Original Article

Serological Evaluation of H9-RBD-Pichia, a Novel Recombinant Influenza Vaccine, in BALB/c Mice

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ABSTRACT

Background: The H9N2 influenza virus is one of the most important subtypes of influenza that has caused irreparable damage to the poultry industry, and many efforts are underway to develop its recombinant vaccines.

Objectives: This study aims to evaluate the effectiveness of H9-RBD-Pichia, a novel recombinant subunit vaccine, in stimulating the immune system and producing hemagglutinin-inhibiting antibodies in mice.

Methods: A total of 24 female BALB/c mice were purchased and randomly divided into six groups: Group 1 received oral administration of lysed yeast twice, group 2 received one injection of lysed yeast, group 3 received two injections of lysed yeast, group 4 received one injection of pure recombinant protein, group 5 received two injections of pure recombinant protein and group 6 was negative control. Prime and booster immunizations were performed on days 14 and 28, respectively. Sera samples were collected on day 42. Hemagglutination (HA) and hemagglutination inhibition (HI) assays were performed on antigen and sera samples, respectively.

Results: HA titers of 1:4, 1:4, and 1:32 were seen in the positive control, intact, and bead-treated yeasts, respectively, showing the functionality of the expressed H9-RBD recombinant protein. HI assay showed this vaccine could stimulate the immune system and produce anti-hemagglutinin antibodies in mice. The highest antibody titer (1:512) was observed when yeast lysate was injected twice on days 14 and 28.

Conclusion: The H9-RBD-Pichia vaccine has been produced based on genetic engineering technology and could produce high titers of hemagglutinin-inhibiting antibodies in mice.

Keywords: H9N2, Influenza, *Pichia pastoris*, Recombinant vaccine

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Introduction

The most common subtype of low-pathogenicity avian influenza in poultry is H9N2. In the 1960s, the first isolation of the H9N2 virus was conducted from turkeys in the United States of America (Iqbal et al., 2013). This subtype has been recognized in several mammalian species. Humans, swine, dogs, weasels, and mink are just a few examples in which the H9N2 viruses have been identified. In most cases, the H9N2 virus or its antibodies have been detected during regular surveillance in healthy mammals, but in some instances, they have been linked to clinical illness (Song & Qin, 2020; Sekhavati, 2024).

H9N2 viruses have raised concerns about zoonotic infection; hence, numerous animal models, especially mice, have been employed to assess their propensity to infect humans (Pusch & Suarez, 2018). In Iran, H9N2 avian influenza virus serological evidence has been found among poultry workers, veterinarians, and occupations associated with poultry (Hadipour, 2010; Heidari et al., 2016).

Nili and Asasi (2003) showed that the mortality rates on H9N2-infected farms typically ranged from 20% to 60% in Iran. Although the isolate in Iran's current H9N2 AI outbreak was classified as having low pathogenicity, mortality in the field on several farms reached 65%. Such a significant fatality rate could be attributed to coinfection with other respiratory diseases. Clinical symptoms included anorexia, decreased water consumption, depression, sneezing, coughing, dyspnea, and body weight loss in both field and experimental investigations. In field cases, sinus swelling and ocular-nasal discharge were frequent (Nili & Asasi, 2003).

The major antigen that activates a protective immune response against the influenza virus is hemagglutinin (HA), making it a crucial vaccine target. In addition to mediating the fusion of the viral envelope with the endosomal membrane, HA is expressed on the viral surface as trimeric glycoproteins that bind to sialic acid on target cells to facilitate host cell entrance (Wu & Wilson, 2020).

One of the primary objectives for the health of humans and animals is immune protection against infectious diseases (Lopez & Legge, 2020; Mascola & Fauci, 2020). It has been shown that immunization against influenza can diminish viral shedding and tissue spread in challenged birds (Abdi Haji et al., 2021). Today, in addition to the scientific literature, specific immunization tactics and vaccine-induced immune responses are extensively investigated and widely debated in the media. It is typically difficult to rapidly include the entire infectious agent in a safe and protective vaccine formulation, whether in inactivated or attenuated versions, especially in cases of influenza pandemics. This fact is also supported by the biological risks that are inevitably present when pathogens are chemically inactivated on a large scale or when attenuated strains have the potential to revert to their virulence (Delrue et al., 2012; Lee et al., 2012).

