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The Effect of *Tsukamurella inchonensis* Bacterin on the Immune Response Against Influenza and Newcastle Disease Vaccines in Broiler Chickens

Forough Talazadeh[1*], Mansour Mayahi[2], Katayoon Nofouzi[3], Ebrahim Golzari[4], Rahil Chegini[4]

[1]Assistant Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2]Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3]Associate Professor, Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[4]Graduate from Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

***Corresponding Author:**
Forough Talazadeh, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
Tel: +98-6113330073;
Fax: +98-6113360807,
Email: ftalazade@gmail.com

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Keywords: Avian influenza virus, *Tsukamurella inchonensis* bacterin, Immune response, Newcastle disease virus, Broiler chickens

Abstract

Background: In poultry production, improving immunity is very important in preventing infectious diseases. One solution to improve the immunity of animals and to decrease their susceptibility to infectious diseases is administration of immunostimulants. Surveys have indicated that some bacteria such as *Mycobacterium vaccae* can work as immunomodulators, promote Th1-mediated mechanisms, and switch off pre-existing Th2 preponderance.[1]

Objectives: The aim of this study was to examine the effect of *Tsukamurella inchonensis* bacterin on the immune response against influenza and Newcastle disease (ND) vaccines in broiler chickens.

Materials and Methods: A total of 170 one-day-old broiler chicks were purchased and divided randomly into 5 equal groups. Chickens of group A received 10^6 bacterin subcutaneously 2 days before vaccination against ND and avian influenza (AI). Chickens of group B received 10^6 bacterin subcutaneously 6 days after the first injection of bacterin. Chickens of group C received 10^6 bacterin subcutaneously 6 days after the second injection of bacterin. Chickens of group D were vaccinated against ND and AI but did not receive bacterin. Chickens of group E were not vaccinated against ND and AI and did not receive bacterin. All groups except group E were vaccinated with live Newcastle vaccine and AI-ND killed vaccine (subtype H9N2). Blood samples were collected and antibody titer against ND vaccine and AI vaccine was determined by hemagglutination inhibition (HI) test.

Results: The results of present study showed that receiving *T. inchonensis* bacterin for 3 times significantly increased the specific antibody response to AI subtype H9N2 vaccine. Moreover, the specific antibody response to Newcastle vaccine was significantly increased at days 21 and 28 after vaccination.

Conclusion: Receiving *T. inchonensis* bacterin can enhance immune response against ND virus and AI virus.

Keywords: Avian influenza virus (AIV), *Tsukamurella inchonensis* bacterin, Immune response, ND virus, Broiler chickens

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Background

The successful poultry production is based on feeding, breeding, marketing, management, and the well-developed immune status of the birds. Immunity means the power of resistance against the pathogenic microorganisms. Such power of defense has two main sources; natural and induced. Schat and Myers[2] stated that the natural immunity in the avian is transmitted through egg yolk, which directly releases maternal antibodies into the intestine and causes protection against infections in the young chicks. Efforts have been made to compensate the natural immune status of the broilers.

Influenza viruses

Influenza viruses belong to the family of Orthomyxoviridae. They have negative-strand RNA, are segmented, and have three types, A, B, and C.[3] Avian influenza viruses (AIVs) belong to type A, but all three types of influenza

are found in humans. H9N2 virus shares similar receptor binding epitopes with human influenza viruses, so H9N2 virus infects a wide range of hosts, including humans. H9N2 AIV infection has also a latent feature and can easily be ignored, so there is a rising chance to infect humans.[3]

Newcastle Disease

Newcastle Disease (ND) is a deadly viral disease that can infect poultry. It was first isolated in England in 1926. This viral disease is reckoned as one of the great economic dangers to birds because it causes high mortality and morbidity that, depending on the type of ND virus, varies between 90%-100%.[4] It is an acute, contagious infection of domestic birds, free living, and pet. Avian paramyxovirus type 1 causes ND. Firstly, ND was reported in Southeast Asia in 1926.[5] Since then, it has become one of the very serious economic dangers to birds all over the world.[6] ND virus belongs to the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Rubulavirus*.[5,7,8] The virus is negative-sense, enveloped, single-stranded RNA genome.[9] The use of safe and good vaccines is very important in controlling the ND.

Tsukamurella ssp.

