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Could Trivalent LAIV Protect Against Both Genetic Lineages of Influenza B Virus?

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ABSTRACT

Introduction: The global co-circulation of two influenza B virus genetic lineages known as B/Yamagata and B/Victoria may lead to a mismatch between the circulating virus and the strain recommended for use in influenza vaccines. Little is known about the protective efficacy of unmatched influenza B strains, especially when it comes to live attenuated influenza vaccine. The main purpose of this study was to demonstrate the viability of using live attenuated influenza vaccine developed on B/USSR/60/69 backbone to protect against heterologous influenza B challenge infection. **Methods:** To estimate the potential cross-protective activity of mono- and trivalent live attenuated vaccines based on B/Victoria or B/Yamagata genetic lineage virus against a heterological challenge, ferrets were given one dose of vaccine and then were challenged with influenza B virus. The ferrets were then monitored for clinical signs associated with influenza infection. Samples of the ferrets' airways were tested for the presence of the challenge virus. **Results:** Mono- and trivalent live attenuated influenza vaccines were shown to be safe and cross-protective against genetically different influenza B viruses based on virological and histological data and clinical signs. A lower titer of heterologous challenge virus in the airways of the vaccinated ferrets compared to mock-vaccinated animals inoculated with the challenge virus was detected. Interestingly, B/Victoria-based vaccines were more cross-protective compared with B/Yamagata-based vaccines. **Conclusion:** In the case of mismatches of B component of the trivalent live attenuated influenza vaccine and lineage of the circulating influenza B viruses, one of the options could be using trivalent preparation containing a B/Victoria lineage component.

INTRODUCTION

Immunization with an influenza vaccine is the best intervention to prevent influenza infection [1]. Since 1999, the World Health Organization (WHO) has made annual recommendations on the composition of influenza virus vaccines for use during the Northern and Southern hemisphere influenza seasons, based on global surveillance programs and the prognosis of the most likely strains to be circulating [2,3]. The efficacy of influenza vaccination depends primarily on the antigenic match between the circulating and the vaccine strains. At the same time, influenza vaccines, in particular, live attenuated influenza vaccine (LAIV), may induce broad-spectrum and long-lasting immune responses, providing protection against drifted influenza viruses [4–7]. In the 1970s, influenza B viruses were classified into two major antigenically distinct lineages, B/Victoria and B/Yamagata [8]. The first B/Victoria-like strains were dominant worldwide since the late 1980s and then were almost replaced by B/Yamagata lineage viruses. In 2001–2002 B/Victoria lineage re-emerged and was detected in several countries in Europe, Asia, and in the USA. As of today, these two lineages have co-circulated among humans all over the world [9–14]. Notable changes in the currently circulating influenza B viruses included an increasing prevalence of B/Victoria or B/Yamagata lineage in different countries and areas. Importantly, either B/Victoria or B/Yamagata lineage is the most predominant in specific countries and areas; hence, predicting which genetic lineage of influenza B virus will dominate in the circulation in particular area during the next influenza season is not always possible [15–19]. For instance, the influenza B strains recommended for the Northern hemisphere seasonal influenza vaccine were incorrect during 5 out of 10 influenza seasons of 2001–2002 to 2010–2011 [16]. A mismatch between the circulating influenza viruses and the vaccine components may dramatically affect the vaccine efficacy [2,18,20–22].

This problem could be solved by including an additional influenza B virus strain in the trivalent vaccine which includes one influenza A (H1N1) strain, one influenza A (H3N2) strain and one influenza B strain. Theoretically, such quadrivalent influenza vaccine should provide the best match between the vaccine strains and the circulating influenza B viruses. In 2012, WHO first suggested that influenza vaccines might contain two influenza B viruses (one of each B lineage), allowing countries

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to decide if they would prefer to produce the trivalent or the quadrivalent influenza vaccines [23]. The quadrivalent LAIV (LAIV-4) is licensed in the USA, Canada, and Europe and in some other countries. However, a number of pro et contra opinions on the use of the quadrivalent influenza vaccine have been raised [21,24] and some countries including Russia still produce trivalent preparations, IIV-3 or/and LAIV-3 only.

The potential mismatch between the circulating influenza B strains and the trivalent vaccine makes broadly effective influenza B vaccine components an important public health urgency [25–27]. Contemporary realities dictate the need for an extensive study in countries that still produce the trivalent influenza vaccine to assay the potential cross-protective activity of vaccines containing the B/Victoria or B/Yamagata lineage viruses against a heterological influenza B virus challenge. A potential cross-protectivity of influenza vaccines against antigenically distinct influenza B viruses through local and cellular immune responses have been reported in some studies [28–30]. In our study, the cross-protectivities of LAIV-1 and LAIV-3 in naïve ferrets against challenge with influenza B virus of genetically different lineages were evaluated. As a control, the protection provided by the quadrivalent LAIV (LAIV-4) has also been estimated.

MATERIALS and METHODS

Viruses

(I) Wild-type (WT) influenza B viruses, B/Phuket/3073/2013 (B/Yamagata lineage, Yam) and B/Brisbane/60/08 (B/Victoria lineage, Vic) were used. (II) A list of LAIVs used in this study is shown in Table 1.

Table 1. LAIVs containing the following influenza B virus components

LAIV	Influenza B virus component
LAIV-1 (Yam)	B/Phuket/3073/2013 (Yam)
LAIV-1 (Vic)	B/Brisbane/60/08 (Vic)
LAIV-3 (Yam)	B/Phuket/3073/2013 (Yam)
LAIV-3 (Vic)	B/Brisbane/60/08–like (Vic)
LAIV-4	B/Phuket/3073/2013 (Yam), B/Brisbane/60/08–like (Vic)

All LAIV candidates with the genomic backbone of Russian master donor viruses, A/Leningrad/134/17/57 (H2N2) and B/USSR/60/69, were developed as described in [31]. Experimental series of monovalent LAIVs contained $10^{7.0}$ EID_{50}/mL of vaccine virus. The LAIV-3 and LAIV-4 were provided by the Serum Institute of India Private Limited (Pune, India) and contained individual vaccine components in the range of $10^{8.0}$–$10^{8.7}$ EID_{50}/mL.

Animals

Two studies were performed in ferrets to assess the protective efficacy of the LAIVs against influenza B viruses belonging to different genetic lineages. All procedures for the use and care of animals were approved by the Local Bioethical Committee of the Institute of Preclinical Research Ltd., St Petersburg, Russia (No. 1.4/17 from 18 January 2017 for Study #1 of LAIV-1 and No. 1.33/17 from 5 June 2017 for Study #2 of LAIV-3 and LAIV-4). All experimental procedures with ferrets were carried out according to the principles of European Union legislation [32] under short-term anesthesia. Every effort was made to reduce the discomfort and suffering of the animals. At the end of the experiments, the ferrets were humanely euthanized according to IACUC guidelines.

Using a routine HI test [33], adult male and female ferrets (Mustela putorius furo), 5–6 months of age, were determined to be negative for human influenza B viruses being tested in this study. Animals were inoculated intranasally with a single dose of LAIV or PBS (mock immunized) in a volume of 0.5 mL/nostril under short-term anesthesia induced by inhalation of Isoflurane (Study #1) or under intramuscular injection of Zoletil 100 at a dose of 12.5 mg/kg of body weight (Study #2). At the end of the experimental period, the ferrets were euthanized with a combination of Zoletil 100 and Xylazine.

Study Design

The study population was used to estimate the cross-protective potential of LAIV-1 and LAIV-3 against infection with influenza B viruses of genetically different lineages. Groups comprised 3 ferrets each were inoculated intranasally with the vaccine, on study day 0. Additional study groups were used as mock-vaccinated controls and received PBS on day 0. Three weeks later, the vaccinated and the mock-vaccinated animals of Study #1 and 4 weeks later, the vaccinated and the mock-vaccinated animals of Study #2 were inoculated with $10^{6.0}$ EID_{50}/mL of B/Phuket/3073/2013 (Yam) or B/Brisbane/60/2008 (Vic) WT virus at a volume of 1.0 mL. The WT challenge virus titer was determined by routine titration at the temperature of 37 ºC in embryonated chicken eggs and calculated by Reed and Munch method [34]. An additional group of animals did not receive either the vaccine or the challenge virus (mock-inoculated animals). LAIV-4 was used as a control of protection.

Assessment of morbidity outcomes and clinical signs

Animals were randomly selected and housed in individual cages. Daily clinical assessment of inoculated and control ferrets was performed 1 week before inoculation and for 3 days after vaccination and challenge. Ferrets were assessed for symptoms of respiratory infection and level of activity. In the preliminary Study #1, the body (rectal) temperature was measured using a digital thermometer. The body temperature of the ferrets of Study #2 was recorded by ultra-small temperature data loggers (Star-Oddi, Reykjavik, Iceland), which were implanted in the peritoneal cavity and recorded temperature every 30 minutes. The area under the temperature curve (AUTC) was calculated for periods of 3 days after the challenge for each animal using appropriate tools in GraphPad Prism software. We considered 38.0 °C as the cut-off temperature for AUTC calculation.