Nowadays, recombinant subunit vaccines are considered an alternative to conventional vaccine strategies because they are safe, and there is no need for large quantities of eggs or live viruses. In addition, producing these vaccines is economical and can be done swiftly during a pandemic (Athmaram et al., 2011; Lei et al., 2016; Ghadimipour et al., 2014). Additionally, tampering with subunit vaccinations might eliminate the need for biosafety level 3 laboratories and high-biosafety facilities.

There are several types of systems for the production of recombinant vaccines. As shown in Figure 1, one of the most interesting systems researchers have widely used in the production of new recombinant subunit vaccines using an expression host system. The most prevalent cell factories utilized for recombinant protein production with appealing characteristics include *Escherichia coli*, a few yeast species, insect cells, mammalian cells, and plant cells (Wang et al., 2019). The yeast cells have been extensively used for the production of different proteins of animal or human origin (Karbalaei et al., 2020). It should be mentioned that there are differences among studies, and the process of producing a recombinant vaccine depends on the type of host, pathogen, and laboratory technique.

Pichia pastoris has become a highly successful expression system due to its increasing popularity, which can be attributed to several factors such as high growth rate, the ease of genetic manipulation, high yield expression of heterologous proteins, and the capability of performing eukaryotic post-translational modifications (Balamurugan et al., 2010). The *P. pastoris* system is much easier to perform and cheaper than bacterial expression systems (Liu et al., 2013). *P. pastoris* usually produces a higher yield of recombinant proteins and is less demanding in terms of time and effort relative to complex eukaryotic systems (Maccani et al., 2014).

The H9-RBD-Pichia is a novel recombinant subunit vaccine produced based on the surface display of the receptor binding site (RBD) of the hemagglutinin antigen in *Pichia pastoris* yeast using genetic engineering technology. This study aims to evaluate the effectiveness of this vaccine in stimulating the immune system and producing hemagglutinin-inhibiting antibodies in mice.

Material and Methods

Preparation of the recombinant vaccine

Our laboratory's new H9 subunit vaccine was used to immunize mice.

Transgenic *P. pastoris* cells containing the H9-RBD gene were cultured, precipitated, and disrupted using the glass bead lysis method described previously (Zhang et al., 2020). In addition, the purification of pure H9-RBD antigen was performed using the HisPur™ Ni-NTA Resin (Thermo Scientific, Catalog Number: 88221) according to the manufacturer's instructions.

Culture condition

Specific media for the culture of *P. pastoris* and the expression of the recombinant antigen, including yeast extract peptone dextrose, buffered complex glycerol medium, and buffered methanol-complex medium, were prepared according to the Pichia expression kit manual (Thermo Fisher Scientific, Catalog Number: K171001).

Mice immunization

Six to eight-week-old healthy female BALB/c mice were purchased from the Mashhad University of Medical Sciences, Mashhad City, Iran, and randomly divided into six groups, with four mice in each group.

Group 1: Oral administration of lysed yeast twice.

Group 2: One subcutaneous injection of lysed yeast.

Group 3: Two subcutaneous injections of lysed yeast.

Group 4: One subcutaneous injection of pure recombinant protein.

Group 5: Two subcutaneous injections of pure recombinant protein

Group 6: Negative control (administration of untransformed yeast)

The animals were housed in a controlled environment at standard room temperature with a 12-hour day/night cycle of light and received a commercially formulated diet and water ad libitum until the end of the experiment.

P. pastoris cells expressing the H9-RBD and untransformed yeast cells, as the negative control, were bead-treated, diluted in 200 µL of sterile endotoxin-free phosphate-buffered saline (PBS) (1×10^9 CFU/mouse), and injected subcutaneously in each group in the neck region. The purified recombinant protein (100 µg/mouse) was also injected without adjuvant. In another group, *P. pastoris* cells were precipitated after expression, washed twice with PBS, resuspended in PBS, and orally administered using a stainless-steel mouse gavage needle. Prime and booster immunizations were performed on days 14 and 28, respectively. Sera samples were collected on day 42. To determine the potential adverse effects of the vaccine, water consumption, food intake, and mice weight were monitored in all groups until the end of the experiment. It should be mentioned that the in vivo experiment was performed according to the animal research reporting in vivo experiments (ARRIVE) guidelines.

