Acta virologica **66**: 197 – 205, 2022 doi: 10.4149/av_2022_304

Evaluation of Quil-A, *E. coli* DNA and Montanide™ ISA 206 adjuvant combination on the antibody response to foot-and-mouth disease vaccine in sheep

Can Çokçalişkan[1], Tunçer Türkoğlu[1], Beyhan Sareyyüpoğlu[1], Pelin Tuncer-Göktuna[2], Banu Bayri Özbilge[1], Ergün Uzunlu[1], Ayça Kürkçü[1], Eylem Aras Uzun[1], Veli Gülyaz[3]

[1]Republic of Turkey, Ministry of Agriculture and Forestry, Institute of Foot and Mouth Disease (Şap), Ankara, Turkey; [2]Republic of Turkey, Ministry of Agriculture and Forestry, Pendik Veterinary Control Institute, Istanbul, Turkey; [3]Harran University, Faculty of Veterinary Medicine, Virology Department, Şanlıurfa, Turkey

Received September 24, 2021; revised April 11, 2022; accepted May 4, 2022

Summary. – Vaccination is one of the basic strategies in the fight against foot-and-mouth disease (FMD) in endemic regions. Today, commercially available FMD vaccines are prepared with inactive whole virion, which has low immunogenicity. Therefore, considerable effort has been devoted to finding novel adjuvants. Although mineral oils are among the most common adjuvants, it is still difficult to provide a long-term and robust immune response. Combined adjuvant systems are currently being studied to solve the problem. Saponins and CpG-ODNs have been shown to increase the immune response to vaccines individually in various studies. In this study, the effect of different adjuvants and their combinations (Quil-A, *E. coli* DNA, and Montanide™ ISA 206) on total and neutralizing antibody response in sheep was investigated. According to the results, the Quil-A group induced the highest antibody level, followed by the combination of Quil-A and the *E. coli* DNA group. The group containing *E. coli* DNA also caused a higher antibody response than the group containing only Montanide™ ISA 206 for certain days of sampling. These affordable alternatives of saponin and CpG sources can be used individually to increase the potency of the FMD vaccine for mass vaccinations of sheep.

Keywords: foot-and-mouth disease; vaccine; adjuvant; Quil-A; E. coli DNA; combination of adjuvants

Introduction

Foot-and-mouth disease (FMD) is one of the most important viral diseases in cloven-hoofed animals. One of the control strategies for the disease is vaccination. Vaccines are generally used in countries where the disease is endemic (Dar *et al*., 2013). Although vaccination plays an important role in controlling FMD, conventional vaccines used against FMD cannot provide a sterile and long-term immunity (Belsham, 2020). In order to overcome these disadvantages of the conventional vaccines, various adjuvants have been studied (Li *et al*., 2013; Park *et al*., 2014; Ren *et al*., 2011; Song *et al*., 2009). Utilization of an adjuvant allows the amount of antigen per dose to be reduced and prolongs the immunity (Dar *et al*., 2013; Rajput *et al*., 2007). An adjuvant also affects the nature of the immune response and determines whether it has Th1 or Th2 character (Mahakapuge *et al*., 2015). On the other hand, some adjuvants may cause side effects which limit their use. An ideal adjuvant should be potent without causing any side effects. The combining of adjuvants has been studied to reduce these limitations and increase the efficacy of the vaccine (Garg *et al*., 2017). There are many studies on the combined use of different adjuvants (Park *et al*., 2014; Ren *et al*., 2011; Song *et al*., 2009; Smitsaart *et*

E-mail: cancokcaliskan@gmail.com, can.cokcaliskan@tarimorman.gov.tr; phone: +903122873600-2541.

Abbreviations: FMD = foot-and-mouth disease; CpG-ODN = cytosine-phosphorothioate-guanine oligodeoxynucleotides; QS = *Quillaja saponaria;* OIE = World Organization for Animal Health; pv = post-vaccination

al., 2004; Xiao *et al*., 2007; Ioannou *et al*., 2002). Among them are antigen-carrying systems consisting of 40 nm particles called ISCOMATRIX™, formulated by using cholesterol, phospholipids and Quil-A. Similarly, two combined adjuvant systems, namely AS01 and AS02, have been developed using QS-21 and monophosphoryl lipid A for human vaccines (Dubois and Wagner, 2017).

Saponins, as natural steroids or triterpene glycosides, have various biological and pharmacological effects. While used for different purposes, they also gain importance and interest as potential vaccine adjuvants, mainly in stimulating the mammalian immune system (Rajput *et al*., 2007). Quil-A is the leading adjuvant among saponins, and it has been used in both commercial and experimental vaccines. It can induce Th1 type immune response as well as cytotoxic T lymphocytes. It was claimed that both Th1 and Th2 type immune responses with an increase in all subclasses of IgGs have been induced by using Quil-A in a commercial FMD vaccine in pigs (Xiao *et al*., 2007). Smitsaart *et al*. (2004) also showed the adjuvanticity of saponin in oil vaccines against FMD in cattle, pigs and goats. It was also demonstrated by the authors of this study that the addition of QS-21 to Montanide™ ISA 206 adjuvanted FMD vaccine increased early specific antibody response in cattle (Çokçalışkan *et al*., 2016). QS-21 is one of the fractions extracted from *Quillaja saponaria* bark, or Quil-A, and purified with reverse phase chromatography. However, as the price of QS-21 is ten times more than Quil-A (https://www.creative-biolabs.com), hence too high to use in sheep vaccination, an increase of antibody response in sheep by adding Quil-A to Montanide™ ISA 206 adjuvanted FMD vaccine was targeted in this study. In order to increase the synergistic effect, another molecule showing adjuvant activity, bacterial DNA, was considered as well.

Bacterial DNA has all the properties that are expected from an antigen. It is a large, structurally diverse molecule that can cross-link the receptors. These traits not only stimulate B cells but also stimulate cytokine response and production of specific antibodies (Pisetsky, 1997). Cytosine-phosphorothioate-guanine oligodeoxynucleotides (CpG-ODNs) are new types of adjuvants. These unmethylated nucleotide sequences are commonly found in bacterial DNA and activate Toll-like receptor-9 found in the phagoendosomes of antigen-presenting cells, especially dendritic cells. CpG-ODNs help the maturation and activation of dendritic cells and expression of antigens in the response. Further, CpG-ODN stimulates the interferon-gamma secretion of Natural Killer cells, as bacterial DNA, and induces a cell-mediated immune response (Nichani *et al*., 2004). Morshedi *et al*. (2010) obtained eight times higher neutralizing antibody titres with a CpG-ODN adjuvanted FMD vaccine compared to the conventional alum gel FMD vaccine.

Sheep are an important source of livelihood in many countries and they play an essential role in the epidemiology of FMD. Although it has been proven that they have potential to transmit the disease from country to country, authorities hesitate to include sheep in vaccination campaigns because of their large population. Instead, regional and strategic vaccinations targeting sheep are generally performed. However, the disease's mild clinical symptoms can give the impression that the vaccination programs are successful, while the virus sometimes continues to circulate in the population (Kitching, 1996).

Although cattle and sheep are closely related species, the same adjuvant may have different biological effects in both species, since the stimulation of the innate immune system has two different mechanisms in each animal (Nichani *et al*., 2004). The adjuvants should be economical, abundant, and potent for sheep vaccination campaigns. For this reason, we have selected affordable alternatives of previously proven potent adjuvants. This study investigated the effect of adding Quil-A and *E. coli* DNA separately or together to the Montanide™ ISA 206 adjuvanted FMD vaccine on the specific antibody response in sheep.