Assessment of the Virus Replication in Ferrets' Airways in Embryonated Chicken Eggs

Three days after the vaccination and challenge, nasal wash specimens were collected for virological analysis. At the end of the study, on day 3 post-challenge, lung tissue samples were taken for histopathological and virological analyses. Determination of the virus replication was assessed by endpoint titration of tested samples in 10–11-day-old embryonated chicken eggs at a temperature of 32 °C for 72 h. The virus titer was detected by routine hemagglutination test [33] with 1.0% chicken erythrocytes and calculated as EID_{50}/mL for the nasal washes and as EID_{50}/mL per gram of the lung tissue samples [34].

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Assessment of the virus replication by real-time PCR

Samples of the nasal lavages and the lung tissues were tested by real-time PCR for the detection of influenza B virus RNA, as described in [35] using a threshold cycle (Ct) comparison method. The RNA extraction from the tested samples was performed using a RIBO-sorb reagent kit (InterLabService). Primers and probes for influenza virus RNA amplification were obtained from the CDC (Atlanta, USA). One-step qRT-PCR System was supplied by Invitrogen.

Hemagglutination Inhibition (HI) Test

Serum antibody response in the non-vaccinated and vaccinated ferrets was detected by a routine HI test [33] with 1.0% chicken erythrocytes. To remove non-specific agglutinin serum, samples collected from the ferrets were treated with a receptor-destroying enzyme (DENKA SEIKEN Co. Ltd) according to the manufacturer's instructions. A fourfold rise in titers in paired sera was considered as seroconversion.

Gross Pathology

Complete macroscopic examinations (gross pathology) of trachea and lung tissues of the ferrets were performed at the time of necropsy. The severity of the lesions (color, number of damaged areas) was assessed in points.

Histopathology

One lobe of the lung and trachea from each sacrificed ferret were taken for histological analysis. After fixation in 10% buffered formalin, samples of the lung tissue and the trachea were embedded in paraffin. Tissue sections of 5μm were prepared and stained with Alcian blue (pH 2.5), followed by hematoxylin and eosin staining to reveal goblet cells on microscopic examination. Histological analysis was performed for the following lung tissue damage parameters: exudate in lung and bronchiole lumen, hypertrophy, and hyperplasia of the bronchial and bronchoalveolar epithelium, necrosis of bronchial and bronchoalveolar epithelium, hyperemia, emphysema, hemorrhages, inflammatory damage of bronchi, alveoli, interstitial tissue, and blood vessels. Each of these parameters was scored as follows: absent – 0; minimal – 1; slight – 2; moderate – 3; strong – 4; and severe – 5, on the 3 sections from each lung as described in [36].

Statistics

The Shapiro-Wilk test was used (normality test) to assess the distribution parameters. Differences between groups were analyzed by a post hoc Tukey test, one-way analysis of variance (one-way ANOVA), Kruskal-Wallis ANOVA, Mann-Whitney U-test by StatSoft Statistica 10.0 (USA) or GraphPad Prism 7. Data are presented as mean values ± SEM unless otherwise indicated. Differences were considered to be statistically significant at $p \leq 0.05$.

RESULTS

Clinical Examinations of the Ferrets;

Effect of Anesthesia on a Macroscopic View of Lung Tissue of the Ferrets

The ferrets received 5 applications of anesthesia in total, namely 3 days prior to the vaccination when screening for seronegative animals to the viruses being tested, on day 0 for the vaccination, on day 3 post-vaccination, on the day of the challenge, and on day 3 post-challenge. In Study #1, inhalation of the anesthesia was maintained by Isoflurane which did not affect the main clinical symptoms, body weight, body temperature, behavior, and the viral replication. However, localized pulmonary hemorrhages were detected after 5 inhalations in all study groups including ferrets which had been intranasally inoculated with PBS (Fig. 1 a,b). As a result, microscopic examination of the ferrets' airways was not performed. Further, in Study #2, we eschewed inhalation of anesthetic and used intramuscular anesthesia which was induced by injection with Zoletil 100.

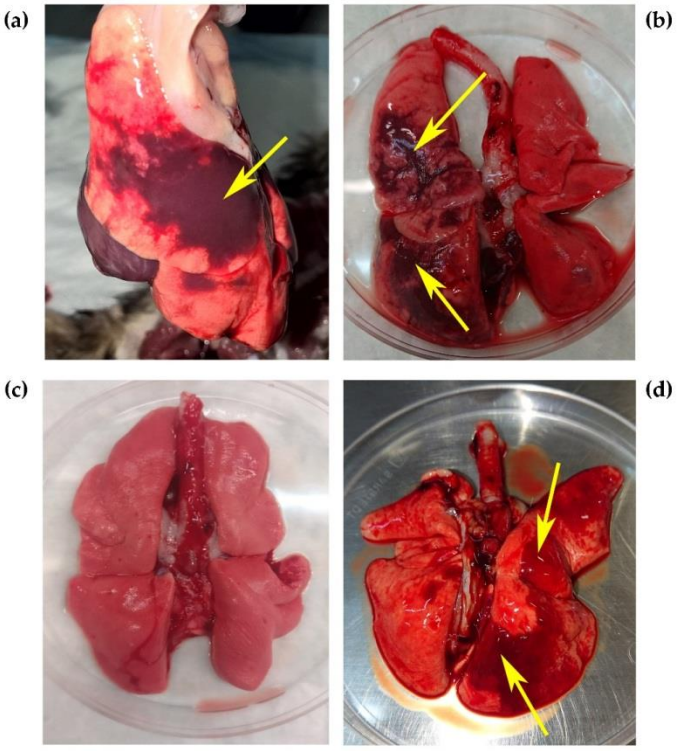

Fig. 1. Macroscopic view of lungs of the ferrets after multiple rounds of anesthesia. Yellow arrows – pulmonary hemorrhages. The ferrets received 5 applications of anesthesia. Isoflurane maintained inhalation anesthesia, intramuscular anesthesia was induced by injection with Zoletil 100. At the end of the experiment, the ferrets in all groups were humanely euthanized with a combination of Zoletil 100 and Xylazine. (a) Placebo group (ferrets that received only PBS)—Isoflurane; (b) B/Victoria challenge virus control group—Isoflurane; (c) placebo group (PBS)—Zoletil 100; (d) B/Victoria challenge virus control group—Zoletil 100.

Activity and Respiratory Symptoms

No clinically significant features were observed in immunized ferrets of Study #1 and Study #2. On days 1–3 post-vaccination, monitoring revealed a slight decrease in behavioral activity. No clinical signs of respiratory infection were detected in the vaccinated ferrets (data not shown).

All ferrets of both studies survived the 3-day follow-up observations, post-challenge period. After the challenge, the mock-vaccinated animals showed pronounced behavioral activity loss, compared with those from the vaccinated groups. One dose of LAIV significantly reduced clinical signs in ferrets challenged with WT viruses; the physiological state of the vaccinated animals post-challenge was close to normal (data not shown).

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Bodyweight

Fig. 2 shows body weight changes in ferrets after vaccination and challenge with WT virus. The mock-inoculated ferrets, challenged with the mock inoculation showed a slight increase in their body weights on days 0–3 (Study #1) or a slight decrease (Study #2). Vaccination with LAIV-1, LAIV-3, or LAIV-4 did not influence the body weights. Meanwhile, changes in body weights of the immunized ferrets were not significantly different from those in the mock-inoculated group.