Hemagglutination (HA) assay

The recombinant protein expressing *P. pastoris* (OD600 of 10) was washed twice with PBS and used as antigens in an HA assay, along with bead-treated yeast cells, to assess the ability of the expressed recombinant protein to hemagglutinate chicken RBCs. A two-fold serial dilution of antigens was made using PBS in a microtiter plate, followed by adding 25 µL of 1% chicken RBCs and incubating at room temperature for 30 minutes (Sano and Ogawa, 2014).

Hemagglutination inhibition (HI) assay

After heat inactivation at 56 ˚C for 30 min, sera samples were serially diluted twofold and incubated with four HA units of commercially available H9 antigen (Pasouflu, Pasouk, Mahdasht, Iran) in a 96-well U-bottom microtiter plate for 30 minutes at room temperature. Then, 25 µL of 1% PBS-washed chicken erythrocytes were added to each well and incubated for 30 minutes. The HI titer was the maximum serum dilution that could inhibit the hemagglutination reaction (Sano & Ogawa, 2014).

Statistical analysis

The data were statistically analyzed by the one-way analysis of variance (ANOVA) followed by the Tukey test for post hoc comparisons using the SPSS software,

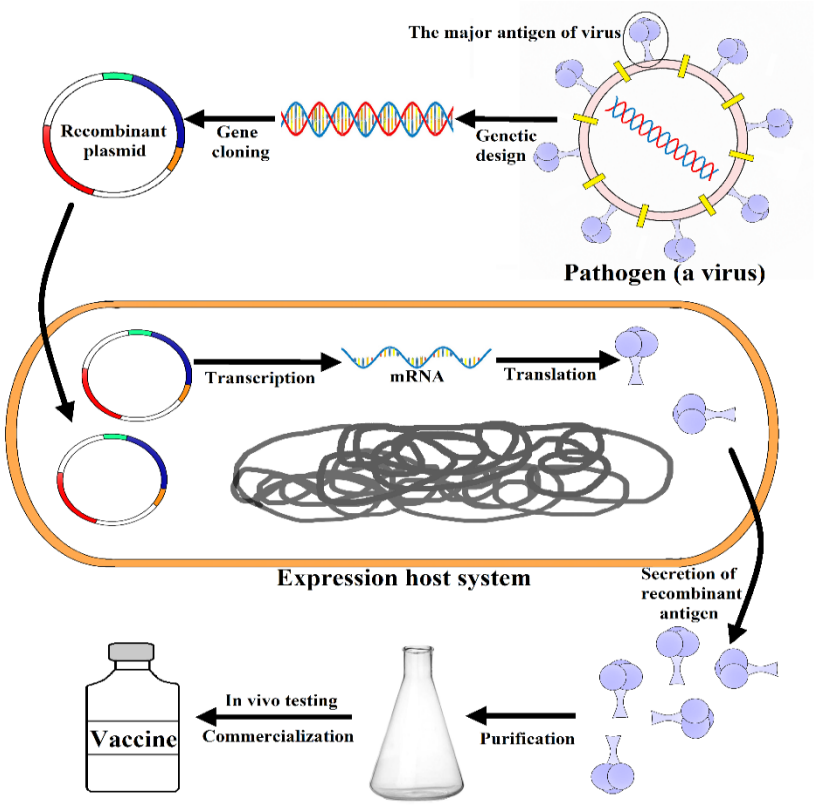

Figure 1. The general process of recombinant vaccine production using the expression system

Notes: The main antigen of a pathogen that stimulates the immune system and produces neutralizing antibodies should be detected, and its nucleotide sequence should be determined. After that, the sequence of this specific gene must be codon optimized and cloned into an expression vector through biotechnological methods such as restriction enzyme digestion and ligation processes. The recombinant plasmid can be transformed into an expression host system. After transcription and translation processes inside the transformed cells, the recombinant antigen can be secreted into the culture medium or attached to the cell surface. In the case of attachment on the surface, the whole cell might be used as a source of antigen, either with or without an adjuvant.

version 22 (SPSS Inc, Chicago, Illinois, USA). $P<0.05$ was considered significant for all tests.