Materials and Methods

Quil-A. Purified, lyophilized saponin Quil-A (Vac-Quil Brenntag Denmark, distributed by InvivoGen, USA) was purchased in ready-to-use powder form.

E. coli DNA. E. coli TOP10 bacterial cells (Invitrogen C4040-50) were thawed from a -80°C freezer. The cells were allowed to grow for 48 h in a 2l sterile antibiotic-free Luria-Bertani broth. When the OD_{600} value exceeded 0.5 at the end of 48 h, the cells were collected by low-speed centrifugation. The pellet was lysed with 50 ml lysis buffer (934 ml TE buffer, 60 ml 10% SDS, 6 ml proteinase K) for 1 hr at room temperature. Following this stage, freeze-thaw processes were done twice in a -20°C and 37°C water bath, respectively. The lysate was mixed equally with the 50–50% phenol-chloroform mixture. It was centrifuged at 5000 rpm for 5 min. The supernatant was collected. This step was repeated three times. After adding chloroform, the samples were centrifuged and incubated for 1 hr with 99% ice-cold ethanol and precipitated with 70% ethanol at -20°C for 1 h. The visible pellet was diluted with 3 ml of TE buffer and treated with 10 mg RNase A at 37°C for 30 min. DNA was measured with a spectrophotometer as 350 ng/µl (DeNovix, DS-11, USA).

Vaccines. Montanide™ ISA 206 (Seppic, France) oil adjuvant was used to form a double oil emulsion (w/o/w). The water phase consists of FMDV O/TUR/07, A/ASIA/G-VII and ASIA/TUR/15 antigen mixture. The antigens were produced by using suspension BHK-21 cell cultures, inactivated by binary ethyl-

eneimine, concentrated by PEG 6000. Desired adjuvant/antigen (v/v) ratio (55:45 (oil/water) (v/v)) was obtained by dilution of the antigen phase with phosphate buffer solution (pH = 7.6). Both phases were kept at 30°C in an incubator to balance the temperature. Heidolph Hei-Torque 100 homogenizer (Heidolph Instruments, Germany) was used for the preparation of emulsion formulations. The blending was performed by adding the water phase to the oil phase at the rate of 2 ml/min under constant stirring (500 rpm). Then stirring was increased to 1000 rpm for additional 5 min. The obtained emulsion was divided into two parts of equal volumes. The concentrated stock solution of Quil-A was added to one, and the same volume of the PBS was added to the other part. Additional stirring for 5 min. at 500 rpm was performed for each sample. The same procedure was applied to prepare the vaccine formulations with *E. coli* DNA. The only difference was that the antigen phase was diluted using the same volume of PBS containing *E. coli* DNA instead of PBS alone.

Thus, four different vaccine combinations were obtained. The first one was a traditional vaccine formulation with Montanide™ ISA 206 alone as an adjuvant. The second one was a vaccine formulation containing Quil-A in combination with Montanide™ ISA 206. The third one was a vaccine formulation containing *E. coli* DNA in combination with Montanide™ ISA 206, and the fourth one was a vaccine formulation containing both Quil-A and *E. coli* DNA in combination with Montanide™ ISA 206 (Table 1). These vaccine formulations were administered to four groups of animals, with final *E. coli* DNA concentrations of 135 µg per dose in the group 3 and the group 4 and the final Quil-A concentrations of 130 µg per dose in group 2 and group 4. The quality of emulsions was checked by the droplet test and measurements of conductivity and viscosity.

Animals, groups and vaccination. A total of 57 FMDV seronegative, eight-month-old male merino sheep were provided from a state farm. Before purchasing, the animals were bled and the sera were tested for the presence of structural and non-structural virus protein antibodies with Liquid Phase Blocking ELISA and non-structural protein ELISA, as described in the ELISA section. Randomly divided into four groups of 13, each sheep received a subcutaneous injection of 1 ml of experimental vaccine as described in the vaccine section. The remaining five animals were separated as the unvaccinated control group. The groups of animals were according to the groups of adjuvant and vaccine combinations.

The animals were kept in paddocks at the institute and fed with grain, green clover, and water ad libitum. Serum samples were collected on days 0, 7, 24, 31, 60, 90, 120 and 150 post-vaccination (pv). The study was carried out according to the approval given by the local ethics committee of the Foot-and-mouth (Şap) Institute (Ethics Committee Decision No: 24.01.16/03-3).

Virus neutralization test (VNT). The assay was done according to the OIE's FMD manual (OIE, 2016). Briefly, the sera were inactivated at 56°C for 30 min in a water bath before the test. Log_2 dilutions of 50 µl of sera were performed in microplates starting from 1:4 to 1:512. Dilutions were made by an automated dilution robot (Integra Viaflo Assist, Integra Biosciences, Switzerland) to minimize pipetting errors. Fifty µl of 100 $TCID_{50}$ homologous virus suspensions were added to the sera dilutions. Plates were incubated in a 5% CO_2 incubator for 1 h. Following the incubation, 50 µl of BHK-21 cell suspension containing 600,000 cells/ml was added to the wells. The plates were kept in a CO_2 incubator for 48 h, after which the wells were examined under a microscope for CPE formation following crystal violet staining. Log_{10} values of reciprocals of serum dilution which neutralized CPE on 50% of the wells were expressed as the VN titre of the serum.

Liquid phase blocking ELISA (LPB-ELISA). The test was carried out according to the method reported by Hamblin *et al*. (1986). Briefly, on the first day, 96-well ELISA plates were coated with rabbit anti-FMDV O/TUR/07, A/ASIA/G-VII and ASIA1/TUR/15, polyclonal sera produced in-house. In the meantime, two-fold dilutions of test and control sera were prepared in U-bottom carrier microplates starting at 1:16 dilutions. The antigen was added, and the plates were stored at +4°C overnight. On the second day, the ELISA plates were washed three times and 50 µl of serum/antigen mixture was transferred to the ELISA plates, then incubated at 37°C for 1 h. After the washing step, in-house produced FMDV- O/TUR/07, A/ASIA/G-VII and ASIA1/TUR/15 specific guinea pig antibodies were added and placed into the incubator. Another wash step was performed, and HRP-labelled polyclonal anti-guinea pig IgG conjugate (Dako, P0141, Agilent, USA) was added to the plate, then kept in the incubator for 1h. After incubation, three washes were performed and the chromogen substrate (OPD + 1:2,000 H_2O_2) was added. The reaction was stopped by 1.25 M sulphuric acid after 15 min at room temperature. The colour development was analysed in the ELISA reader (VersaMax, Molecular Devices, USA) at a wavelength of 492 nm. The

Table 1. The groups of antigen and adjuvant combinations

Group	Adjuvants	FMD antigens
1	Montanide™ ISA 206	O/TUR/07 + A/ASIA/GVII + ASIA1/TUR/15
2	Quil-A + Montanide™ ISA 206	O/TUR/07 + A/ASIA/GVII + ASIA1/TUR/15
3	*E. coli* DNA + Montanide™ ISA 206	O/TUR/07 + A/ASIA/GVII + ASIA1/TUR/15
4	Quil-A + *E. coli* DNA + Montanide™ ISA 206	O/TUR/07 + A/ASIA/GVII + ASIA1/TUR/15

antibody titres were expressed as the log10 of the reciprocal of the last dilution of serum, giving 50% of the mean OD of the positive control wells.