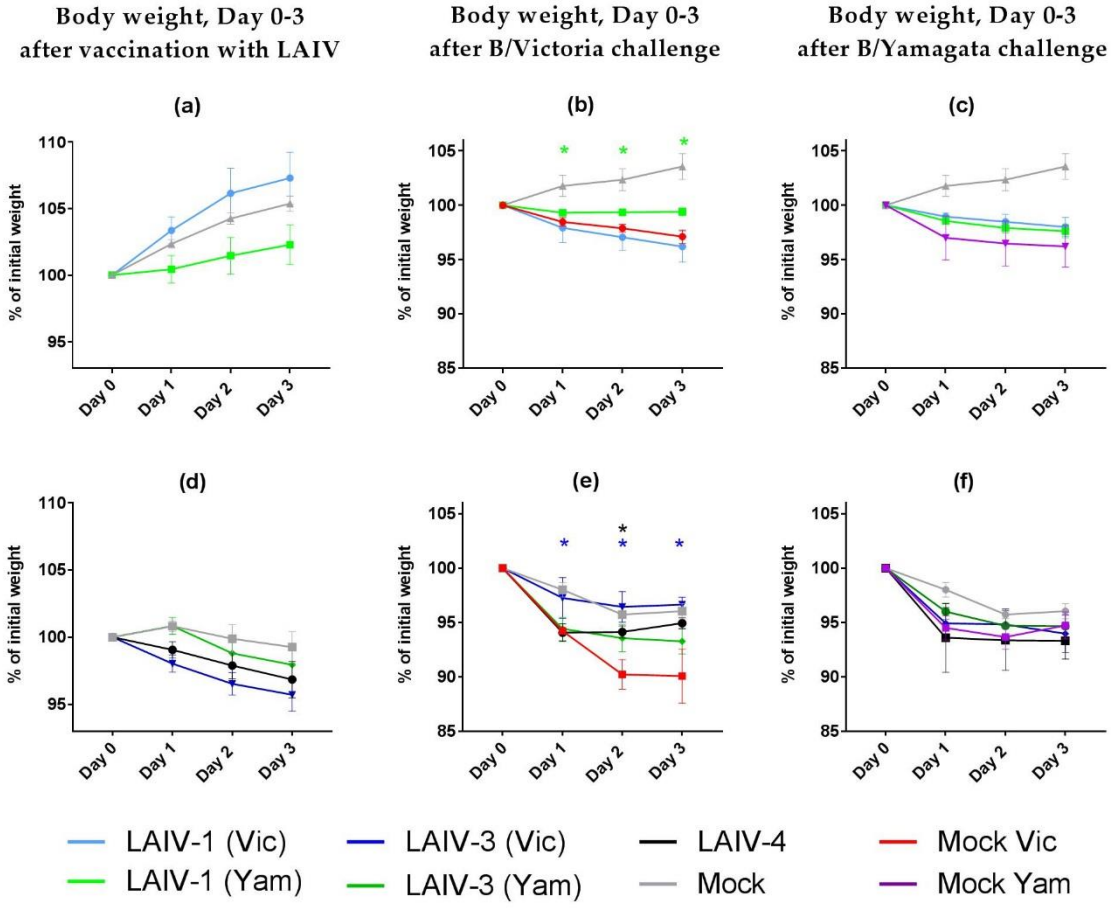

Fig. 2. Bodyweight changes in the vaccinated and the mock-vaccinated animals before and after challenge with homologous or heterologous WT influenza B virus. Data presented as % of initial (Day 0) body weight (mean ± SEM). Upper panel **(a–c)**: body weights of animals post-vaccination with LAIV-1 or PBS and subsequent challenge; lower panel **(d–f)**: body weights post-vaccination with LAIV-3, LAIV-4 or PBS and subsequent challenge. **(a)** Day 0-3 post-vaccination with LAIV-1 or the mock inoculation; **(d)** Day 0–3 post-vaccination with LAIV-3, LAIV-4 or the mock inoculation; **(b, e)** Day 0–3 post-challenge with B/Victoria; **(c, f)** Day 0–3 post-challenge with B/Yamagata. *Significantly different from the control of challenge virus (Mann-Whitney U test, $p \leq 0.050$)

The data of bodyweight changes from day 0 to day 3 post-challenge followed a normal distribution. Bodyweights of the immunized animals after the challenge did not differ significantly from that of the mock-inoculated ferrets. A minimum weight loss (2.7–3.6%) was seen in ferrets vaccinated with the LAIV-3 (Vic) after challenge with homologous WT B/Victoria virus. The utmost decrease in body weighst was recorded in the mock-vaccinated animals of Study #2, inoculated with B/Victoria (5.8–9.9%) or B/Yamagata (5.3–6.3%) challenge viruses.

Body Temperature

Body temperature changes, together with respiratory symptoms, is one of the most accurate and objective indicators of influenza infection in ferrets. The absence of statistically significant effects of the administration of LAIV-1, LAIV-3, and LAIV-4 on body temperature on any day between vaccination and challenge was confirmed by one-way ANOVA (Fig.3 a,d).

A short-term depression of body temperatures of the ferrets followed by intramuscular anesthesia with Zoletil was noticed (Fig. 3, lower panel). However, it did not cause any non-specific pulmonary hemorrhages (Fig. 2c) as Isoflurane did (Fig. 2a). In Study #1, a slight increase in body temperature (0.5–0.7 °C, average 39.2 °C) was detected in the control groups on day 1 post-inoculation with B/Victoria or B/Yamagata challenge viruses and persisted for 1–2 days. In both studies, post-challenge monitoring revealed that the vaccinated ferrets did not develop clinical signs of influenza infection with the exception of a rise of body temperature in the group vaccinated with LAIV-3 (Yam) after its challenge with B/Vic WT virus (Study #2, one-way ANOVA, F = 11.34, p = 0.0010) (Fig. 3). A significant rise in body temperatures was also seen in the mock-vaccinated animals after challenge with

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B/Victoria virus (one-way ANOVA, F = 11.34, p = 0.0010). In addition, AUTC was calculated for the period after the challenge for all groups (since a moment of body temperature normalization after anesthetic treatment). Significant differences in AUTC were detected for the mock-vaccinated ferrets and for ferrets vaccinated with LAIV-3 (Yam) after B/Victoria challenge (Fig. 4). Differences between other groups were not significant.

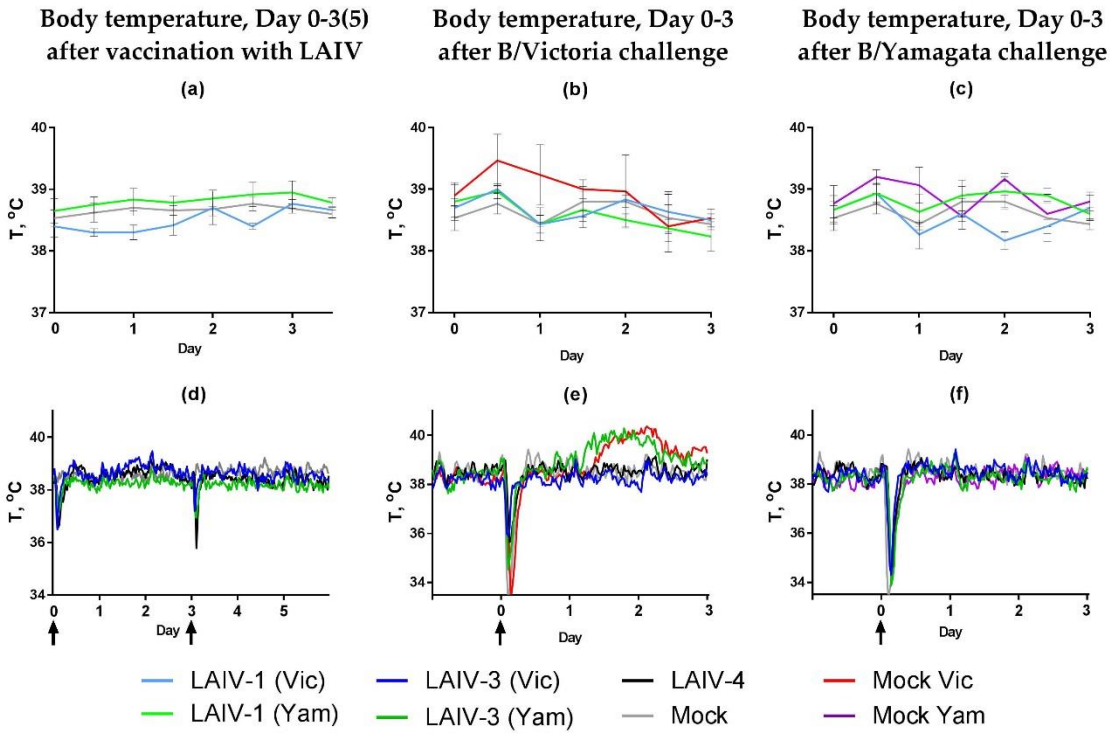

Fig. 3. Body temperature changes among studied groups of the vaccinated ferrets before and after challenge with homologous or heterologous WT influenza B virus. Upper panel **(a–c)**: rectal temperature of the animals post-vaccination with LAIV-1 or PBS and subsequent challenge (temperature was measured two times per day by digital thermometer), mean ± SEM; lower panel **(d–f)**: body temperatures post-vaccination with LAIV-3, LAIV-4 or PBS and subsequent challenge (temperature was recorded every 30 min by temperature loggers), mean values. **(a)** Day 0-3 post-vaccination with LAIV-1 or mock; **(d)** Day 0–5 post-vaccination with LAIV-3, LAIV-4 or mock; **(b, e)** Day 0–3 post-challenge with B/Victoria; **(c, f)** Day 0–3 post-challenge with B/Yamagata. Black arrows indicate administration of Zoletil 100.