Tsukamurella ssp., which belong to the family *Nocardiaceae*, are weakly or variably acid-fast, non-spore-forming, gram-positive, nonmotile, obligate, rod-shaped, and aerobic actinomycetes. They are environmental saprophytes; soil, sludge, and arthropods are their natural habitats. *Tsukamurella* ssp., pathogenic to humans, include *T. paurometabolum*,[10,11] *T. inchonensis*,[12,13] *T. pulmonis*,[14] and *T. tyrosinosolvens*.[15]

Description of *Tsukamurella inchonensis*

Tsukamurella inchonensis does not have capsules. It is acid-alcohol-fast bacterium. Colonies are brownish orange, and rough on Loewen stein Jensen medium. At 24, 31, 37, and 45°C, growth of *T. inchonensis* occurs. *T. inchonensis* has meso-diaminopimelic acid in addition to diamino acid, arabinose, and galactose as determined with whole-cell hydrolysates; the cell wall chemotype is chemotype IV (12).

Objectives

The aim of this study was to examine the effect of *T. inchonensis* bacterin on the immune response against influenza and ND vaccines in broiler chickens.

Materials and Methods

Chickens

A total of 170 one-day-old broiler chicks were purchased and 20 chicks were bled for determination of maternal antibody and remaining chicks were divided randomly into 5 equal groups and each group was divided into 3 equal subgroups.

Vaccinia

Hitchner B1 vaccine Cevac®, and AI- ND killed vaccine (subtype H9N2) were supplied.

Tsukamurella inchonensis

Tsukamurella inchonensis was supplied from BioEos Ltd (Kent, UK).

Experimental Design

Chickens of group A received 10^6 bacterin subcutaneously 2 days before vaccination against ND and avian influenza (AI). Chickens of group B received 10^6 bacterin subcutaneously 6 days after the first injection of bacterin. Chickens of group C received 10^6 bacterin subcutaneously 6 days after the second injection of bacterin. Chickens of group D were vaccinated against ND and AI, but did not receive bacterin. Chickens of group E were not vaccinated against ND and AI, and did not receive bacterin. All groups except group E were vaccinated with live Newcastle vaccine (B1 strain) intraocularly and AI-ND killed vaccine (subtype H9N2) subcutaneously, at neck back at 9 days old.

Blood Collection and Serological Tests

Blood samples were collected before vaccination as well as on days 14, 21, and 28 post-immunization. Ten chickens of each group were bled randomly and antibody titer against ND and Influenza virus vaccines was determined by Hemagglutination inhibition (HI) test. Brachial vein was used and blood samples were drained from, and sera were frozen at -20˚C. HI test was performed to detect antibodies against AIV and NDV in serum samples according to Alexander et al.[16]

Microplate hemagglutination inhibition test

Beta procedure of micro-plate HI test was performed in U-bottomed 96-well µL plates with 1% chicken RBC to determine the antibody level of the sera samples. Four HA unit AIV and 4 HA unit ND virus were used in this test.

Statistical Analysis

SPSS version 18.0 was used to analyze the titers obtained by HI test. After vaccination, the significant differences in HI titres of chickens of each group were determined by one-way analysis of variance (ANOVA) LSD test. Means were compared at a significance level of 5%.

Results

As shown in Table 1, the results indicated that 14 days after vaccination, there was a significant difference between all groups and group E (unvaccinated control). But 14 days after vaccination, there was not any significant difference between those groups that received injection of bacterin and group D (vaccinated control). Twenty-one days after vaccination, there was a significant difference between all groups and group E (unvaccinated control), and between all groups and group D (vaccinated control), but there was not any significant difference between those groups that received injection of bacterin. Twenty-eight days after vaccination, there was a significant difference

Table 1. The Effect of *Tsukamurella inchonensis* on HI Antibody Titer Against ND Vaccine in Broiler Chicks

Groups	Days Post-vaccination			
	0	14	21	28
A	6.75 ± 0.88	5 ± 0.93^{e*}	8.09 ± 0.96^{ed}	8.5 ± 0.93 ^{ed}
B	6.75 ± 0.88	5 ± 0.95 ^{e}	7.84 ± 0.98 ^{ed}	9 ± 0.85^{ed}
C	6.75 ± 0.88	5.35 ± 0.84 ^{e}	8.83 ± 0.93^{ed}	9 ± 0.8 ^{ed}
D (vaccinated)	6.75 ± 0.88	4.7 ± 0.85^{e}	6 ± 0.47^{abce}	6.2 ± 0.98^{abce}
E (unvaccinated)	6.75 ± 0.88	1.7 ± 0.82^{abcd}	-^{abcd}	-^{abcd}

Abbreviations: ND, Newcastle disease; HI, hemagglutination inhibition.
The groups with different superscripts are significantly different in each column ($P < .05$).
*Mean ± standard deviation.

