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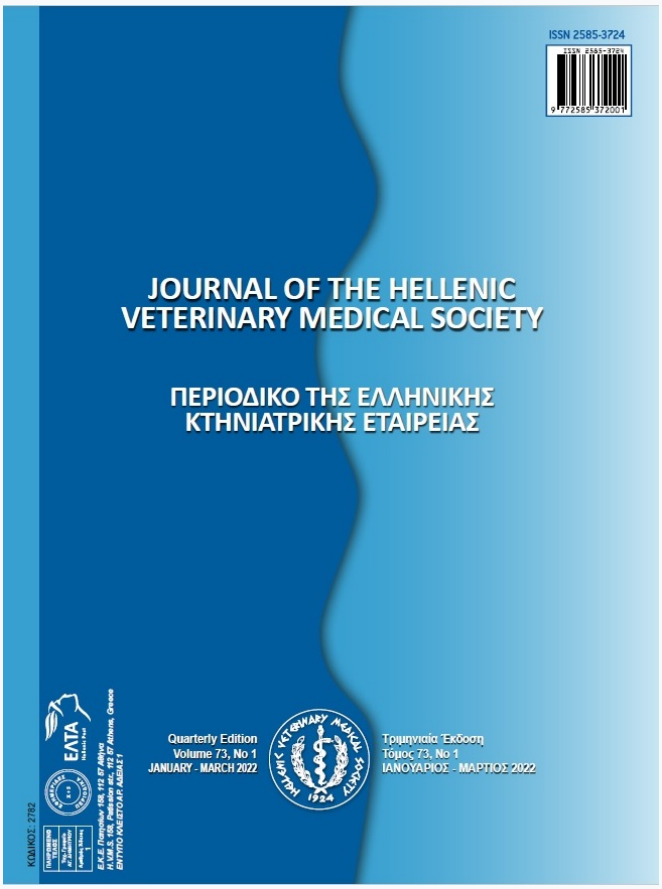

The effect of the challenge with Newcastle disease virus on the gastrointestinal bacterial population in Japanese quail (Coturnix japonica)

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Research article
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The effect of the challenge with Newcastle disease virus on the gastrointestinal bacterial population in Japanese quail (*Coturnix japonica*)

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ABSTRACT: Newcastle disease (ND), caused by virulent strains of Newcastle disease virus (NDV), is a devastating disease of poultry worldwide. The effect of a challenge with NDV on bacterial population in quail is poorly documented, so for this purpose, a total of 100 day-old Japanese quail were purchased and divided into 2 equal groups randomly. Each group was divided into 2 subgroups. The birds in group A challenged with a velogenic chicken isolate of NDV. The birds in group B did not challenge with NDV as the control group. For the determination of *lactobacillus* counts in the intestine and crop of Japanese quail, at the end of the period, 10 birds of each subgroup were chosen randomly. One gram of the crop and ileocecal content were taken and cultured on MRS for determination of *lactobacillus* counts. The colony-forming units of *Escherichia coli* in digesta of ileocecal on Mac Conkey agar were investigated. The results of this study showed that the challenge with a velogenic chicken isolate of NDV could increase colony-forming units of *Escherichia coli* in group A compared to the control group. Also, it reduced lactobacillus counts of intestine and crop compared to the control group. So it concluded that velogenic chicken isolate of NDV influences microflora of intestine and crop of Japanese quail.

Keywords: birds, *Escherichia coli*, gut, *lactobacillus*, velogenic NDV.