Statistical analysis. In this study, a priori power analysis was achieved with G*Power software (3.1.9.7 version, Kiel, Germany) to calculate the minimum number of animals for each group. The analysis was carried out by setting the power of the test as 80%, the effect size of medium as 25%, and the significance level as 5%. The Kruskal-Wallis H test was used for comparison of the data of the groups. To compare pairwise data, the Mann-Whitney U-test was used. SPSS software (IBM SPSS Statistics for Windows, Version 19.0 Armonk, USA) was used for analysis of the data.

Results

Virus neutralization test

Low levels of maternal antibodies to all serotypes have been detected in all the samples taken at day 0 pv.

Serotype O

The neutralizing antibodies increased in all groups from day 7 pv. The peak values were obtained on the 60th day in groups 1, 2 and 3, and on the 90th day in group 4, where the combination of adjuvants was used for formulation. The highest mean antibody titre was obtained on the 60th day in group 2 (a formulation with Quil-A plus Montanide™ ISA 206). On the same day, the lowest titre was detected in group 1.

The differences between group 1 and group 2 on days 24, 31, 60, 90 and 120 pv were statistically significant ($p < 0.05$). The virus neutralizing antibody response to group 3 was significantly higher than that of group 1 on days 90 and 120 pv ($p < 0.05$). The response to group 4 was significantly higher than that of group 1 on days 90 and 120 pv. It was also detected that group 2 induced higher antibody titres on days 24 and 31 pv than those of groups 3 and 4 ($p < 0.05$) (Fig. 1a).

Serotype A

The neutralizing antibodies increased in all groups from day 7 pv. The peak titres were obtained on day 90 pv. The highest mean antibody titre was obtained by group 2 on day 90 pv among the groups and the lowest titre was detected in group 1 for the same day. The difference was significant between group 1 and group 2 on days 7, 60, 90, 120 and 150 pv ($p < 0.05$). The virus neutralizing antibody response to group 3 was significantly higher than that of group 1 on day 150 pv. The response to group 4 was significantly higher than that of group 1 on days 7, 60, 90, 120 and 150 pv ($p < 0.05$) (Fig. 1b).

Serotype ASIA1

The difference between groups 2 and 3 was significant on day 24 pv. On day 90 pv the titres of group 4 were significantly higher than those of groups 1 and 3 ($p < 0.05$) (Fig. 1c).

Liquid phase blocking ELISA

Low levels of maternal antibodies to all serotypes except to serotype A have been detected in samples obtained at day 0. There was an increase in the total FMD-specific antibodies in all groups from day 7 pv. Detectable levels of antibodies were produced by all groups for all serotypes.

Serotype O

The peak antibody levels were obtained for all groups on day 31 pv, whereas the peak titre levels were obtained on day 90 pv for serotypes A and ASIA1. The highest mean antibody titre on day 31 was found in group 2 for serotype O. On the same day, the lowest titre was found in group 1. From day 31 pv, antibody titres in all groups decreased gradually. Statistically significant differences were found between groups 1 and 2 on days 31 and 90 pv, and between groups 2 and 3 on day 31 pv ($p < 0.05$) (Fig. 2a).

Serotype A

A significant difference was detected on day 90 pv between groups 1 and 2. Also, the titres of group 4 were significantly higher than those of group 1 for the same day ($p < 0.05$) (Fig. 2b).

Serotype ASIA1

No significant differences of LPB-ELISA titres were found between the groups for all sampling days (Fig. 2c).

Discussion

The ongoing studies on the new adjuvants are designed to increase and prolong immune response against FMD vaccines (Ren *et al.*, 2011; Park *et al.*, 2014; Bazid *et al.*, 2021; Rathogwa *et al.*, 2021). Although Montanide™ ISA 206 has been used as an adjuvant in FMD vaccines for a long time in Asia and South America, novel adjuvants are being investigated to stimulate a robust immunity (Dar *et al.*, 2013). Unfortunately, a single adjuvant may not have all

the desired features. Thus, adjuvant combinations or multiple adjuvant systems that simultaneously stimulate various immune system cells, such as dendritic cells, macrophages, and lymphocytes, are being studied (Garg *et al.*, 2017). The importance of humoral immune response in protection against FMD is well known by researchers. Although attempts to measure the level of protection with alternative methods – interferon response, etc. (Parida *et al.*, 2006), the *in vitro* gold test has still been the virus neutralization test (Mc Cullough *et al.*, 1992;

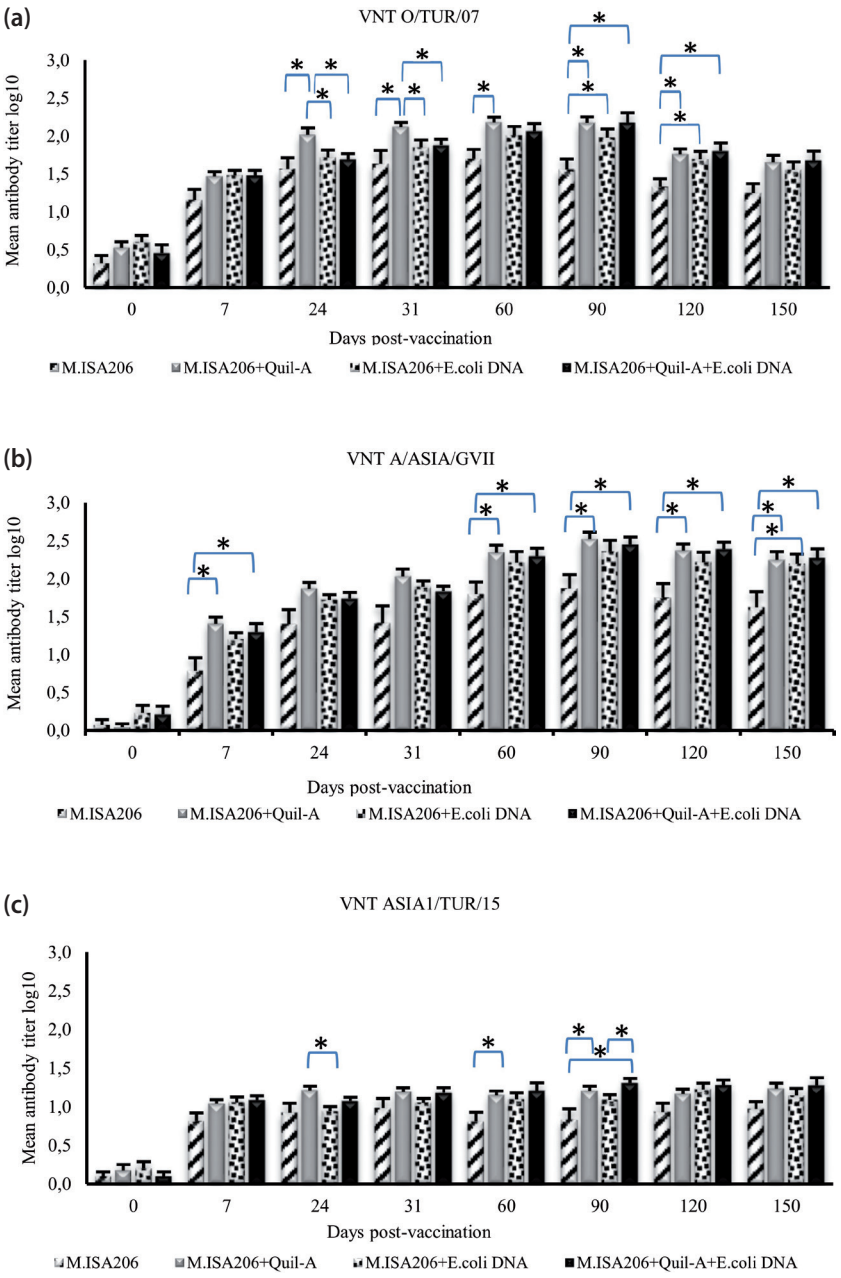


Fig. 1

Virus neutralization test (VNT) results

Arithmetic mean log10 homologous VN serum antibody titres against O/TUR/07 **(a)**, A/ASIA/G-VII (**b**), and ASIA1/TUR/15 **(c)** of vaccinated sheep with standard trivalent vaccine containing Montanide™™™ ISA 206 (group 1), Quil-A + Montanide™ ISA 206 (group 2), *E. coli* DNA + Montanide™ ISA 206 (group 3) and Quil-A + *E. coli* DNA + Montanide™ ISA 206 (group 4) on days 0, 7, 24, 31, 60, 90, 120 and 150 pv. The error bars indicate standard deviation.