Results

Growth parameter

There was no significant difference between the control and immunized groups regarding food intake or the mice's weight ($P>0.05$). In addition, no mortality was observed in the control or immunized groups.

Hemagglutination assay

HA assay was carried out to ensure the expressed H9-RBD recombinant protein's functionality indirectly. A functional antigen prevents chicken erythrocytes from settling by agglutination. Therefore, settling RBCs at the bottom of the well indicates the lack of hemagglutinin activity in the sample. Settling patterns were observed in negative control wells containing PBS or untransformed *P. pastoris*. HA titers of 1:4, 1:4, and 1:32 were seen in the positive control, intact, and bead-treated yeasts, respectively. The HA titer in the bead-treated yeast was higher than in intact yeast cells (Figure 2). It is plausible that some recombinant protein is retained in the cell or periplasmic space and released after cell lysis (Buckholz & Gleeson, 1991; Ferrara et al., 2006).

HI assay

This experiment found that injection and oral administration of the recombinant vaccine candidate produced in this study could stimulate the immune system and produce anti-hemagglutinin antibodies in mice. The highest antibody titer (1:512) was observed when yeast lysate

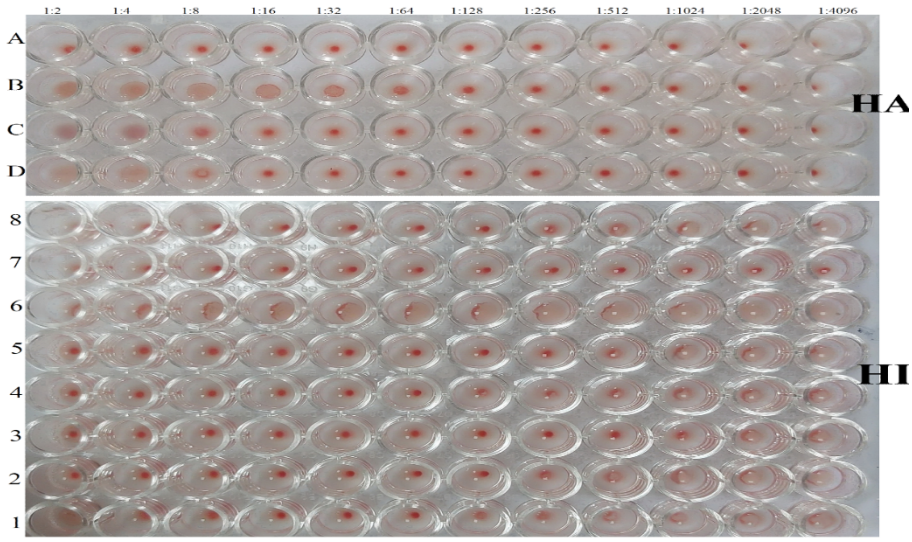

Figure 2. The results of HA and HI tests

Notes: In the HA experiment, the results showed that bead-treated yeasts (B) with 1:16 titer, intact transgenic yeast (C) with 1:4 titer, chimerical HA antigen (D) with 1:4 titer have considerable hemagglutinin activity in chicken red blood cells—negative control (A). Hemagglutinin activity in group C indicated surface-display partial H9 antigen in transgenic yeast. In the HI experiment, the highest antibody titer was observed when yeast lysate was injected twice. Numbers 1-6 are representatives of groups 1-6, respectively. 7: Positive control 8: The second positive control (chicken serum with a titer of 1:128).

was injected twice on days 14 and 28. Administering the booster vaccine increased the antibody titer significantly compared to the groups that received just one dose of the vaccine ($P<0.05$). The antibody titer in injection groups was significantly higher than in oral vaccine groups. No hemagglutination-inhibiting activity was observed in the control serum.