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INTRODUCTION

The microflora in the gastrointestinal tract of birds plays an important role in growth performance, nutrition, detoxification of certain compounds, and protection against pathogenic bacteria (Roto et al., 2016; Varmuzova et al., 2016; Sikandar et al., 2020). There is an active and complex microbial community in the animal's gastrointestinal tract, which plays key roles in the immune system, nutrition absorption, pathogenesis, and further in the health and physiological functions of the host; thus, the use of intestinal microflora as probiotic has become one of the hot topics in the international research, especially the use of lactobacillus (Yadav and Jha, 2019). Some studies suggest that various strains of lactobacilli have a stimulating effect on antibody-mediated response in chicken and such effect is dependent on the strain of *Lactobacillus* used and the type (layer- or meat-type) and age of the chicken (Brisbin et al., 2011).However, it remains to be elucidated how probiotics enhance antibody-mediated immune response. It is speculated that probiotics can stimulate the production of Th2 cytokines (e.g., IL-4 and IL-10), which may subsequently enhance the immune response mediated by antibodies (Haghighi et al., 2005). Lactobacillus is one of the predominant bacteria in the animal's gastrointestinal tract (Li et al., 2020).Lactobacillus can produce abundant lactic and acetic acids to lower the pH value in the gastrointestinal tract (Zhao et al., 2021), or compete for the nutrients and epithelial adhesion sites with pathogens to inhibit the growth of pathogens or have the excellent properties of acid and bile salt tolerance as well as the power capacity of colonization and adhesion, and so on (Monteagudo-Mera et al., 2019). Lactobacilli used as probiotics are non-pathogenicgram-positive bacteria that live in the animal intestine. In the chicken, as well as the ability to limit food-borne pathogens and to improve production parameters, the administration of various members of the Lactobacillus species could stimulate multiple aspects of the immune response. These activities include improving systemic antibody response, increasing the number of intestinal epithelial lymphocytes (IELs) expressing CD3, CD4, CD8, and T cell receptors (TCR), modulation of chicken chemokine and cytokine gene expression, and improving the function of T cells (Talazadehet al., 2016).Several lactobacilli strains have been shown to decrease the population of *Salmonella*, *Campylobacter,* and some other non-beneficial bacterial groups in the chicken gut (Nakphaichit et al., 2011).Researchers also demonstrated that multispecies probiotics containing *L. salivarius*, and *L. reuteri* significantly reduced cecal colonization by *C. jejuni*, indicating that probiotic products can also be used to improve food safety by reducing the population of human pathogens, such as *C. jejuni*, in chicken (Ghareeb et al., 2012).Despite the interest in the administration of probiotics in commercial poultry production, to date, there is little information about the mechanisms of stimulation of chicken immune response by probiotic bacteria (Talazadeh et al., 2016). The diagnosis of lactobacilli is carried out by culture-dependent techniques (isolation and culture, microscopic examination, physiological and biochemical events) (Karami et al., 2017). Controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut is important. The intestinal microflora is relatively stable under common circumstances but is easily influenced by various diseases. Since bacteria may play a role in the disease process due to the modification of intestinal innate immunity by dysbiosis and translocation of bacteria, changes of normal microflora in the digestive tract are important sites in the susceptibility of birds to bacterial infection (Cui et al., 2018). ND is a lethal viral disease of poultry and is caused by specified viruses of the avian Paramyxovirus serotype 1 (PMV-1), the serotype of the genus *Rubulavirus*, belonging to the family *Paramyxoviridae.* There are nine serotypes of avian paramyxoviruses designated APMV-1 to APMV-9 (Talazadeh et al., 2016). There is a wide variation in the infectivity of the disease produced by NDV in birds. The pathogenicity of various NDV strains varies from apathogenic strains to velogenic highly virulent strains (Cattoli et al., 2011). The virulence of NDV strains varies greatly with the host, but breed or genetic stock does not appear to have a significant effect on the susceptibility of chickens to the disease (Talazadeh and Mayahi, 2013). NDV has also been categorized into five pathotypes based on clinical signs in infected chickens, including a) viscerotropic velogenic, b) neurotropic velogenic, c) mesogenic, d) lentogenic or respiratory and e) asymptomatic (Getabalew et al., 2019).Viscerotropic velogenic ND (VVND) has been reported in Brazil in ducks, pigeons, quail *(Coturnix),* turkeys, teal, and guan.Although Japanese quails are more resistant to NDV than chickens, the severity of the disease may increase under stress conditions (Mazlan et al., 2017). Today, quail is extensively reared in several countries of the world for human consumption. In the poultry world, quail meat production is negligible compared