Patil *et al.*, 2002). Dar *et al.* (2013) suggested that if an adjuvant was potent, it should generate a faster, stronger neutralizing antibody response with long duration. This study aimed to evaluate the effect of the use of two different Th1 inducers individually or in combination on the antibody response.

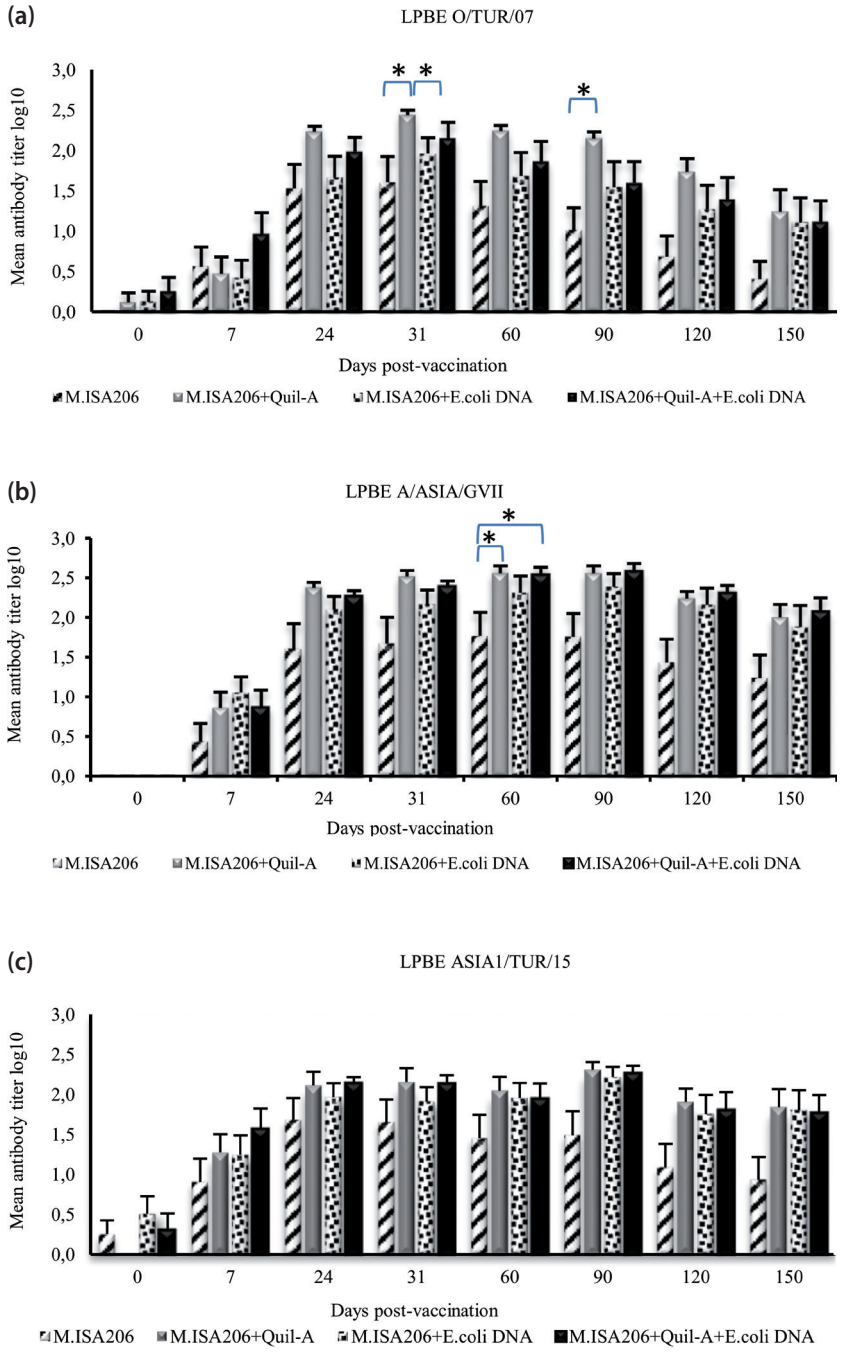


Fig. 2

Liquid-phase blocking ELISA (LPBE) test results

Arithmetic mean log10 homologous virus-specific total antibody titres against O/TUR/07 **(a)**, A/ASIA/G-VII **(b)**, and ASIA1/TUR/15 **(c)** of vaccinated sheep with standard trivalent vaccine containing Montanide™ ISA 206 (group 1), Quil-A + Montanide™ ISA 206 (group 2), *E. coli* DNA + Montanide™ ISA 206 (group 3) and Quil-A + *E. coli* DNA + Montanide™ ISA 206 (group 4) on days 0, 7, 24, 31, 60, 90, 120 and 150 pv. The error bars indicate standard deviation.

The results revealed that Quil-A and *E. coli* DNA increase the antibody response when added individually to the oil adjuvant FMD vaccine, but surprisingly, if both are added together, they cannot exceed the level of the antibody obtained in the Quil-A only group. Starting from day 24 pv to the end of the study, there were significantly higher neutralizing antibody titres for serotype O in the Quil-A added group than in the Montanide™ ISA 206 group. These results confirm that Quil-A, as shown in other studies, is a potent adjuvant. It has been previously shown that the use of saponin fractions such as Quil-A or QS-21 with oil adjuvant increases the immune response to FMD vaccine (Smitsaart *et al*., 2004; Çokçalışkan *et al*., 2016; Bazid *et al*., 2021; Rathogwa *et al*., 2021). Although the action mechanism of saponins has not been fully clarified, it was thought that it has shown its effect by generating danger signals by changing the membrane integrity of the cells (Bonam *et al*., 2017). In a study in which Quil-A was tested using OVA antigen in sheep, it was revealed that five-fold more potent and a balanced Th1/Th2 immune response than the Alum adjuvant could be obtained (Mahakapuge *et al*., 2015). In another study with the inactive FMDV ASIA1 antigen, 10 μg of Ginseng stem-leaf saponins together with mineral oil have been shown to induce higher IgG antibodies in mice measured by ELISA (Song *et al*., 2009). Bayry *et al*. (1999) obtained higher VN and ELISA antibodies with ISCOMs containing saponin for FMD virus recombinant proteins than Montanide™ ISA in a guinea pig model. According to our findings, formulations containing Quil-A also resulted in higher ELISA antibody titres than the formulation containing only Montanide™ ISA 206 as an adjuvant.