Discussion

P. pastoris is a safe organism whose genome has been completely sequenced and widely used to express endogenous, secreted membrane proteins and produce recombinant subunit vaccines (Juturu & Wu, 2018). For instance, Wasilenko et al. (2010) expressed the HA protein from the influenza H5N1 subtype in the *P. pastoris* GS115 strain and reported oral administration of the transformed yeast-neutralized antibodies in white leghorn chickens. In another study, Pietrzak et al. (2016) transformed a recombinant pPICZαC vector containing H5N1 hemagglutinin into the *P. pastoris* KM 71 strain. Their study showed that the recombinant protein produced in the yeast expression system could be an effective vaccine and protect layer chickens from the lethal challenge. Furthermore, a published paper by De Sá Magalhães and Keshavarz-Moore (2021) showed that *P. pastoris* is a strong and cost-effective tool for producing recombinant vaccines in low- and middle-income countries.

As mentioned before, surface-display technology has produced the H9-RBD-Pichia vaccine. Therefore, the main epitopes of influenza HA antigen have been placed on the external surface of *P. pastoris* yeast, and the resultant vaccine can be administered orally or injected subcutaneously.

In this study, the transgenic yeasts were treated with glass beads. Although some researchers have used the sonication cell lysis method for the disruption of yeast cells, the lysis of *P. pastoris* cells with 0.5 mm glass beads was a more effective method in this study, which is in agreement with Zhang et al. (2020), who showed that bead lysis is a more productive method in comparison to sonication and high-pressure homogenization approaches.

Adjuvants are chemical or biological substances that boost the immune system. According to a study by Radmehri et al. (2021), a combination of the ISA70VG adjuvant and nano-selenium could enhance chickens' immunological response to the bivalent ND+AI vaccine. Analysis of serological tests revealed that the antibody titer in mice injected with bead-treated transgenic yeast is higher than in mice injected with pure antigens. The natural adjuvant activity of *P. pastoris* cell wall components may cause this. It has been previously demonstrated that administering recombinant protein accompanied by yeast cell wall components is more immunogenic than administering pure protein (Rios-Hernandez et al., 1994; Stubbs et al., 2001).

High antibody titers were seen in the groups that received the booster vaccine, which agrees with previous studies. Lei et al. (2020) used surface display technology to produce an oral vaccine against H7N9 influenza. *Saccharomyces cerevisiae* yeast expressing recombinant HA antigen was administered orally to BALB/c mice on days 1 and 14. A single vaccine administration led to the production of low levels of detectable antibodies. However, a significant increase in the level of antibodies was observed after booster immunization.

In the end, it should be pointed out that although vaccination of susceptible species, particularly poultry, is a practical approach for avian influenza control, it should not be considered a single strategy and a combination of different measures, including biosecurity, management, diagnosis of influenza infections, Active and passive surveillance, and reducing the susceptibility of the host, must be used (Swayne, 2012). Besides, veterinarians, farmers, and poultry-related professionals should receive proper education since teaching is one of the most important factors in AI control. Our subsequent study will investigate various aspects of this recombinant vaccine and its protective effects in chickens.

Conclusion

In the present study, the effectiveness of H9-RBD-Pichia, a novel recombinant subunit vaccine, was evaluated in mice and a high antibody titer was observed in HI assay. The vaccine stimulated the immune system of mice, resulting in the production of neutralizing antibodies. Interestingly, administration of lysed transgenic *P. pastoris* resulted in higher antibody titer in mice rather than pure protein, emphasizing the inherent adjuvant activity of *P. pastoris* cell wall components. Our research team is now investigating experiments regarding the culture of this transgenic *P. pastoris* in an industrial medium.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the University of Tehran, Tehran, Iran (Code: 141578).

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This paper was extracted from the PhD dissertation of Amir Asghari Baghkheirati, approved by the Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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References

Abdi, H. M., Mayahi, M., Boroomand, Z., & Shoshtari, A. (2021). Avian influenza-killed vaccine on tissue distribution and shedding of avian influenza virus H9N2 in ducklings. *Archives of Razi Institute, 76*(3), 437–444. [DOI:10.22092/ari.2020.342078.1452] [PMID]

Athmaram, T. N., Saraswat, S., Santhosh, S. R., Singh, A. K., Suryanarayana, W. S., & Priya, R., et al. (2011). Yeast expressed recombinant Hemagglutinin protein of novel H1N1 elicits neutralising antibodies in rabbits and mice. *Virology Journal, 8*, 524. [DOI:10.1186/1743-422X-8-524] [PMID]