to broilers, but occupies a relevant place in poultry breeding and contributes to the variety in poultry meat production (Jeke et al., 2018). Quails are bred for egg and meat production, and the relative importance of their two products varies between countries (Jeke et al., 2018). In recent years, commercial production of quail has increased in some regions of Iran, and a part of the protein demands of Iranian people is provided with the meat of this bird. As the quail industry has developed in the world and also in Iran, therefore it is necessary to study more about the effect of a challenge with NDV in this bird.In the present survey, we intend to determine the effects of a challenge with a velogenic chicken isolate of NDV on the *lactobacillus* numbers (as beneficial microflora) in intestine and crop, and also *Escherichia coli* numbers (as important opportunistic pathogen) in the intestine of Japanese quail.

MATERIAL AND METHODS

Ethics statement

I declare all ethical standards have been respected in the preparation of this article. Ethical permission was granted by the Shahid Chamran University of Ahvaz Ethical Commission for Animal Experiments (22 May 2020) under verification number EE /99.3.02.47185/scu.ac.ir

Virus

Based on the nucleotide sequence, the velogenic NDV used in this experiment was previously characterized as genotype VII (subgenotype VIId) and assigned an accession number of NDa: KP347437. Initially, the virus was propagated twice in 9-day-old embryonated chicken eggs through inoculation into the chorioallantoic sac. The 50% embryo infective dose (EID50) was calculated for the second passage according to the method of Reed and Muench, and the harvested allantoic fluid was used as inoculum as specified in the experimental design.

Vaccination program: At 20 days of age, the chicks of each group were vaccinated with Newcastle B_1 strain (commercial vaccines Avishield® ND B1 was provided by Genera Inc. (Croatia)) via eye-drop.

Animal husbandry, experimental design, and diets

A total of 100 day-old Japanese quail (average body weight 9.53g), were divided into two equal groups randomly.Each group was divided into two subgroups of 25 quails. The birds in group A challenged with a velogenic chicken isolate of NDV. The birds in group B did not challenge with NDV as the control group. Chicks were reared in standard conditions (temperature, ventilation, and light) for 56 days and throughout the trial, birds had free access to water and feed. A standard basal diet in pellet form was provided to the birds. There was no mortality in any of the groups during the study period.They were housed in cages separately in the animal research unit of Shahid Chamran University of Ahvaz (Iran) and received feed and water ad libitum during the experiment. At 34 days of age, when the sera were negative for maternal antibodies in conventional hemagglutination- inhibition (HI) test, the birds in groups A were inoculated with 100 µL (50 µL/eye) of NDV-infected allantoic fluid containing 10^5 EID50 of viral inoculum, whereas the birds in groups B received distilled water by the same route.

Determination of *lactobacillus* counts in intestine and crop

For the determination of *Lactobacillus* counts, 10 and 20 days postinoculation, 10 birds of each subgroup (20 birds of each treatment) were chosen randomly. The contents of the distal part of the small intestine (10 cm anterior to the junction with caecum and rectum) and crop were separately collected, and used for microbial assays. The populations of lactobacillus were estimated as CFU g^{-1}. Sterilized phosphate-buffered saline (PBS) (9 mL) was added to 1 g of fresh materials (1:10), and then subsequent dilutions were prepared. 50 microliters of each dilution were cultured on MRS at 37°C for 48 hours, under microaerophilic conditions, and the presence of bacteria was then determined (Talazadeh et al., 2016).