CpG synthetic oligonucleotides produce a Th1-type immune response. The use of CpG-ODNs with FMDV antigen has been tried in several studies to increase the immune response (Ren *et al*., 2011; Morshedi *et al*., 2010; Gungor *et al*., 2014). In this study, the group containing *E. coli* DNA in the neutralizing antibody titres on the 90th and 120th days was significantly higher than the group containing only Montanide™ ISA 206, revealing the limited adjuvant effect of *E. coli* DNA. However, the co-administration of both *E. coli* DNA and Quil-A did not reveal the expected synergistic effect on the neutralizing antibody response, and combination group titres did not exceed the titre levels of the group Montanide™ ISA 206 plus Quil-A. As a result, the synergistic effect of these two adjuvants was not observed in the ISA 206 emulsion. This finding is consistent with that of Vreman *et al*. (2021) who demonstrated that the antibody response after booster vaccination against porcine reproductive and respiratory syndrome virus in neonates was related to emulsion type and was not dependent on the presence of Toll-like receptor agonists.

In this study, *E. coli* DNA, as a low-cost alternative to CPG-ODNs, was used in sheep. Montanide™ ISA 206 provides the depot effect at the site of injection for the antigen and for the nucleotide, as Ren *et al*. (2011) suggested. Similarly, it has been demonstrated that insect DNA increases the amount of a specific antibody to an antigen if it was given in mineral oil (IFA) (Sun *et al*., 1998). However, it was rapidly degraded by nuclease enzymes when given in soluble form *in vivo*. Gungor *et al*. (2014) claimed that it was possible to construct nano-ring structures of ODNs by using cationic peptides to increase the resistance against nucleases and generate a robust response to FMD in mice. The absence of those secondary structures of DNA in this study could be another reason for not receiving the synergistic effect of combined adjuvants.

Both substances used in this study were found to increase antibody titres in sheep. Quil-A or *E. coli* DNA can be added to the antigen phase to form a vaccine emulsion during production without a substantial change in the process. Although almost the same amounts were used in the formulations as micrograms per ml, it is difficult to compare directly both adjuvants with each other, however, the higher antibody responses on days 24 and 31 in group 2 may indicate that Quil-A induces humoral immunity better than *E. coli* DNA. Nevertheless, Quil-A has some drawbacks. It would be more reasonable to use synthetic alternatives or triterpenoid saponins discovered from other plants because the extraction of Quil-A, from a bark of tree originating in South America will put pressure on natural resources as demand increases. It can also be difficult to produce *E. coli* DNA in large volumes for vaccine manufacturing. In order to facilitate production, the use of whole *E. coli* extracts can be considered. Thus, other components that can serve as adjuvants such as flagella, lipopolysaccharides, etc., can also be utilized to provoke an immune response.

Finally, when three or more adjuvants are used as a combination, further studies are needed to demonstrate the mechanism of action of each adjuvant and their interaction with each other and with the antigen.

Acknowledgments. The authors thank Ertan Ağtürk and Mehmet Karakaya for their guidance. Ayca Babak, Abdullah Arslan and Ömer Şişman are acknowledged for their technical help. This study was supported as a part of Research Project (TAGEM/HSGYAD/16/A02/P02/69) by General Directorate of Agricultural Research and Policy (GDAR) of Turkish Republic Ministry of Food and Agriculture and livestock.

Supplementray information is available in the online version of the paper.

References

Bayry J, Prabhudas K, Gopalakrishna S, Patil PK, Ramakrishna C, Misra LD, Suryanarayana VV (1999): Protective immune response to 16 kDa immunoreactive recombinant protein encoding the C-terminal VP1 portion of foot and mouth disease virus type Asia 1. Microbiol. Immunol. 43, 765–771. https://doi.org/10.1111/j.1348-0421.1999.tb02468.x

Bazid AI, El-Alfy HA, El-Didamony G, Elfeil WK, El-Sayed MM, Fawzy M (2021): Adjuvant effect of saponin in an oil-based monovalent (serotype O) foot-and-mouth disease virus vaccine on the antibody response in guinea pigs and cattle. Arch. Virol. 166, 1977–1984. https://doi.org/10.1007/s00705-021-05043-9

Belsham GJ (2020): Towards improvements in foot-and-mouth disease vaccine performance. Acta Vet. Scand. 62, 20. https://doi.org/10.1186/s13028-020-00519-1

Bonam SR, Partidos CD, Halmuthur SKM, Muller S (2017): An Overview of Novel Adjuvants Designed for Improving Vaccine Efficacy. Trends Pharmacol. Sci. 38, 771–793. https://doi.org/10.1016/j.tips.2017.06.002

Çokcaliskan C, Turkoglu T, Sareyyupoglu B, Uzunlu E, Babak A, Ozbilge BB, Gulyaz V (2016): QS-21 enhances the early antibody response to oil adjuvant foot-and-mouth disease vaccine in cattle. Clin. Exp. Vaccine Res. 5, 138–147. https://doi.org/10.7774/cevr.2016.5.2.138

Dar P, Kalaivanan R, Sied N, Mamo B, Kishore S, Suryanarayana VV, Kondabattula G (2013): Montanide ISA™ 201 adjuvanted FMD vaccine induces improved immune responses and protection in cattle. Vaccine 18, 3327–3332. https://doi.org/10.1016/j.vaccine.2013.05.078

Dubois MA, Wagner H (2017): New perspectives for natural triterpene glycosides as potential adjuvants. Phytomedicine 37, 49–57. https://doi.org/10.1016/j.phymed.2017.10.019

Garg R, Babiuk L, Littel-van den Hurk SD, Gerdts V (2017): A novel combination adjuvant platform for human and animal vaccines. Vaccine 35, 4486–4489. https://doi.org/10.1016/j.vaccine.2017.05.067

Gungor B, Yagci FC, Tincer G, Bayyurt B, Alpdundar E. Yildiz S, Ozcan M, Gursel I, Gursel M (2014): CpG ODN nanorings induce IFNa from plasmacytoid dendritic cells and demonstrate potent vaccine adjuvant activity. Sci. Transl. Med. 6, 235ra61. https://doi.org/10.1126/scitranslmed.3007909

Hamblin C, Barnett IT, Hedger RS (1986): A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. I. Development and method of ELISA. J. Immunol. Methods 23, 115–121. https://doi.org/10.1016/0022-1759(86)90441-2

Ioannou XP, Gomis SM, Karvonen B, Hecker R, Babiuk LA, van Drunen Littelvan, den Hurk S (2002): CpG-containing oligodeoxynucleotides, in combination with conventional adjuvants, enhance the magnitude and change the bias of the immune responses to a herpesvirus glycoprotein. Vaccine 21, 127–137. https://doi.org/10.1016/S0264-410X(02)00378-X

Kitching RP (1996): A recent history of foot-and-mouth disease. J. Comp. Pathol. 118, 89–108. https://doi.org/10.1016/S0021-9975(98)80002-9

Li D, Zhou C, She D, Li P, Sun P, Bai X, Chen Y, Xie B, Liu Z (2013): The comparison of the efficacy of swine FMD vaccine emulsified with oil adjuvant of ISA 201 VG or ISA 206 VG. J. Biosci. Med. 1, 22–25. https://doi.org/10.4236/jbm.2013.13005

Mahakapuge TAN, Every AL, Scheerlinck JP (2015): Exploring local immune responses to vaccines using efferent lymphatic cannulation. Expert Rev. Vaccines 14, 579–588. https://doi.org/10.1586/14760584.2015.1002475

McCullough KC, De Simone F, Brocchi E, Capucci L, Crowther JR, Kihm U (1992): Protective immune response against foot-and-mouth disease. J. Virol. 66, 1835–1840. https://doi.org/10.1128/jvi.66.4.1835-1840.1992

Morshedi A, Daghighi M, Mahravani H (2010): CpG-DNA enhancement the immune elicited as adjuvant of foot-and- mouth disease vaccine. Arch. Razi Inst. 65, 1–7.