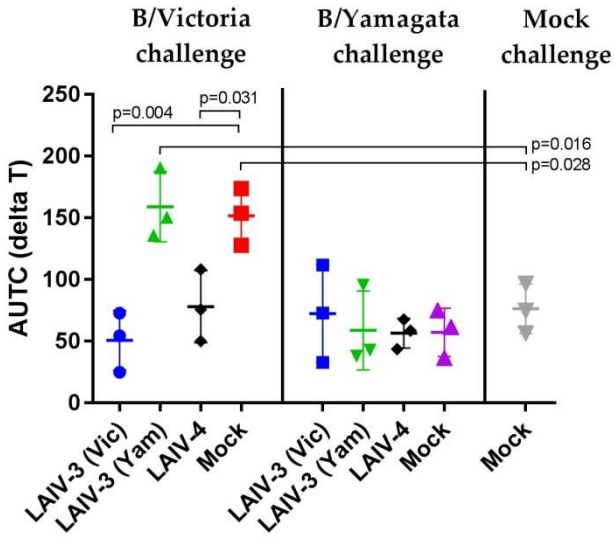

Fig. 4. AUTC of the ferrets for the period of 3 days after challenge with homologous or heterologous WT influenza B virus. Statistically significant differences with the control groups (mock-vaccinated, mock-inoculated) and the study groups are indicated (*p* value, Tukey post hoc test, one-way ANOVA, *p* = 0.0010)

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Replication of the Vaccine Virus in Embryonated Chicken Eggs

The replication of the vaccine virus in the upper respiratory tract (URT) of the immunized ferrets was assessed on day 3 post-vaccination by end-point titration of the nasal lavages in embryonated chicken eggs. No virus was found in the non-vaccinated ferrets. In contrast, the vaccine virus titers of LAIV-1 ranged from $10^{3.7}$ to $10^{3.9}$ EID_{50}/mL. The total virus titer of LAIV-3 and LAIV-4 varied from $10^{4.20}$ to $10^{4.53}$ EID_{50}/mL (Fig. 5a).

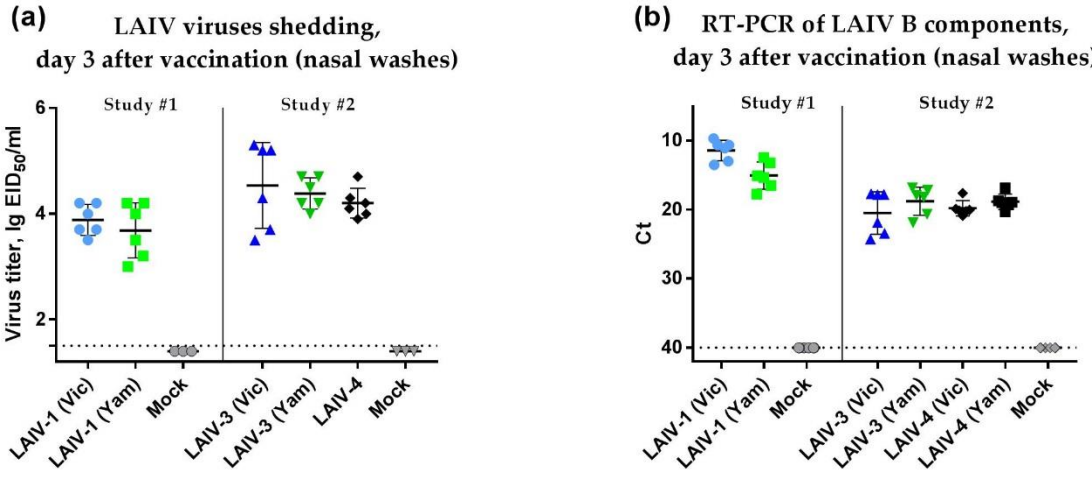

Fig. 5. Detection of the vaccine virus in embryonated chicken eggs and by RT-PCR. *P* values indicated a significant difference from the control of challenge virus by Mann-Whitney U test. **(a)** LAIV virus shedding detected by culture in embryonated chicken eggs; threshold limit value was estimated as < $10^{1.5}$ EID_{50}/mL (dotted line); **(b)** assessment of the vaccine virus replication by RT-PCR; Ct: the number of PCR cycles which is necessary to achieve a given level of fluorescence; estimated threshold limit value of Ct was suggested as high as 40 (dotted line); samples displayed Ct value ≥ 40 were considered as negative.

Assessment of the Vaccine Virus Replication by Real-Time PCR

Expressed replication of each influenza B vaccine virus in URT of the ferrets has been verified by real-time PCR. Cycle quantification values of B components of all vaccines tested in nasal washes on day 3 post-vaccination ranged from the minimum value of 11.41 for LAIV-1 (Vic) to the maximum value of 20.47 (LAIV-3 (Vic) (Fig. 5b).

Replication of the Challenge Virus in Embryonated Chicken Eggs

In Study #1 the mock-vaccinated control ferrets challenged with WT virus showed average lung viral titers of $10^{3.01}$ (Vic) and $10^{0.96}$ (Yam) EID_{50}/mL/g, respectively. In Study #2 the lung viral titers were higher – $10^{6.10}$ (Vic) and $10^{3.37}$ (Yam) EID_{50}/mL/g, respectively (Fig. 6). In both studies, the vaccinated animals shed the heterologous challenge virus in their URT at significantly lower levels than the mock-vaccinated animals (Mann–Whitney U, $p < 0.05$). None of the vaccinated ferrets had a detectable replication of the challenge virus in the lungs with only one exception where the animals inoculated with LAIV-3 (Yam) showed B/Victoria challenge virus lung titer of $10^{4.70}$ EID_{50}/mL per gram of tissue that was not significantly different from the lung virus titer in the control group of the mock-vaccinated ferrets ($10^{4.7}$ EID_{50}/mL vs. $10^{6.1}$ EID_{50}/mL, Mann–Whitney U test, $p = 0.127$).

Assessment of the Challenge Virus Replication by Real-Time PCR

Pronounced replication of the WT challenge virus in the airways of the mock-vaccinated ferrets was confirmed by real-time PCR. Cycle quantification values in nasal washes ranged from 14.92 to 20.69 Ct for B/Victoria and from 17.89 to 21.75 Ct for B/Yamagata, in the first and the second study, respectively. In the lungs, Ct values varied from 29.18 to 20.45 for B/Victoria and from 27.86 to 24.72 for B/Yamagata, in the first and the second study, respectively. Vaccination inhibited replication of the challenge viruses in the upper and low airways of ferrets vaccinated with monovalent LAIV. It should be noted that LAIV (Vic) had a more pronounced effect on the replication of the heterologous challenge virus than immunization with LAIV (Yam) while B/Victoria challenge virus was detected in the lungs of animals vaccinated with LAIV-1 (Yam) or LAIV-3 (Yam) (Fig. 7). These results were found to be close to those obtained by end-point titration of the nasal washes and the lung tissue samples in eggs. Mann-Whitney U test. (a, b) Ct for B/Vic challenge virus on day 3 after challenge; (c, d) Ct for B/Yam challenge virus on day 3 after challenge; (a, c) nasal washes; (b, d) lung tissue.

Serum Antibody Response to B Components of LAIVs

All the ferrets were seronegative for the tested influenza B viruses. Particularly, all of the ferrets had HI antibody titers < 1:10 to B/Victoria or B/Yamagata influenza viruses prior to the vaccination. Titers < 1:10 were set to 5 for calculation of the geometric mean titer (GMT) as described in [37,38]. Twenty-one days after the vaccination with LAIV-1 or 28 days after the vaccination with LAIV-3 or LAIV-4, all the ferrets presented a fourfold or higher increase in HI antibody titers to the homologous WT virus (Table 2). The antibody titer increase ranged from 3.6 to 7.2-fold for B/Victoria component and from 4.5 to 18.0-fold for B/Yamagata component, respectively. In contrast, no cross-lineage antibody response to the heterologous challenge virus was seen.

Gross Pathology

Macroscopic examination of the organs of the vaccinated and non-vaccinated animals showed no pathological changes in their trachea or lungs. The administration of the LAIV or

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vaccination with LAIV followed by challenge with homologous or heterologous influenza B virus did not result in damage to the trachea or lungs of the ferrets. Thus, gross pathologic examination revealed that LAIV provided protection from infection with WT not only for homologous but also for heterologous influenza B virus in the ferrets.