Balamurugan, V., Venkatesan, G., Sen, A., Annamalai, L., Bhanuprakash, V., & Singh, R. K. (2010). Recombinant protein-based viral disease diagnostics in veterinary medicine. *Expert Review of Molecular Diagnostics, 10*(6), 731–753. [DOI:10.1586/erm.10.61] [PMID]

Buckholz, R. G., & Gleeson, M. A. (1991). Yeast systems for the commercial production of heterologous proteins. *Bio/technology (Nature Publishing Company), 9*(11), 1067–1072. [DOI:10.1038/nbt1191-1067] [PMID]

de Sá Magalhães, S., & Keshavarz-Moore, E. (2021). Pichia pastoris (Komagataella phaffii) as a cost-effective tool for vaccine production for low-and middle-income countries (LMICs). *Bioengineering (Basel, Switzerland), 8*(9), 119. [DOI:10.3390/bioengineering8090119] [PMID]

Delrue, I., Verzele, D., Madder, A., & Nauwynck, H. J. (2012). Inactivated virus vaccines from chemistry to prophylaxis: Merits, risks and challenges. *Expert Review of Vaccines, 11*(6), 695–719. [DOI:10.1586/erv.12.38] [PMID]

Ferrara, M. A., Severino, N. M., Mansure, J. J., Martins, A. S., Oliveira, E. M., & Siani, A. C., et al. (2006). Asparaginase production by a recombinant Pichia pastoris strain harbouring Saccharomyces cerevisiae ASP3 gene. *Enzyme and Microbial Technology, 39*(7), 1457-1463. [DOI:10.1016/j.enzmictec.2006.03.036]

Ghadimipour, R., Ghadimipour, I., Ameghi, A., Masoudi, S., Sedigh-Eteghad, S., & Ebrahimi, M. M. (2014). Monitoring virus harvesting time in embryonated chicken eggs inoculated with avian influenza H9N2 vaccine strain. *Archives of Razi Institute, 69*(1), 35-39. [Link]

Hadipour, M. M. (2010). H9N2 avian influenza virus antibody titers in human population in fars province, Iran. *Brazilian Journal of Poultry Science, 12*(3), 160-164. [DOI:10.1590/S1516-635X2010000300004]

Heidari, A., Mancin, M., Nili, H., Pourghanbari, G. H., Lankarani, K. B., & Leardini, S., et al. (2016). Serological evidence of H9N2 avian influenza virus exposure among poultry workers from Fars province of Iran. *Virology Journal, 13*, 16. [DOI:10.1186/s12985-016-0472-z] [PMID]

Iqbal, M., Yaqub, T., Mukhtar, N., Shabbir, M. Z., & McCauley, J. W. (2013). Infectivity and transmissibility of H9N2 avian influenza virus in chickens and wild terrestrial birds. *Veterinary Research, 44*(1), 100. [DOI:10.1186/1297-9716-44-100] [PMID]

Juturu, V., & Wu, J. C. (2018). Heterologous protein expression in Pichia pastoris: Latest research progress and applications. *Chembiochem: A European Journal of Chemical Biology, 19*(1), 7–21. [DOI:10.1002/cbic.201700460] [PMID]

Karbalaei, M., Rezaee, S. A., & Farsiani, H. (2020). Pichia pastoris: A highly successful expression system for optimal synthesis of heterologous proteins. *Journal of Cellular Physiology, 235*(9), 5867–5881. [DOI:10.1002/jcp.29583] [PMID]

Lee, S. W., Markham, P. F., Coppo, M. J., Legione, A. R., Markham, J. F., & Noormohammadi, A. H., et al. (2012). Attenuated vaccines can recombine to form virulent field viruses. *Science, 337*(6091), 188. [DOI:10.1126/science.1217134] [PMID]

Lei, H., Jin, S., Karlsson, E., Schultz-Cherry, S., & Ye, K. (2016). Yeast surface-displayed H5N1 avian influenza vaccines. *Journal of Immunology Research, 2016,* 4131324. [DOI:10.1155/2016/4131324] [PMID]