Escherichia coli counts in the intestinal contents

10 and 20 days postinoculation, for determination of populations of *Escherichia coli* in intestinal digesta of birds, 10 birds of each subgroup (20 birds of each treatment) were chosen randomly. The contents of the distal part of the small intestine (10 cm anterior to the junction with caecum and rectum) of birds were collected and used for microbial assays. The populations of *E. coli* were estimated as CFU g^{-1}. Sterilized phosphate-buffered saline (PBS) (99 mL) was added to 1 g of fresh material (1:100), and then subsequent dilutions were prepared. Samples were cultured on Mac Conkey agar (Merck, Germany), at 37 °C for 24 hours, and the presence of *E. coli* then determined. The original data for *Escherichia coli* counts were

transformed to log10 CFU g^{-1} of intestinal content for statistical analysis (Talazadeh et al., 2016).

Statistical analysis

The data were submitted to analysis of variance using the Statistical Package for Social Sciences (SPSS) version 18.0. Mean differences among treatments were evaluated through the One Way ANOVA LSD Test at $P< 0.05$.

RESULTS

Table 1. The effect of velogenic NDV on lactobacillus counts in ileocecal contents of quails in MRS Agar

Medium / groups	10 days postinoculation	20 days postinoculation
A (challenge) (10^5)	25±3.9[b]	38±3[b]
B (control) (10^5)	48±4.1[a]	57±5.5[a]

*CFU/g± standard deviation of means
Columns with different superscripts (a and b) are significantly different ($P < 0.05$).

Table 2. The effect of velogenic NDV on lactobacillus counts in the crop of quails in MRS Agar

days / groups	10 days postinoculation	20 days postinoculation
A (challenge) (10^5)	16.5±0.7[b]	26±3.5[b]
B (control) (10^5)	44±1.4[a]	59±5.2[a]

*CFU/g± standard deviation of means
Columns with different superscripts (a and b) are significantly different ($P < 0.05$).

Table 3. The effect of velogenic NDV on *E.coli* numbers in ileocecal contents of quails on Mac Conkey agar

days / groups	10 days postinoculation	20 days postinoculation
A (challenge)	9±1.3[b]	20±1.5[b]
B (control)	6.5±2.5[a]	16±3.2[a]

log CFU g^{-1} ± standard deviation of means
Columns with different superscripts (a and b) are significantly different ($P < 0.05$).

According to these tables, the results of this study showed that challenge with velogenic NDV decreased significantly lactobacillus counts in crop and ileocecal contents of quails compared to the control group and increased significantly *E.coli* numbers in ileocecal contents of quails compared to the control group (Table 1-3).

DISCUSSION

Our results suggest that the challenge with a velogenic chicken isolate of NDV decreased the proliferation of beneficial bacteria and increased the presence of gram-negative bacteria. This study indicates that velogenic NDV infection interferes with the intestinal microbiome in quails. The intestinal microflora is relatively stable under common circumstances but is easily influenced by various diseases (Ma et al., 2017).NDV is the causative agent of ND, which is one of the most highly contagious diseases in chickens, and result in severe economic losses to the poultry industry worldwide (Turmagambetova et al., 2017). In Iran, NDV is endemic in different parts of the country, causing enormous losses due to high mortality, sub-optimal production, slaughterhouse condemnation of carcasses, and high prevention and treatment expenses. In recent years, outbreaks of ND have been occasionally observed in different avian species in Iran, including Japanese quail (Momayez et al., 2007), ostrich (Ghiamirad et al., 2010), exotic caged birds (Madadgar et al., 2013), and broiler chickens (Mehrabanpour et al., 2014). Cui et al. (2018) showed that the NDV infection may be associated with the dysbiosis of gut flora and NDV infection interferes with the formation of the intestinal microbiome in newly hatched chicks and loss of a subset of bacteria along with decreased richness and diversity were observed in the gastrointestinal tract of NDV infected newly hatched chicks (Cui et al., 2018). Similar phenomena are also found in many other important poultry diseases like *Eimeria tenella* (Zhou et al., 2017), Marek's disease virus (Perumbakkam et al., 2014), avian leukosis virus (Ma et al., 2017), etc. The normal microbiota of the gastrointestinal tract of chickens plays an important role in inhibiting the establishment of intestinal pathogens