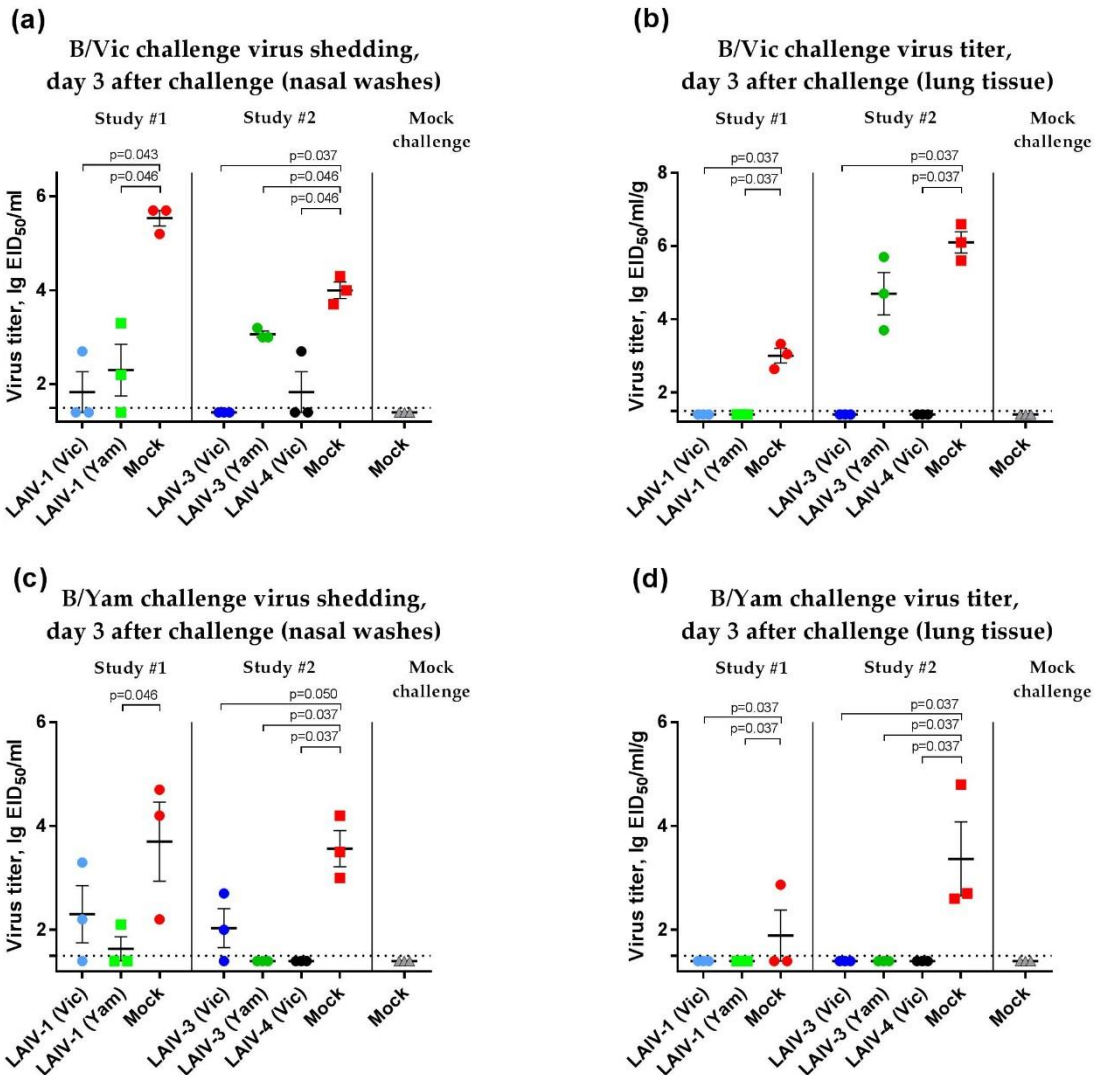

Fig. 6. Shedding of the challenge virus was detected in embryonated chicken eggs. Threshold limit value was estimated as < $10^{1.5}$ EID_{50}/mL (dotted line). *P* values indicated a significant difference from the control of challenge virus by Mann-Whitney U test. **(a, b)** B/Vic challenge virus on day 3 after challenge; **(c, d)** B/Yam challenge virus on day 3 after challenge; **(a, c)** nasal washes; **(b, d)** lung tissue.

Histopathology

The semi-quantitatively examination of histological changes is presented in Table 3 as an average sum of the scores per ferret ± standard error of the mean. Histopathological analysis of the mock-inoculated control group established that there was little to no change in the lung tissue. The mean score for the lung lesions in the control group was 12.7. The histological examination of the tracheas of all the ferrets showed no damages.

In the mock-vaccinated ferrets challenged with influenza B viruses, more severe changes were defined: mild to moderate inflammatory damage to the bronchi, blood vessels, alveolar, and interstitial tissue. Histological examination also showed diffuse infiltration of mononuclear cells, hyperemia of the alveolar septa, alveolar emphysema, and focal hemorrhages. Bronchiolitis, peribronchitis, and hyperplasia of the pneumocytes were also detected. Collectively, the mean score for the lung lesions was significantly higher than in the mock-inoculated control (35.7 compared to 12.7, Tukey test, p =0.004). In the lungs of the mock-vaccinated ferrets challenged with B/Yamagata lineage, severe pathological changes were defined. Histological examination revealed moderate to severe catarrhal bronchitis and bronchiolitis, with detection of a cell exudate in the lumen of the bronchi and bronchioles. Hyperplasia and hypertrophy of the bronchial epithelium with focal necrosis and lymphocytic infiltration were also detected. The mean score for the lung lesions was significantly higher than the mock-inoculated control (32.7 compared to 12.7, Tukey test, p = 0.004). Histological examination of LAIV-vaccinated ferrets showed less pronounced changes in the lung tissue compared with the lungs of the mock-vaccinated animals after the challenge with the WT virus. Slight damage to the bronchial epithelium and a low degree of lymphocytic and mononuclear infiltration in the interstitial tissue were also

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found. The acute inflammation in the lungs of LAIV-vaccinated ferrets was not defined. It is also relevant that ferrets vaccinated with T-LAIV (B/Yam) had fewer goblet cells of bronchial epithelium after B/Victoria challenge compared with the mock group. The mean scores in the groups of LAIV-vaccinated ferrets had values between 16.3 and 22.0 (compared 12.7 in the mock-inoculated control group).

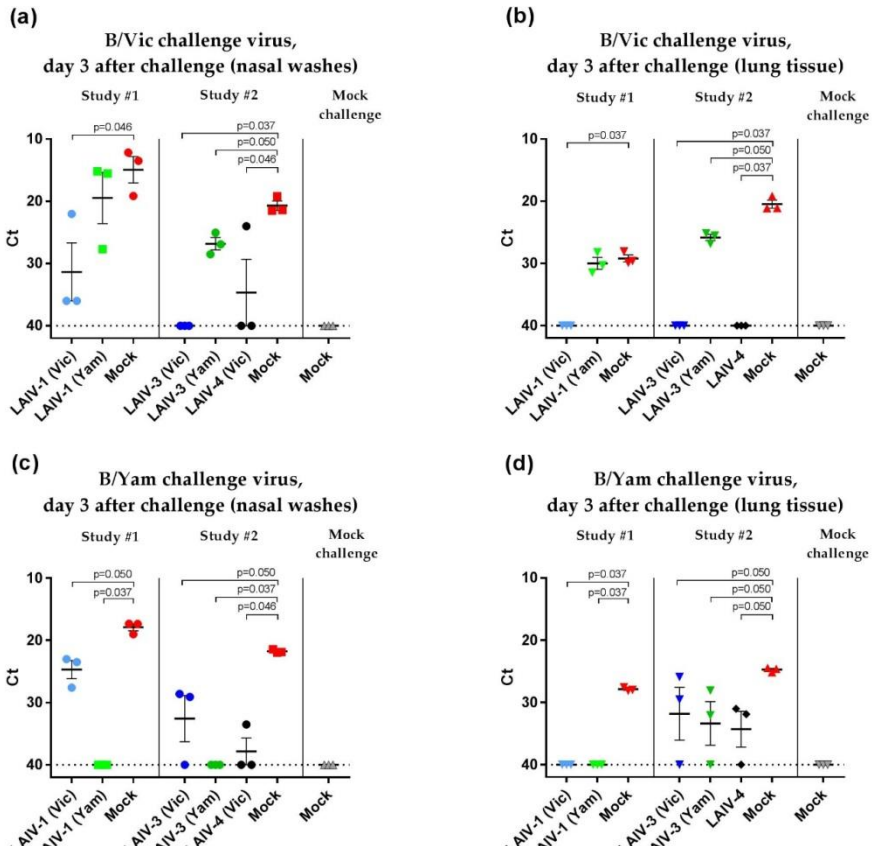

Fig. 7. Shedding of challenge viruses was detected by RT-PCR. Ct: the number of PCR cycles which is necessary to achieve a given level of fluorescence. Estimated threshold limit value of Ct was suggested as high as 40 (dotted line). Samples displayed Ct value ≥ 40 were considered as negative. *P* values indicated for significantly different from the control of challenge virus by Mann-Whitney U test. **(a, b)** Ct for B/Vic challenge virus on day 3 after challenge; **(c, d)** Ct for B/Yam challenge virus on day 3 after challenge; **(a, c)** nasal washes; **(b, d)** lung tissue.

Table 2. Humoral immune response to B components of LAIVs (HI test).