Lei, H., Xie, B., Gao, T., Cen, Q., & Ren, Y. (2020). Yeast display platform technology to prepare oral vaccine against lethal H7N9 virus challenge in mice. *Microbial Cell Factories, 19*(1), 53. [DOI:10.1186/s12934-020-01316-1] [PMID]

Liu, Y., Wu, C., Wang, J., Mo, W., & Yu, M. (2013). Codon optimization, expression, purification, and functional characterization of recombinant human IL-25 in Pichia pastoris. *Applied Microbiology and Biotechnology, 97*(24), 10349–10358. [DOI:10.1007/s00253-013-5264-4] [PMID]

Lopez, C. E., & Legge, K. L. (2020). Influenza A virus vaccination: Immunity, protection, and recent advances toward a universal vaccine. *Vaccines, 8*(3), 434. [DOI:10.3390/vaccines8030434] [PMID]

Maccani, A., Landes, N., Stadlmayr, G., Maresch, D., Leitner, C., & Maurer, M., et al. (2014). Pichia pastoris secretes recombinant proteins less efficiently than Chinese hamster ovary cells but allows higher space-time yields for less complex proteins. *Biotechnology Journal, 9*(4), 526-537. [DOI:10.1002/biot.201300305] [PMID]

Mascola, J. R., & Fauci, A. S. (2020). Novel vaccine technologies for the 21st century. *Nature Reviews Immunology, 20*(2), 87-88. [DOI:10.1038/s41577-019-0243-3] [PMID]

Nili, H., & Asasi, K. (2003). Avian influenza (H9N2) outbreak in Iran. *Avian Diseases, 47*(3 Suppl), 828–831. [DOI:10.1637/0005-2086-47.s3.828] [PMID]

Pietrzak, M., Macioła, A., Zdanowski, K., Protas-Klukowska, A. M., Olszewska, M., & Śmietanka, K., et al. (2016). An avian influenza H5N1 virus vaccine candidate based on the extracellular domain produced in yeast system as subviral particles protects chickens from lethal challenge. *Antiviral Research, 133,* 242-249. [DOI:10.1016/j.antiviral.2016.08.001] [PMID]

Pusch, E. A., & Suarez, D. L. (2018). The multifaceted zoonotic risk of H9N2 avian influenza. *Veterinary Sciences, 5*(4), 82. [DOI:10.3390/vetsci5040082] [PMID]

Radmehri, M., Talebi, A., Ameghi Roudsari, A., Mousaviyan, S. M., Gholipour, M. A. J., & Taghizadeh, M. (2021). Comparative study on the efficacy of MF 59, ISA70 VG, and nano-aluminum hydroxide adjuvants, alone and with nano-selenium on humoral immunity induced by a bivalent newcastle+ avian influenza vaccine in chickens. *Archives of Razi Institute, 76*(5), 1213-1220. [PMID]

Ríos-Hernández, M., Dos-Santos, N. J., Silvia-Cardoso, Bello-Gárciga, J. L., & Pedroso, M. (1994). Immunopharmacological studies of beta-1, 3-glucan. *Archives of Medical Research, 25*(2), 179-180. [PMID]

Sekhavati, M. H. (2024). Antimicrobial peptides, their significance and challenges in veterinary medicine. *Journal of Poultry Sciences and Avian Diseases, 2*(4), 54-55. [DOI: 10.61838/kman.jpsad.2.4]

Sano, K., & Ogawa, H. (2014). Hemagglutination (Inhibition) assay. *Methods in Molecular Biology (Clifton, N.J.), 1200,* 47–52. [DOI:10.1007/978-1-4939-1292-6_4] [PMID]

Song, W., & Qin, K. (2020). Human-infecting influenza A (H9N2) virus: A forgotten potential pandemic strain? *Zoonoses and Public Health, 67*(3), 203-212. [DOI:10.1111/zph.12685] [PMID]

Stubbs, A. C., Martin, K. S., Coeshott, C., Skaates, S. V., Kuritzkes, D. R., & Bellgrau, D., et al. (2001). Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nature Medicine, 7*(5), 625–629. [DOI:10.1038/87974] [PMID]