(Güntheret al., 2016). Indeed, some important pathogens like *Rhodoplanes* were found to be enriched in both the duodenum and the ceca of the NDV infected chicks. It is resumed that *Rhodoplanes sp.* might be an emerging human pathogen involved in unknown febrile conditions and could cause local infection of any tissues or organs (Zhang et al., 2011). Besides, the duodenum showed relatively homogeneous flora among individuals, but NDV infection made a preference for implantation of known conditionedpathogens. Similarly, the ceca of normal chicks were dominated by *Paenibacillus* or *Enterococcus* at hatch. Several *Paenibacillus species* produce antimicrobial substances that affect a wide spectrum of microorganisms such as fungi, soil bacteria, plant pathogenic bacteria, and even important anaerobic pathogens such as *Clostridium botulinum* and *Paenibacillus pasadenensis* (Passera et al., 2017). In line with this, more *Epulopiscium* and *Clostridium* were established accompanied by the complete loss of *Paenibacillus* after the infection of NDV in newly hatched individuals. *Clostridium* contains around 100 species that include common free-living bacteria, as well as important pathogens. A previous study conferred that bacterial infections could also be enhanced by NDV in a mice model (Cuiet al., 2018). Therefore, NDV infection increased the chance of secondary infection since the intestinal gut might be a great source of conditionedpathogens. Bacterial dysbiosis has been linked to altered immune function and/or persistent inflammation. Understanding the roles of microbiota in intestinal mucosal immunity should offer novel insight into gastrointestinal disease pathophysiology and deliver new immunotherapy strategies (Chang and Lin, 2016).Microbial dysbiosis and translocation are associated with systemic immune activation in HIV and SIV infections, which in turn helps the increase of virus load in the host (Marchetti et al., 2011).It is increasingly observed that the NDV infection may be associated with the dysbiosis of gut flora. NDV infection is associated with mucosal damage in chickens. Thus, it is imperative to better understand the interplay between intestinal microbiota changes and the pathogenesis of NDV infection. The data will be useful for future studies related to the pathophysiology of NDV in birds and for experiments evaluating the interactions of NDV and bacteria, and other mixed infections in poultry. It has been reported that NDV infection of chicks induced disproportion of gastrointestinal tract microbial population (Cui et al., 2018). A similar phenomenon was also found in our study.Indeed, in the present study, the velogenic chicken isolate of NDV, induced disproportion of the gastrointestinal tract microbial population. So *Escherichia coli* as an important opportunistic pathogen was found to be enriched in the intestine of the NDV infected quails. Therefore, a velogenic chicken isolate of NDV increased the chance of secondary infection since the intestinal gut might be a great source of conditioned pathogens. Also, in the present study, a velogenic chicken isolate of NDV decreased lactobacillus counts of intestine and crop compared to the control group. This study showed that the challenge with a velogenic chicken isolate of NDV decreased the proliferation of beneficial microflora and increased the presence of gram-negative bacteria. This study indicates that velogenic NDV infection interferes with the intestinal microbiome in quails. The data will be useful for future studies related to the pathophysiology of NDV in quails and for experiments evaluating the interactions of NDV and bacteria, and other mixed infections in poultry.

CONCLUSIONS

In the present study, the velogenic chicken isolate of NDV, induced disproportion of the gastrointestinal tract microbial population. So *Escherichia coli* as an important opportunistic pathogen was found to be enriched in the intestine of the NDV infected quails. Therefore, a velogenic chicken isolate of NDV increased the chance of secondary infection since the intestinal gut might be a great source of conditioned pathogens. Also, in the present study, a velogenic chicken isolate of NDV decreased lactobacillus counts of the intestine and crop as beneficial microflora. So it concluded that NDV may have serious consequences in quail farms and according to these findings vaccination against NDV in quail farms of Iran is highly recommended. Controlling factors like isolation of the farms, applying bio-security, decreasing stress conditions, certification of quail movement, and particularly enforcement of vaccination programs, etc., must be considered to improve disease security and reduce danger of spreading of infection.

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CONFLICT OF INTEREST

None was declared by the authors.

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