriocin, perfrin, with netB among isolates from healthy and diseased ostriches and broiler chickens. Forty-six C. perfringens isolates from broiler chickens and ostriches collected from 2010 to 2014 were included in this study and subjected to PCR to detect netB and perfrin genes. Six (60%) and 9 (25%) isolates were positive for both netB and perfrin genes in broilers and ostriches, respectively. Statistical analysis found a significant difference between healthy and diseased flocks for perfrin both in broilers and ostriches. For netB, the significant difference was only found between healthy and diseased ostrich flocks. This is the first report of the presence of perfrin in netB-positive C. perfringens strains in ostriches.

RESUMEN. Nota de investigación- Detección de una bacteriocina, perfrina recientemente descrita entre aislamientos de Clostridium perfringens de avestruces y pollos de engorde sanos y enfermos en Irán.

La enteritis necrótica debida a cepas de Clostridium perfringens que albergan el gene netB es un trastorno bien conocido en avicultura. El objetivo de este estudio fue investigar la asociación entre una nueva bacteriocina, perfrina, con el gene netB entre aislamientos de avestruces y de pollos de engorde sanos y enfermos. Cuarenta y seis aislados de C. perfringens de pollos de engorde y avestruces que fueron recolectados entre el 2010 al 2014 se incluyeron en este estudio y se sometieron a PCR para detectar los genes netB y perfrin. Seis (60%) y nueve (25%) aislamientos fueron positivos para ambos genes netB y perfrin en los aislamientos en pollo de engorde y avestruces, respectivamente. El análisis estadístico encontró una diferencia significativa entre las parvadas sanas y enfermas para el gene de la perfrina tanto en pollos de engorde como en avestruces. Para el gene netB la diferencia significativa sólo se encontró entre las parvadas de avestruces sanas y enfermas. Este es el primer reporte de la presencia de perfrina en cepas de C. perfringens positivas para netB en avestruces.

Key words: Clostridium perfringens, perfrin, netB, poultry, necrotic enteritis

Abbreviations: NE = necrotic enteritis, netB = necrotic enteritis toxin B gene, Tpel = C. perfringens Tpel. toxin-encoding gene

Necrotic enteritis (NE) is one of the most important diseases of broiler chickens and turkeys due to resulting economic losses. It is caused by the gram-positive, non-motile anaerobic spore-forming bacillus, Clostridium perfringens (13,14). Several toxigenotypes of C. perfringens have been characterized but only types A and C are known to cause NE in broiler chickens and turkeys. This bacterium lives in the normal intestine flora of a wide range of animals. Under certain conditions, it becomes pathogenic, causing gastrointestinal disorders in human and many kinds of animals (1,12). The pathogenicity of C. perfringens field isolates is based on their toxin secreting ability and the type of toxins that are produced. Four major lethal toxins (alpha, beta, epsilon, iota) have been identified that has classified C. perfringens into five toxinotypes from A to E (3,4). In addition to 4 major toxins, up to 16 other toxins and extracellular enzymes such as C. perfringens alpha toxin gene (CPE), C. perfringens beta toxin gene (CPB2), necrotic enteritis toxin B gene (netB), and C. perfringens Tpel. toxin encoding gene (Tpel) that are secreted by C. perfringens isolates have been detected (4).

In contrast to early studies suggesting the alpha toxin as the main virulence factor involved in pathogenesis of NE, recent investigations have revealed that other toxins, especially netB and Tpel, may play more important roles in the virulence of NE (5,11).

Recently, a novel protein called perfrin has been identified in netB-positive C. perfringens type A strains associated with NE in broiler chickens (13). Perfrin is a bacteriocin that can prevent the growth of other C. perfringens isolates and, therefore, enables perfrin-positive isolates to outcompete other isolates. If these isolates are capable of secreting other toxins, they could easily cause gut lesions in experimental models exposed to other predisposing factors (13).

In the present study, presence of this bacteriocin in netB-positive and netB-negative isolates originating from ostriches and broiler chickens in Iran was investigated.

MATERIALS AND METHODS

Bacteria. Forty-six isolates of C. perfringens type A (10 isolates from 10 broiler flocks and 36 isolates from 36 ostrich flocks) collected from 2010 to 2014 in our laboratory and frozen in 50%