Table 2. The Effect of *Tsukamurella inchonensis* on HI Antibody Titer Against AI Disease Vaccine

Groups	Days Post-vaccination			
	0	14	21	28
A	6 ± 0.9	4.33 ± 0.49 ^{ce*}	5.09 ± 0.94^{e}	5.66 ± 0.98^{e}
B	6 ± 0.9	4.41 ± 0.51 ^{e}	4.61 ± 0.93^{ce}	5.33 ± 0.9^{e}
C	6 ± 0.9	4.75 ± 0.45 ^{ade}	5.83 ± 0.9^{bde}	6 ± 0.91^{de}
D (vaccinated)	6 ± 0.9	4.18 ± 0.4 ^{ce}	4.36 ± 0.92^{ce}	4.5 ± 0.89^{ce}
E (unvaccinated)	6 ± 0.9	1.4 ± 0.32 ^{abcd}	- ^{abcd}	- ^{abcd}

Abbreviations: AI, avian influenza ; HI, hemagglutination inhibition.
The groups with different superscripts are significantly different in each column ($P < .05$).
*Mean ± standard deviation.

between all groups and group E (unvaccinated control), and between all groups and group D (vaccinated control), but there was not any significant difference between those groups that received injection of bacterin. As seen in Table 2, the results indicated that 14 days after vaccination, there was a significant difference between all groups and group E (unvaccinated control). Fourteen days after vaccination, there was also a significant difference between groups A and C, and between groups D and C, and group C (receiving 3 injections of bacterin) had the highest antibody titer compared to group A (receiving one injection of bacterin) and group D (vaccinated control). Twenty-one days after vaccination, there was a significant difference between all groups and group E (unvaccinated control). Additionally, 21 days after vaccination, there was a significant difference between groups B and C, and between groups D and C, and group C (receiving 3 times of bacterin injection) had the highest antibody titer compared to group B (receiving 2 injections of bacterin) and group D (vaccinated control). Twenty-eight days after vaccination, there was a significant difference between all groups and group E (unvaccinated control). And 28 days after vaccination, there was a significant difference between groups D and C, and group C (receiving 3 times of bacterin injection) had higher antibody titer than group D (vaccinated control).

Discussion

Studies have indicated that some bacteria such as *Mycobacterium vaccae* can work as immunomodulators promote Th1-mediated mechanisms, and switch off pre-existing Th2 preponderance.[1] Recently, some other aerobic Actinomycetales species closely related to mycobacteria, including *Rhodococcus coprophilus* (Rc), *Gordonia bronchialis* (Gb), and *Tsukamurella inchonensis* (Ti), which have immunomodulatory or adjuvant activities when injected as suspension of killed bacilli, have been identified.[17,18] Immunotherapy can change a person's immune response to reduce infection either by reducing the severity of the condition or protecting against it.[19] Then, sensitivity to an allergen that causes allergic signs, such as asthma[20] or allergic rhinitis[21] in allergen immunotherapy, is reduced. For modification of the response to venoms, such as those of wasps or bees, for people with acute reactions, subsequent to prior exposure, venom immunotherapy is used.[22] Immunotherapy has been tested for parasitic diseases, chronic bacterial diseases, and chronic viral diseases.[23-25] The host immune response affects the damage caused by the infection in tuberculosis.[26] A mixed lymphocyte response including both type 1 and type 2 responses are more likely to be associated with death of local tissues than a type 1 lymphocyte response in experiments that are performed in animals.[27] In tuberculosis, a type 1 response has an important role in immunity against infection that is characterized by production of gamma interferon.[28] A fast-growing mycobacterium species that is found in the environment is *M. vaccae*. As immunotherapy for tuberculosis, this bacterium has been used as a whole cell killed vaccine.[29,30] It has been proposed that immunotherapy allows immune identification of antigens common to all mycobacteria or that immunotherapy shifts T-lymphocyte responses to a type 1 pattern. Some indirect experimental evidence indicates that the pattern occurs in humans.[26] Recently, experimental

evidence has demonstrated that by vaccination with *M. vaccae*, through induction of regulatory T cells, allergic responses can be reduced.[1] Immunotherapy may cause a more rapid recovery from disease, because it allows the host to ruin infectious agents.

In conclusion, the results of the present study indicated that 21 and 28 days after vaccination, receiving bacterin could improve antibody titer against ND vaccine compared to vaccinated control group. The present study also indicated that 3 times of bacterin injection could improve antibody titer against AI disease vaccine.

Authors' Contribution

Study concept and design: FT, MM, KN, EG, and RC; Analysis and interpretation of data: FT and MM; Drafting of the manuscript: FT; Critical revision of the manuscript for important intellectual content: FT and MM; Statistical analysis: FT.

Conflict of Interest Disclosures

None.

Ethical Approval

Ethical issues have been completely observed by the authors.

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