Study	Test article	B/Victoria WT antigen		B/Yamagata WT antigen	
		HI GMT[1]	Fold increase	HI GMT[1]	Fold increase
#1	LAIV-1 (Vic)	5.0 / 36.0	7.2	5.0 / 5.0	1.0
	LAIV-1 (Yam)	5.0 / 5.0	1.0	5.0 / 90.0	18.0
	Intact group	5.0 / 5.0	1.0	5.0 / 5.0	1.0
#2	LAIV-3 (Vic)	5.0 / 35.6	7.1	5.0 / 5.0	1.0
	LAIV-3 (Yam)	5.0 / 5.0	1.0	5.0 / 31.7	6.3
	LAIV-4	5.0 / 17.8	3.6	5.0 / 22.4	4.5
	Mock-inoculated animals	5.0 / 5.0	1.0	5.0 / 5.0	1.0

[1]Day 0/day 21 for study #1 and day 0/day 28 for study #2 (twofold dilutions)

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Table 3. Histopathology of the ferret lung tissues after challenge with homologous or heterologous WT influenza B virus.

Group	Lung examination[1] on day 4 after challenge with	
	B/Victoria	B/Yamagata
LAIV-3 (Vic)	16.3 ± 2.73	18.7 ± 2.91
LAIV-3 (Yam)	22.0 ± 2.08	17.3 ± 2.03
LAIV-4	19.3 ± 2.73	19.0 ± 3.21
Control of challenge virus (mock-vaccinated animals)	35. 7 ± 1.76[2]	32.7 ± 2.73[2]
Mock-inoculated animals	12.7 ± 1.76	

[1]Semi-quantitative analysis of the lung tissue, the average sum of scores per ferret ± SEM. [2]Significantly different from non-vaccinated mock-inoculated control by one-way ANOVA, post hoc Tukey test, control of B/Victoria challenge virus $p = 0.004$, control of B/Yamagata challenge virus, $p = 0.010$.

DISCUSSION

The quadrivalent influenza vaccine is likely to be more effective compared with the trivalent vaccine [39]. In seasons with relatively high influenza B activity, the quadrivalent inactivated influenza vaccine (IIV-4) has appeared to be more protective than IIV-3 [40]. In addition, LAIV-4 and IIV-4 maintained a similar level of protection against a new antigenic variant H3N2. However, in children 2–17 years of age, LAIV-4 has provided significantly better protection against a drifted influenza B virus [41]. The quadrivalent influenza vaccine compared to the trivalent preparation may lead to a substantial decrease in an epidemiological burden and influenza-associated costs [42,43]. On the other hand, if the vaccine strain matches with circulating influenza B virus, there is no need for the quadrivalent vaccine. In addition, a number of different factors are influenced by the development and use of the quadrivalent vaccine in different countries. For instance, the quadrivalent vaccine is more expensive than the trivalent preparation [39], the limited production capacity may result in fewer doses of the quadrivalent vaccine compared with the trivalent vaccine [21] and there is a longer time of production for the quadrivalent preparation [15]. Obviously, matched vaccines provide the best protection; however, little is known about the protectivity of unmatched vaccines.

Evidence of cross-protectivity of unmatched influenza B components of vaccines is still contradictory and sparse, especially for LAIV-3. The main purpose of our study was to demonstrate the viability of using Russian LAIV-3 to protect ferrets from heterologous influenza B challenge infection. In our work, a single dose of LAIV-4 was used as a control of vaccine protectivity. In our study, LAIV-4 had no adverse effect on the clinical condition, body temperature, or bodyweight of the ferrets. The immunization of the animals with LAIV-4 followed by challenge with B/Victoria or B/Yamagata virus led to a significant reduction of clinical signs of influenza infection. The overall efficiency of protection was sustained. As assessed by challenge virus detection in ferrets' airways, immunization with LAIV-4 proved to be equally effective against both influenza B virus lineages. This implies that LAIV-4 is the best option for protection against influenza B virus infection.

The evaluation of clinical symptoms in ferrets and examination of virological data demonstrated the cross-protectivity of LAIV-1 and LAIV-3. The clinical manifestation of respiratory infection after challenge with homologous and heterologous influenza B lineage viruses was significantly milder in the immunized ferrets than in the mock-vaccinated animals; the physiological condition of vaccinated animals was close to the normal state. In mock-vaccinated ferrets, challenge infection with B/Victoria or B/Yamagata influenza viruses led to nasal discharge, a decrease in body weight and behavioral activity, and to some rise in body temperature. In Study #1, the clinical manifestation of the challenge infection with B/Yamagata in the mock-vaccinated animals was less pronounced than infection with B/Victoria lineage. These data may correspond to the findings of a study [44] that reported preferential binding of the B/Yamagata lineage to α2-6 sialic acid linkages, which are predominant in the ferret URT [45]. In contrast to our Study #1, B/Yamagata challenge virus of Study #2 was less reactogenic for the animals than B/Victoria strain.

Interestingly, cross-protection against challenge with B/Yamagata virus after vaccination with B/Victoria-containing LAIVs was stronger than cross-protection against challenge with B/Victoria virus after vaccination with B/Yamagata-containing LAIVs. These results correlate with the results of clinical trials on children immunized with IIV-3. Skowronski and co-authors [46] have demonstrated that IIV-3 (Yam) did not sufficiently prime young children for the response to subsequent immunization with B/Victoria-contained vaccine. In our study, ferrets immunized with LAIV-3 (Yam) had a lower level of B/Victoria challenge virus in the respiratory tracts were compared with the mock-vaccinated animals. However, cross-protection was less expressed. This data corresponds well with the clinical results of other authors. In an earlier study, serologically negative children were not protected against infection with B/Victoria lineage after vaccination with B/Yamagata lineage [47]. However, these findings need to be further studied.

One dose of LAIV-1 or LAIV-3 stimulated intensive production of specific antibodies to homologous influenza B virus. In contrast, the antibody response to the heterologous lineage of influenza B virus was not detected. It should be taken into account that the humoral immune response is not the only indicator of vaccine efficacy [4]. This cross-protectivity may be related to other mechanisms, in particular, cell-mediated or local immune responses. It is well known that the live attenuated influenza vaccine provides broad-spectrum immune responses against the circulating strains, including heterotypic protective immunity [4,6,7]. In contrast, the immune response to IIV is strain-specific. Virus-specific CD8+ T-cell-response and local immunity stimulated by LAIV also play an important

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role in preventing influenza infection [4,48–53]. It has been demonstrated that influenza B viruses contain cross-reactive cytotoxic T-lymphocyte (CTL) epitopes; CTL response directed to one lineage of influenza B virus may cross-react with another lineage [54].

Histopathological examinations have an important role in preclinical animal studies of vaccines. Ketamine is widely used in veterinary practices as a surgical anesthetic for general anesthesia. However in Russia, Ketamine is included in the list of narcotic drugs, psychotropic substances, and their precursors, which are subject to control; hence, its usage is strictly limited. In Russian veterinary practice, Isoflurane and Zoletil 100 are broadly used as anesthetic agents. However, in Study #1, we faced an unexpected problem with inhalation of the anesthesia, induced by Isoflurane. The efficacy and safety of Isoflurane have been shown in a number of nonclinical [55–57] and clinical [58] trials; however, these studies have not included post-mortem exploration of the animal lungs. In our experiments, a five-fold inhalation of Isoflurane dramatically affected the lung tissue and caused injuries in form of hemorrhagic lung edema. Gross necropsy examination revealed gross lung lesions in a mock-inoculated group, similar to the mock-vaccinated animals challenged with WT virus. This finding corresponded to a previous study [59] in which the authors have reported that general anesthesia with volatile agents including Isoflurane provoked lung injury, acute inflammatory response, and leukocytic infiltration in rats. In contrast, the absence of any non-specific lung damages in ferrets caused by the injection of Zoletil 100 was reported in studies of influenza virus [60,61].

Hypothermia is a well-known side effect of general anesthesia described for a number of mammals [55,57,58,62,63] and humans [64]. In our second ferret study, Zoletil 100 provoked a temporary decrease in the body temperatures. However, it did not affect the lung tissue as Isoflurane in Study #1 did. Macroscopic and histological inspection of the lung tissue of the mock-inoculated control animals of Study #2 showed very little or no pathological changes. In the mock-vaccinated ferrets challenged with WT influenza B viruses, histopathological changes in the lungs were significantly higher than in the mock-inoculated ferrets.