Swayne, D. E. (2012). Impact of vaccines and vaccination on global control of avian influenza. *Avian Diseases, 56*(4 Suppl), 818–828. [DOI:10.1637/10183-041012-Review.1] [PMID]

Wang, S. C., Liao, H. Y., Zhang, J. Y., Cheng, T. R., & Wong, C. H. (2019). Development of a universal influenza vaccine using hemagglutinin stem protein produced from Pichia pastoris. *Virology, 526,* 125-137. [DOI:10.1016/j.virol.2018.10.005] [PMID]

Wasilenko, J. L., Sarmento, L., Spatz, S., & Pantin-Jackwood, M. (2010). Cell surface display of highly pathogenic avian influenza virus hemagglutinin on the surface of Pichia pastoris cells using α-agglutinin for production of oral vaccines. *Biotechnology Progress, 26*(2), 542-547. [DOI: 10.1002/btpr.343] [PMID]

Wu, N. C., & Wilson, I. A. (2020). Influenza hemagglutinin structures and antibody recognition. *Cold Spring Harbor Perspectives in Medicine, 10*(8), a038778. [DOI:10.1101/cshperspect.a038778] [PMID]

Zhang, L., Liu, W. Q., & Li, J. (2020). Establishing a eukaryotic Pichia pastoris cell-free protein synthesis system. *Frontiers in Bioengineering and Biotechnology, 8,* 536. [DOI:10.3389/fbioe.2020.00536] [PMID]

مقاله پژوهشی

ارزیابی سرولوژیکی RBD-Pichia-H۹، یک واکسن نوترکیب جدید آنفلوانزا، در موش BALB/c

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چکیده

زمینه مطالعه: ویروس آنفولانزای H9N2 یکی از مهمترین تحت تیپهای آنفلوانزا است که خسارات جبران‌ناپذیری به صنعت طیور وارد کرده است و تلاش‌های زیادی برای تولید واکسن‌های نوترکیب آن در حال انجام است.

هدف: هدف از این مطالعه بررسی اثربخشی واکسن جدید زیرواحد نوترکیب H9-RBD-Pichia در تحریک سیستم ایمنی و تولید آنتی‌بادی‌های مهارکننده هماگلوتینین در موش است.

روش کار: ۲۴ موش ماده BALB/c خریداری و به طور تصادفی به شش گروه تقسیم شدند. گروه ۱: دوبار تجویز خوراکی مخمر لیز شده. گروه ۲: یکبار تزریق مخمر لیز شده. گروه ۳: دوبار تزریق مخمر لیز شده. گروه ۴: یکبار تزریق پروتئین نوترکیب خالص. گروه ۵: دوبار تزریق پروتئین نوترکیب خالص و گروه ۶: کنترل منفی. واکسیناسیون اولیه و یادآور به ترتیب در روزهای ۱۴ و ۲۸ انجام شد. نمونه های سرم در روز ۴۲ جمع‌آوری شدند. آزمایشات هماگلوتیناسیون (HA) و ممانعت ازهماگلوتیناسیون (HI) به ترتیب بر روی نمونه های آنتی ژن و سرم انجام شد.

نتایج: تیترهای HA ۱:۴، ۱:۴ و ۱:۳۲ به ترتیب در مخمرهای کنترل مثبت، دست نخورده و تیمار شده با گوی شیشه ای مشاهده شد که نشان دهنده ی عملکرد پروتئین نوترکیب H9–RBD بیان شده است. با انجام سنجش HI، مشاهده شد که این واکسن می تواند سیستم ایمنی را تحریک کرده و آنتی بادی های ممانعت کننده از هماگلوتینین را در موش تولید کند. بالاترین تیتر آنتی بادی (۱:۵۱۲) زمانی مشاهده شد که مخمر لیز شده، دو بار در روزهای ۱۴ و ۲۸ تزریق شد.

نتیجه‌گیری نهایی: واکسن H9–RBD–Pichia بر اساس فناوری مهندسی ژنتیک تولید شده است و می تواند تیتر بالایی از آنتی بادی های مهارکننده هماگلوتینین را در موش تولید کند.

کلیدواژه‌ها: H9N2، آنفولانزا، پیکیا پاستوریس، واکسن نوترکیب، نمایش سطحی.

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