Nichani AK, Mena A, Popowych Y, Dent D, Townsen, HGG, Mutwiria GK, Hecker R, Babiuk LA, Griebel PJ (2004): In vivo immunostimulatory effects of CpG oligodeoxynucleotide in cattle and sheep. Vet. Immunol. Immunopathol. 98, 17–29. https://doi.org/10.1016/j.vetimm.2003.10.001

OIE (2016): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals Ch. 2.1.8 http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.08 FMD.pdf.

Parida S, Oh Y, Reid SM, Cox SJ, Statham RJ, Mahapatra M, Anderson J, Barnett JV, Charleston B, Paton DJ (2006): Interferon gamma production in vitro from whole blood of foot-and-mouth disease virus (FMDV) vaccinated and infected cattle after incubation with inactivated FMDV. Vaccine 24, 964–969. https://doi.org/10.1016/j.vaccine.2005.08.108

Park ME, Lee SY, Kim RH, Ko MK, Lee KN, Kim SM, Kim BK, Lee JS, Kim B, Park JH (2014): Enhanced immune responses of foot-and-mouth disease vaccine using new oil/gel adjuvant mixtures in pigs and goats. Vaccine 8, 5221–5227. https://doi.org/10.1016/j.vaccine.2014.07.040

Patil PK, Bayry J, Ramakrishna C, Hugar B, Misra LD, Natarajan C (2002): Immune responses of goats against foot-and-mouth disease quadrivalent vaccine: comparison of double oil emulsion and aluminium hydroxide gel vaccines in eliciting immunity. Vaccine 20, 2781–2789. https://doi.org/10.1016/S0264-410X(02)00184-6

Pisetsky DS (1997): Immunostimulatory DNA. A clean and present danger. Nature Med. 3, 829–831. https://doi.org/10.1038/nm0897-829

Rajput ZI, Hu SH, Xiao CW, Arijo AG (2007): Adjuvant effects of saponins on animal immune responses. J. Zhejiang Univ. Sci. B. 8, 153–161. https://doi.org/10.1631/jzus.2007.B0153

Rathogwa, NM, Scott KA, Opperman P, Theron J, Maree FF (2021): Efficacy of SAT2 Foot-and-Mouth Disease vaccines formulated with Montanide ISA 206B and Quil-A saponin adjuvants. Vaccines 9, 996. https://doi.org/10.3390/vaccines9090996

Ren J, Yang L, Xu H, Zhang Y, Wan M, Liu G, Zhao L, Wang L, Yua Y (2011): CpG oligodeoxynucleotide and Montanide ISA 206 adjuvant combination augments the immune responses of a recombinant FMDV vaccine in cattle. Vaccine 29, 7960–7965. https://doi.org/10.1016/j.vaccine.2011.08.072

Smitsaart E, Mattion N, Filippi JL, Robiolo B, Periolo O, La Torre J, Bellinzoni RC (2004): Enhancement of the immune response induced by the inclusion of saponin in oil adjuvanted vaccines against foot-and-mouth disease. Session of the Research Group of the Standing Technical Committee of EuFMD Chania, Crete, Greece. 12–15 October, Appendix 53.

Song X, Bao S, Wu L, Hu S (2009): Ginseng stem-leaf saponins (GSLS) and mineral oil act synergistically to enhance the immune responses to vaccination against foot-and-mouth disease in mice. Vaccine 27, 51–55. https://doi.org/10.1016/j.vaccine.2008.10.030

Sun S, Kishimoto H, Sprent J (1998): DNA as an Adjuvant: Capacity of Insect DNA and Synthetic Oligodeoxynucleotides to Augment T Cell Responses to Specific Antigen. J. Exp. Med. The Rockefeller University Press 187, 1145–1150. https://doi.org/10.1084/jem.187.7.1145

Vreman S, Stockhofe-Zurwieden N, Popma-de Graaf DJ, Savelkoul HFJ, Barnier-Quer C, Collin N, Collins D, McDaid D, Moore AC, Rebel JMJ (2021): Immune responses induced by inactivated porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in neonatal pigs using different adjuvants. Vet. Immunol. Immunopathol. 232:110170. https://doi.org/10.1016/j.vetimm.2020.110170

Xiao CW, Rajput ZI, Hu SH (2007): Improvement of a commercial foot-and-mouth vaccine by supplement of Quil A. Vaccine 25, 4795–4800. https://doi.org/10.1016/j.vaccine.2007.04.027

Acta virologica **66**: 197 – 205, 2022 doi: 10.4149/av_2022_301

SUPPLEMENTARY INFORMATION

Evaluation of Quil-A, *E. coli* DNA and Montanide™ ISA 206 adjuvant combination on the antibody response to foot-and-mouth disease vaccine in sheep

Can Çokçalişkan[1], Tunçer Türkoğlu[1], Beyhan Sareyyüpoğlu[1], Pelin Tuncer-Göktuna[2], Banu Bayri Özbilge[1], Ergün Uzunlu[1], Ayça Kürkçü[1], Eylem Aras Uzun[1], Veli Gülyaz[3]

[1]Republic of Turkey, Ministry of Agriculture and Forestry, Institute of Foot and Mouth Disease (Şap), Ankara, Turkey; [2]Republic of Turkey, Ministry of Agriculture and Forestry, Pendik Veterinary Control Institute, Istanbul, Turkey; [3]Harran University, Faculty of Veterinary Medicine, Virology Department, Şanlıurfa, Turkey

Received September 24, 2021; revised April 11, 2022; accepted Maɣ 4, 2022

E-mail: cancokcaliskan@gmail.com, can.cokcaliskan@tarimorman.gov.tr; phone: +903122873600-2541.

VNT A	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	1	16	128	256	512	1024	512	512
	1	8	128	64	256	192	90	128
	1	6	16	32	64	128	64	64
	1	90	90	128	128	128	64	64
	1	1	1	1	4	8	8	1
	1	1	4	1	11	8	6	6
	1	11	45	32	64	128	256	90
	1	1	4	6	22	16	11	11
	1	11	32	45	64	64	32	16
	1	16	90	90	192	392	392	181
	1	1	8	4	32	16	11	32
	1	8	90	90	90	64	64	32
	8	16	45	128	90	192	256	256
	0	1,20412	2,10721	2,40824	2,70927	3,0103	2,70927	2,70927
	0	0,90309	2,10721	1,80618	2,40824	2,283301	1,954243	2,10721
	0	0,778151	1,20412	1,50515	1,80618	2,10721	1,80618	1,80618
	0	1,954243	1,954243	2,10721	2,10721	2,10721	1,80618	1,80618
	0	0	0	0	0,60206	0,90309	0,90309	0
	0	0	0,60206	0	1,041393	0,90309	0,778151	0,778151
	0	1,041393	1,653213	1,50515	1,80618	2,10721	2,40824	1,954243
	0	0	0,60206	0,778151	1,342423	1,20412	1,041393	1,041393
	0	1,041393	1,50515	1,653213	1,80618	1,80618	1,50515	1,20412
	0	1,20412	1,954243	1,954243	2,283301	2,593286	2,593286	2,257679
	0	0	0,90309	0,60206	1,50515	1,20412	1,041393	1,50515
	0	0,90309	1,954243	1,954243	1,954243	1,80618	1,80618	1,50515
	0,90309	1,20412	1,653213	2,10721	1,954243	2,283301	2,40824	2,40824
ort	0,069468	0,787209	1,400004	1,413927	1,794313	1,870661	1,750846	1,621767
SEM	0,069468	0,170188	0,189125	0,223941	0,157684	0,180786	0,183886	0,204125