In conclusion, our results collectively show that LAIV may protect against infection with genetically distinct influenza B lineages. Overall, the relevance of quadrivalent influenza vaccines is unquestionable. They have a favorable safety profile and provide a sustained level of protection against each vaccine component. However, the quadrivalent influenza vaccines, including LAIV-4 are not on the market worldwide yet. In the case of mismatches of the trivalent vaccine and lineage of the circulating influenza B viruses, one of the options could be using LAIV-3 containing a B/Victoria lineage component which its broad-spectrum and cross-protective potencies may provide substantial protection against heterologous B/Yamagata virus.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. World Health Organization. Global influenza pandemic action plan to increase vaccine supply. Geneva: WHO; 2006 (WHO/IVB/06.13; Available online: http://whqlibdoc.who.int/hq/2006/WHO_IVB_06.13_eng.pdf (accessed on July 28, 2019) [Internet]. 2006 [cited 2019 Jul 28]. Available from: http://whqlibdoc.who.int/hq/2006/WHO_IVB_06.13_eng.pdf.
2. Hannoun C. The evolving history of influenza viruses and influenza vaccines. Expert Rev Vaccines. 2013;12:1085–94. http://dx.doi.org/10.1586/14760584.2013.824709.
3. World Health Organization. WHO recommendations on the composition of influenza virus vaccines. Available online: https://www.who.int/influenza/vaccines/virus/recommendations/en/ (accessed on July 10, 2019) [Internet]. [cited 2019 Jul 19]. Available from: https://www.who.int/influenza/vaccines/virus/recommendations/en/
4. Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. Scand J Immunol. 2004;59:1–15.
5. Tamura S, Tanimoto T, Kurata T. Mechanisms of broad cross-protection provided by influenza virus infection and their application to vaccines. Jpn J Infect Dis. 2005;58:195–207.
6. Rudenko LG, Slepushkin AN, Monto AS, Kendal AP, Grigorieva EP, Burtseva EP, et al. Efficacy of live attenuated and inactivated influenza vaccines in schoolchildren and their unvaccinated contacts in Novgorod, Russia. J Infect Dis. 1993;168:881–7. http://dx.doi.org/10.1093/infdis/168.4.881.
7. Rudenko LG, Alexandrova GI. Current strategies for the prevention of influenza by the Russian cold-adapted live influenza vaccine among different populations. Int Congr Ser. 2001;1219:945–50. http://dx.doi.org/10.1016/S0531-5131(01)00661-6.
8. Yang J-R, Huang Y-P, Chang F-Y, Hsu L-C, Lin Y-C, Huang H-Y, et al. Phylogenetic and Evolutionary History of Influenza B Viruses, which Caused a Large Epidemic in 2011–2012, Taiwan. PLoS ONE [Internet]. 2012 [cited 2017 Nov 30];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3470568/. http://dx.doi.org/10.1371/journal.pone.0047179.
9. Rota PA, Wallis TR, Harmon MW, Rota JS, Kendal AP, Nerome K. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virology. 1990;175:59–68.
10. Shaw MW, Xu X, Li Y, Normand S, Ueki RT, Kunimoto GY, et al. Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000-2001 and 2001-2002 seasons. Virology. 2002;303:1–8.
11. Barr IG, Komadina N, Durrant C, Sjogren H, Hurt AC, Shaw RP. Circulation and antigenic drift in human influenza B viruses in SE Asia and Oceania since 2000. Commun Dis Intell Q Rep. 2006;30:350–7.
12. Centers for Disease Control and Prevention (CDC). Update: influenza activity--United States, September 28, 2008-April 4, 2009, and composition of the 2009-10 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2009;58:369–74.
13. Chen R, Holmes EC. The Evolutionary Dynamics of Human Influenza B Virus. J Mol Evol. 2008;66:655–63. http://dx.doi.org/10.1007/s00239-008-9119-z.
14. Bedford T, Riley S, Barr IG, Broor S, Chadha M, Cox NJ, et al. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. Nature. 2015;523:217–20. http://dx.doi.org/10.1038/nature14460.
15. Barr IG, Jelley LL. The coming era of quadrivalent human influenza vaccines: who will benefit? Drugs. 2012;72:2177–85. http://dx.doi.org/10.2165/11641110-000000000-00000.
16. Ambrose CS, Levin MJ. The rationale for quadrivalent influenza vaccines. Hum Vaccines Immunother. 2012;8:81–8. http://dx.doi.org/10.4161/hv.8.1.17623.
17. Jennings L, Huang QS, Barr I, Lee P, Kim WJ, Buchy P, et al. Literature review of the epidemiology of influenza B disease in 15 countries in the Asia-Pacific region. Influenza Other Respir Viruses. 2018;12:383–411. http://dx.doi.org/10.1111/irv.12522.

[Downloaded from vacres.pasteur.ac.ir on 2026-10-08]

[DOI: 10.29252/vacres.6.1.13]

18. Dos Santos G, Neumeier E, Bekkat-Berkani R. Influenza: Can we cope better with the unpredictable? Hum Vaccines Immunother. 2016;12:699–708. http://dx.doi.org/10.1080/21645515.2015.1086047.
19. Bandell AR, Simões EAF. Live attenuated influenza vaccine tetravalent: a clinical review. Expert Rev Vaccines. 2015;14:963–73. http://dx.doi.org/10.1586/14760584.2015.1034695.
20. Li W-C, Shih S-R, Huang Y-C, Chen G-W, Chang S-C, Hsiao M-J, et al. Clinical and genetic characterization of severe influenza B-associated diseases during an outbreak in Taiwan. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2008;42:45–51. http://dx.doi.org/10.1016/j.jcv.2007.11.026.
21. Reed C, Meltzer MI, Finelli L, Fiore A. Public health impact of including two lineages of influenza B in a quadrivalent seasonal influenza vaccine. Vaccine. 2012;30:1993–8. http://dx.doi.org/10.1016/j.vaccine.2011.12.098.
22. Tricco AC, Chit A, Soobiah C, Hallett D, Meier G, Chen MH, et al. Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. BMC Med. 2013;11:153. http://dx.doi.org/10.1186/1741-7015-11-153.
23. WHO | Recommended composition of influenza virus vaccines for use in the 2013 southern hemisphere influenza season [Internet]. WHO. [cited 2019 Jul 19]. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/2013_south/en/
24. Quinn E, Jit M, Newall AT. Key issues and challenges in estimating the impact and cost-effectiveness of quadrivalent influenza vaccination. Expert Rev Pharmacoecon Outcomes Res. 2014;14:425–35. http://dx.doi.org/10.1586/14737167.2014.908713.
25. Heikkinen T, Ikonen N, Ziegler T. Impact of influenza B lineage-level mismatch between trivalent seasonal influenza vaccines and circulating viruses, 1999-2012. Clin Infect Dis Off Publ Infect Dis Soc Am. 2014;59:1519–24. http://dx.doi.org/10.1093/cid/ciu664.
26. Mosnier A, Caini S, Daviaud I, Bensoussan J-L, Stoll-Keller F, Bui TT, et al. Ten influenza seasons in France: distribution and timing of influenza A and B circulation, 2003-2013. BMC Infect Dis. 2015;15:357. http://dx.doi.org/10.1186/s12879-015-1056-z.
27. Trucchi C, Alicino C, Orsi A, Paganino C, Barberis I, Grammatico F, et al. Fifteen years of epidemiologic, virologic and syndromic influenza surveillance: A focus on type B virus and the effects of vaccine mismatch in Liguria region, Italy. Hum Vaccines Immunother. 2017;13:456–63. http://dx.doi.org/10.1080/21645515.2017.1264779.
28. Levandowski RA, Gross PA, Weksler M, Staton E, Williams MS, Bonelli J. Cross-reactive antibodies induced by a monovalent influenza B virus vaccine. J Clin Microbiol. 1991;29:1530–2.
29. Asahi-Ozaki Y, Yoshikawa T, Iwakura Y, Suzuki Y, Tamura S-I, Kurata T, et al. Secretory IgA antibodies provide cross-protection against infection with different strains of influenza B virus. J Med Virol. 2004;74:328–35. http://dx.doi.org/10.1002/jmv.20173.
30. Camilloni B, Neri M, Lepri E, Iorio AM. Cross-reactive antibodies in middle-aged and elderly volunteers after MF59-adjuvanted subunit trivalent influenza vaccine against B viruses of the B/Victoria or B/Yamagata lineages. Vaccine. 2009;27:4099–103. http://dx.doi.org/10.1016/j.vaccine.2009.04.078.
31. Shcherbik S, Pearce N, Kiseleva I, Larionova N, Rudenko L, Xu X, et al. Implementation of new approaches for generating conventional reassortants for live attenuated influenza vaccine based on Russian master donor viruses. J Virol Methods. 2016;227:33–9. http://dx.doi.org/10.1016/j.jviromet.2015.10.009.
32. Directive 2010/63/EU. Directive 2010/63/EU of the European parliament and of the council of September 22, 2010, on the protection of animals used for scientific purposes [Internet]. 2010 [cited 2017 Dec 1]. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010L0063
33. World Health Organization. WHO Manual on animal influenza diagnosis and surveillance [Internet]. 2002 [cited 2017 Nov 30]. Available from: http://www.wpro.who.int/emerging_diseases/documents/docs/manualonanimalaidiagnosisandsurveillance.pdf
34. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol. 1938;27:493–7. http://dx.doi.org/10.1093/oxfordjournals.aje.a118408.
35. Zang Y, Du D, Ge P, Xu Y, Liu X, Zhang Y, et al. Development of one-step real-time PCR assay for titrating trivalent live attenuated influenza vaccines. Hum Vaccines Immunother. 2014;10:3642–8. http://dx.doi.org/10.4161/hv.34453.
36. Widjojoatmodjo MN, Boes J, van Bers M, van Remmerden Y, Roholl PJ, Luytjes W. A highly attenuated recombinant human respiratory syncytial virus lacking the G protein induces long-lasting protection in cotton rats. Virol J. 2010;7:114. http://dx.doi.org/10.1186/1743-422X-7-114.
37. Cowling BJ, Lim WW, Perera RAPM, Fang VJ, Leung GM, Peiris JSM, et al. Influenza hemagglutination-inhibition antibody titer as a mediator of vaccine-induced protection for influenza B. Clin Infect Dis Off Publ Infect Dis Soc Am. 2018; http://dx.doi.org/10.1093/cid/ciy759.
38. Laurie KL, Horman W, Carolan LA, Chan KF, Layton D, Bean A, et al. Evidence for Viral Interference and Cross-reactive Protective Immunity Between Influenza B Virus Lineages. J Infect Dis. 2018; http://dx.doi.org/10.1093/infdis/jix509.
39. Baxter D. Evaluating the case for trivalent or quadrivalent influenza vaccines. Hum Vaccines Immunother. 2016;12:2712–7. http://dx.doi.org/10.1080/21645515.2015.1091130.
40. Shasha D, Valinsky L, Hershkowitz Sikron F, Glatman-Freedman A, Mandelboim M, Toledano A, et al. Quadrivalent versus trivalent influenza vaccine: clinical outcomes in two influenza seasons, historical cohort study. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2019; http://dx.doi.org/10.1016/j.cmi.2019.05.003.
41. McLean HQ, Caspard H, Griffin MR, Poehling KA, Gaglani M, Belongia EA, et al. Effectiveness of live attenuated influenza vaccine and inactivated influenza vaccine in children during the 2014-2015 season. Vaccine. 2017;35:2685–93. http://dx.doi.org/10.1016/j.vaccine.2017.03.085.
42. Uhart M, Bricout H, Clay E, Largeron N. Public health and economic impact of seasonal influenza vaccination with quadrivalent influenza vaccines compared to trivalent influenza vaccines in Europe. Hum Vaccines Immunother. 2016;12:2259–68. http://dx.doi.org/10.1080/21645515.2016.1180490.
43. de Boer PT, Crépey P, Pitman RJ, Macabeo B, Chit A, Postma MJ. Cost-Effectiveness of Quadrivalent versus Trivalent Influenza Vaccine in the United States. Value Health J Int Soc Pharmacoeconomics Outcomes Res. 2016;19:964–75. http://dx.doi.org/10.1016/j.jval.2016.05.012.
44. Wang Y-F, Chang C-F, Chi C-Y, Wang H-C, Wang J-R, Su I-J. Characterization of glycan binding specificities of influenza B viruses with correlation with hemagglutinin genotypes and clinical features. J Med Virol. 2012;84:679–85. http://dx.doi.org/10.1002/jmv.23219.
45. Belser JA, Katz JM, Tumpey TM. The ferret as a model organism to study influenza A virus infection. Dis Model Mech. 2011;4:575–9. http://dx.doi.org/10.1242/dmm.007823.
46. Skowronski DM, Hottes TS, De Serres G, Ward BJ, Janjua NZ, Sabaiduc S, et al. Influenza B/Victoria antigen induces strong recall of B/Yamagata but lower B/Victoria response in children primed with two doses of B/Yamagata. Pediatr Infect Dis J. 2011;30:833–9. http://dx.doi.org/10.1097/INF.0b013e31822db4dc.
47. Levandowski RA, Regnery HL, Staton E, Burgess BG, Williams MS, Groothuis JR. Antibody responses to influenza B viruses in immunologically unprimed children. Pediatrics. 1991;88:1031–6.
48. Isakova-Sivak I, Rudenko L. Safety, immunogenicity and infectivity of new live attenuated influenza vaccines. Expert Rev Vaccines. 2015;14:1313–29. http://dx.doi.org/10.1586/14760584.2015.1075883.
49. Naykhin AN, Petukhova GD, Chirkova TV, Koren'kov DA, Donina SA, Rudenko LG. Virus-specific CD4+ and CD8+ memory T-cells in young volunteers after immunization with pandemic live attenuated reassortant influenza vaccines A (H5N2) and A (H1N1). Influenza Other Respir Viruses. 2011;195–201.
50. Naĭkhin AN, Donina SA, Petukhova GD, Koren'kov EM, Doroshenko EM, Grigor'eva EP, et al. [Humoral and cell-mediated immune responses in humans to the A/California/07/ 2009 (H1N1) virus, A(H1N1)pdm2009]. Vopr Virusol. 2013;58:38–42.
51. Rudenko L, Kiseleva I, Naykhin AN, Erofeeva M, Stukova M, Donina S, et al. Assessment of Human Immune Responses to H7 Avian Influenza Virus of Pandemic Potential: Results from a Placebo–Controlled, Randomized Double–Blind Phase I Study of Live Attenuated H7N3 Influenza Vaccine. PLoS ONE [Internet]. 2014 [cited 2019 Jul 27];9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3922724/. http://dx.doi.org/10.1371/journal.pone.0087962.
52. Chirkova TV, Naykhin AN, Petukhova GD, Korenkov DA, Donina SA, Mironov AN, et al. Memory T-Cell Immune Response in Healthy Young Adults Vaccinated with Live Attenuated Influenza A (H5N2) Vaccine. Clin Vaccine Immunol CVI. 2011;18:1710–8. http://dx.doi.org/10.1128/CVI.05116-11.
53. Sridhar S, Begom S, Bermingham A, Hoschler K, Adamson W, Carman W, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. Nat Med. 2013;19:1305–12. http://dx.doi.org/10.1038/nm.3350.

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[DOI: 10.29252/vacres.6.1.13]

54. Van de Sandt CE, Dou Y, Vogelzang-van Trierum SE, Westgeest KB, Pronk MR, Osterhaus ADME, et al. Influenza B virus-specific CD8+ T-lymphocytes strongly cross-react with viruses of the opposing influenza B lineage. J Gen Virol. 2015;96:2061–73. http://dx.doi.org/10.1099/vir.0.000156.
55. Imai A, Steffey EP, Farver TB, Ilkiw JE. Assessment of isoflurane-induced anesthesia in ferrets and rats. Am J Vet Res. 1999;60:1577–83.
56. Lichtenberger M, Ko J. Anesthesia and Analgesia for Small Mammals and Birds. Veterinary Clin North Am Exot Anim Pract. 2007;10:293–315. http://dx.doi.org/10.1016/j.cvex.2006.12.002.
57. Lester PA, Moore RM, Shuster KA, Myers DD. Anesthesia and Analgesia. Lab Rabbit Guin Pig Hamst Rodents [Internet]. Elsevier; 2012 [cited 2019 Jun 21]. p. 33–56. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012380920900002X. http://dx.doi.org/10.1016/B978-0-12-380920-9.00002-X.
58. Yasuda N, Lockhart SH, Eger EI, Weiskopf RB, Johnson BH, Freire BA, et al. Kinetics of desflurane, isoflurane, and halothane in humans. Anesthesiology. 1991;74:489–98.
59. Nader-Djalal N, Knight PR, Bacon MF, Tait AR, Kennedy TP, Johnson KJ. Alterations in the course of acid-induced lung injury in rats after general anesthesia: volatile anesthetics versus ketamine. Anesth Analg. 1998;86:141–6.
60. Lee D-H, Kim J-I, Lee J-W, Chung W-H, Park J-K, Lee Y-N, et al. Quantitative measurement of influenza virus replication using consecutive bronchoalveolar lavage in the lower respiratory tract of a ferret model. J Vet Sci. 2014;15:439–42. http://dx.doi.org/10.4142/jvs.2014.15.3.439.
61. Kim HM, Kang YM, Ku KB, Park EH, Yum J, Kim JC, et al. The severe pathogenicity of alveolar macrophage-depleted ferrets infected with 2009 pandemic H1N1 influenza virus. Virology. 2013;444:394–403. http://dx.doi.org/10.1016/j.virol.2013.07.006.
62. Tom Evans A, Springsteen KK. Anesthesia of ferrets. Semin Avian Exot Pet Med. 1998;7:48–52. http://dx.doi.org/10.1016/S1055-937X(98)80057-1.
63. Isakova-Sivak I, Matyushenko V, Kotomina T, Kiseleva I, Krutikova E, Donina S, et al. Sequential Immunization with Universal Live Attenuated Influenza Vaccine Candidates Protects Ferrets against a High-Dose Heterologous Virus Challenge. Vaccines. 2019;7:61. http://dx.doi.org/10.3390/vaccines7030061.
64. Lenhardt R. The effect of anesthesia on body temperature control. Front Biosci Sch Ed. 2010;2:1145–54.

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