	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	1	32	90	90	192	256	128	128
	1	4	11	16	32	64	90	32
	1	11	90	90	90	392	392	128
	1	22	64	90	128	256	192	128
	1	45	128	90	512	512	256	64
	1	32	90	128	192	181	256	181
	1	45	128	512	256	512	256	512
	1	32	128	128	392	90	90	128
	1	64	64	64	90	512	128	90
	1	22	64	128	512	512	1028	1028
	4	16	128	362	392	512	512	256
	1	45	32	64	512	1024	512	512
	1	16	128	128	256	392	128	362
	1	45	64	128	392	512	256	128
	0	1,50515	1,954243	1,954243	2,283301	2,40824	2,10721	2,10721
	0	0,60206	1,041393	1,20412	1,50515	1,80618	1,954243	1,50515
	0	1,041393	1,954243	1,954243	1,954243	2,593286	2,593286	2,10721
	0	1,342423	1,80618	1,954243	2,10721	2,40824	2,283301	2,10721
	0	1,653213	2,10721	1,954243	2,70927	2,70927	2,40824	1,80618
	0	1,50515	1,954243	2,10721	2,283301	2,257679	2,40824	2,257679
	0	1,653213	2,10721	2,70927	2,40824	2,70927	2,40824	2,70927
	0	1,50515	2,10721	2,10721	2,593286	1,954243	1,954243	2,10721
	0	1,80618	1,80618	1,80618	1,954243	2,70927	2,10721	1,954243
	0	1,342423	1,80618	2,10721	2,70927	2,70927	3,011993	3,011993
	0,60206	1,20412	2,10721	2,558709	2,593286	2,70927	2,70927	2,40824
	0	1,653213	1,50515	1,80618	2,70927	3,0103	2,70927	2,70927
	0	1,20412	2,10721	2,10721	2,40824	2,593286	2,10721	2,558709
	0	1,653213	1,80618	2,10721	2,593286	2,70927	2,40824	2,10721
ort	0,043004	1,405073	1,869289	2,031248	2,343685	2,520505	2,3693	2,246913
SEM	0,043004	0,084611	0,079096	0,093054	0,095672	0,087376	0,083154	0,106491

VNT O	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	6	22	128	256	128	90	90	64
	4	11	45	64	128	64	45	45
	4	11	45	90	45	64	22	16
	1	22	64	128	64	45	22	22
	1	6	8	11	11	8	6	4
	1	4	6	8	11	6	8	4
	1	64	45	45	128	90	64	45
	1	11	16	45	45	16	22	22
	4	22	128	64	45	32	11	8
	6	22	64	90	45	90	22	32
	1	1	4	1	11	6	6	4
	1	22	90	90	90	45	22	22
	6	90	128	90	192	192	45	45
	0,778151	1,342423	2,10721	2,40824	2,10721	1,954243	1,954243	1,80618
	0,60206	1,041393	1,653213	1,80618	2,10721	1,80618	1,653213	1,653213
	0,60206	1,041393	1,653213	1,954243	1,653213	1,80618	1,342423	1,20412
	0	1,342423	1,80618	2,10721	1,80618	1,653213	1,342423	1,342423
	0	0,778151	0,90309	1,041393	1,041393	0,90309	0,778151	0,60206
	0	0,60206	0,778151	0,90309	1,041393	0,778151	0,90309	0,60206
	0	1,80618	1,653213	1,653213	2,10721	1,954243	1,80618	1,653213
	0	1,041393	1,20412	1,653213	1,653213	1,20412	1,342423	1,342423
	0,60206	1,342423	2,10721	1,80618	1,653213	1,50515	1,041393	0,90309
	0,778151	1,342423	1,80618	1,954243	1,653213	1,954243	1,342423	1,50515
	0	0	0,60206	0	1,041393	0,778151	0,778151	0,60206
	0	1,342423	1,954243	1,954243	1,954243	1,653213	1,342423	1,342423
	0,778151	1,954243	2,10721	1,954243	2,283301	2,283301	1,653213	1,653213
ort	0,31851	1,152071	1,564253	1,630438	1,700183	1,556421	1,329211	1,247048
sem	0,100803	0,140029	0,145177	0,176302	0,119391	0,136617	0,104414	0,120619

	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	4	22	128	192	192	128	64	45
	1	22	64	64	90	90	45	22
	4	32	90	192	392	256	64	45
	4	22	22	90	128	64	32	22
	4	22	392	392	192	128	64	45
	1	22	90	128	192	392	64	64
	6	32	128	128	90	90	45	45
	6	128	392	192	256	192	45	22
	4	45	90	128	90	64	32	32
	4	45	64	128	128	392	192	181
	4	22	128	128	256	90	64	64
	6	32	128	128	256	392	192	256
	6	22	45	45	45	90	32	22
	1	16	128	128	128	192	32	22
	0,60206	1,342423	2,10721	2,283301	2,283301	2,10721	1,80618	1,653213
	0	1,342423	1,80618	1,80618	1,954243	1,954243	1,653213	1,342423
	0,60206	1,50515	1,954243	2,283301	2,593286	2,40824	1,80618	1,653213
	0,60206	1,342423	1,342423	1,954243	2,10721	1,80618	1,50515	1,342423
	0,60206	1,342423	2,593286	2,593286	2,283301	2,10721	1,80618	1,653213
	0	1,342423	1,954243	2,10721	2,283301	2,593286	1,80618	1,80618
	0,778151	1,50515	2,10721	2,10721	1,954243	1,954243	1,653213	1,653213
	0,778151	2,10721	2,593286	2,283301	2,40824	2,283301	1,653213	1,342423
	0,60206	1,653213	1,954243	2,10721	1,954243	1,80618	1,50515	1,50515
	0,60206	1,653213	1,80618	2,10721	2,10721	2,593286	2,283301	2,257679
	0,60206	1,342423	2,10721	2,10721	2,40824	1,954243	1,80618	1,80618
	0,778151	1,50515	2,10721	2,10721	1,954243	1,954243	1,653213	1,342423
	0,778151	1,342423	1,653213	1,653213	1,653213	1,954243	1,50515	1,342423
	0	1,20412	2,10721	2,10721	2,10721	2,283301	1,50515	1,342423
ort	0,523359	1,46644	2,01381	2,114807	2,178963	2,171318	1,755553	1,650599
sem	0,078613	0,06028	0,087482	0,059698	0,066522	0,076988	0,068423	0,090391

VNT AS	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	1	22	22	32	16	22	22	22
	1	8	16	32	11	22	16	16
	1	6	8	8	8	11	8	11
	1	16	16	11	11	6	6	8
	1	4	4	1	1	1	1	4
	1	1	1	6	1	1	6	4
	1	11	8	8	11	22	22	22
	4	2	2	4	2	2	4	4
	4	6	16	11	11	11	11	11
	1	8	22	32	16	11	16	8
	1	4	4	4	6	2	4	4
	1	8	11	22	8	8	6	6
	1	16	22	11	11	22	32	45
	0	1,342423	1,342423	1,50515	1,20412	1,342423	1,342423	1,342423
	0	0,90309	1,20412	1,50515	1,041393	1,342423	1,20412	1,20412
	0	0,778151	0,90309	0,90309	0,90309	1,041393	0,90309	1,041393
	0	1,20412	1,20412	1,041393	1,041393	0,778151	0,778151	0,90309
	0	0,60206	0,60206	0	0	0	0	0,60206
	0	0	0	0,778151	0	0	0,778151	0,60206
	0	1,041393	0,90309	0,90309	1,041393	1,342423	1,342423	1,342423
	0,60206	0,30103	0,30103	0,60206	0,30103	0,30103	0,60206	0,60206
	0,60206	0,778151	1,20412	1,041393	1,041393	1,041393	1,041393	1,041393
	0	0,90309	1,342423	1,50515	1,20412	1,041393	1,20412	0,90309
	0	0,60206	0,60206	0,60206	0,778151	0,30103	0,60206	0,60206
	0	0,90309	1,041393	1,342423	0,90309	0,90309	0,778151	0,778151
	0	1,20412	1,342423	1,041393	1,041393	1,342423	1,50515	1,653213
ort	0,092625	0,812521	0,922489	0,982346	0,807736	0,829013	0,92933	0,97058
sem	0,062707	0,10369	0,119411	0,120591	0,117885	0,141643	0,112761	0,094613

	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	1	11	32	16	16	11	11	16
	1	11	16	8	6	6	6	11
	1	11	16	11	11	16	11	22
	1	4	22	11	16	16	16	16
	1	22	22	16	22	22	11	8
	4	6	11	16	11	32	16	16
	4	22	8	22	16	11	11	11
	4	16	16	11	11	16	16	16
	1	11	11	16	11	16	22	22
	1	11	11	11	16	16	16	16
	1	11	32	45	45	22	45	32
	1	11	32	32	16	45	32	90
	4	11	11	16	11	8	11	16
	1	8	11	11	11	16	11	8
	0	1,041393	1,50515	1,20412	1,20412	1,041393	1,041393	1,20412
	0	1,041393	1,20412	0,90309	0,778151	0,778151	0,778151	1,041393
	0	1,041393	1,20412	1,041393	1,041393	1,20412	1,041393	1,342423
	0	0,60206	1,342423	1,041393	1,20412	1,20412	1,20412	1,20412
	0	1,342423	1,342423	1,20412	1,342423	1,342423	1,041393	0,90309
	0,60206	0,778151	1,041393	1,20412	1,041393	1,50515	1,20412	1,20412
	0,60206	1,342423	0,90309	1,342423	1,20412	1,041393	1,041393	1,041393
	0,60206	1,20412	1,20412	1,041393	1,041393	1,20412	1,20412	1,20412
	0	1,041393	1,041393	1,20412	1,041393	1,20412	1,342423	1,342423
	0	1,041393	1,041393	1,041393	1,20412	1,20412	1,20412	1,20412
	0	1,041393	1,50515	1,653213	1,653213	1,342423	1,653213	1,50515
	0	1,041393	1,50515	1,50515	1,20412	1,653213	1,50515	1,954243
	0,60206	1,041393	1,041393	1,20412	1,041393	0,90309	1,041393	1,20412
	0	0,90309	1,041393	1,041393	1,041393	1,20412	1,041393	0,90309
ort	0,172017	1,035958	1,208765	1,18796	1,14591	1,202282	1,167412	1,232709
sem	0,075435	0,051821	0,054108	0,054004	0,053128	0,060016	0,05897	0,070878

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	6	64	90	1028	512	392	512
6	32	64	128	1028	1024	512	362
1	11	192	128	512	512	512	512
1	32	32	64	192	1024	512	362
1	22	45	64	90	192	192	256
1	8	90	128	128	128	128	90
1	11	45	64	64	64	128	181
1	16	32	32	32	64	32	32
4	8	32	64	128	90	64	64
1	11	64	64	90	90	45	32
6	32	32	32	45	90	64	90
4	45	45	256	256	1024	392	256

0	0,778151	1,80618	1,954243	3,011993	2,70927	2,593286	2,70927
0,778151	1,50515	1,80618	2,10721	3,011993	3,0103	2,70927	2,558709
0	1,041393	2,283301	2,10721	2,70927	2,70927	2,70927	2,70927
0	1,50515	1,50515	1,80618	2,283301	3,0103	2,70927	2,558709
0	1,342423	1,653213	1,80618	1,954243	2,283301	2,283301	2,40824
0	0,90309	1,954243	2,10721	2,10721	2,10721	2,10721	1,954243
0	1,041393	1,653213	1,80618	1,80618	1,80618	2,10721	2,257679
0	1,20412	1,50515	1,50515	1,50515	1,80618	1,50515	1,50515
0,60206	0,90309	1,50515	1,80618	2,10721	1,954243	1,80618	1,80618
0	1,041393	1,80618	1,80618	1,954243	1,954243	1,653213	1,50515
0,778151	1,50515	1,50515	1,50515	1,653213	1,954243	1,80618	1,954243
0,60206	1,653213	1,653213	2,40824	2,40824	3,0103	2,593286	2,40824

0,230035	1,201976	1,719693	1,893776	2,209354	2,359587	2,215235	2,19459
0,099278	0,084151	0,066996	0,075147	0,143077	0,142749	0,129122	0,12635

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
6	22	256	392	256	192	90	45
6	22	64	64	256	192	128	128
4	45	90	192	256	256	192	90
1	22	22	45	192	128	64	64
4	45	45	45	90	128	45	45
6	32	90	90	90	90	45	45
4	32	32	64	64	64	16	16
1	22	32	45	32	22	16	8
4	16	32	45	45	32	32	16
8	22	22	22	32	45	22	16
6	128	32	45	45	45	45	32
6	22	128	128	392	392	90	64

0,778151	1,342423	2,40824	2,593286	2,40824	2,283301	1,954243	1,653213
0,778151	1,342423	1,80618	1,80618	2,40824	2,283301	2,10721	2,10721
0,60206	1,653213	1,954243	2,283301	2,40824	2,40824	2,283301	1,954243
0	1,342423	1,342423	1,653213	2,283301	2,10721	1,80618	1,80618
0,60206	1,653213	1,653213	1,653213	1,954243	2,10721	1,653213	1,653213
0,778151	1,50515	1,954243	1,954243	1,954243	1,954243	1,653213	1,653213
0,60206	1,50515	1,50515	1,80618	1,80618	1,80618	1,20412	1,20412
0	1,342423	1,50515	1,653213	1,50515	1,342423	1,20412	0,90309
0,60206	1,20412	1,50515	1,653213	1,653213	1,50515	1,50515	1,20412
0,90309	1,342423	1,342423	1,342423	1,50515	1,653213	1,342423	1,20412
0,778151	2,10721	1,50515	1,653213	1,653213	1,653213	1,653213	1,50515
0,778151	1,342423	2,10721	2,10721	2,593286	2,593286	1,954243	1,80618

0,600174	1,473549	1,715731	1,846574	2,011058	1,974747	1,693386	1,554504
0,085748	0,06984	0,095932	0,098173	0,113578	0,11187	0,099631	0,103582

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	22	8	16	32	11	11	22
1	11	8	11	45	22	64	32
1	16	11	11	22	11	22	45
1	11	8	11	11	22	32	32
4	22	11	11	16	22	22	11
1	8	16	11	11	16	11	11
4	8	6	11	6	6	8	6
1	8	8	11	11	8	8	6
4	6	4	6	6	8	8	8
1	8	6	6	6	6	16	6
4	32	8	11	8	11	22	16
1	6	22	32	11	22	22	16

0	1,342423	0,90309	1,20412	1,50515	1,041393	1,041393	1,342423
0	1,041393	0,90309	1,041393	1,653213	1,342423	1,80618	1,50515
0	1,20412	1,041393	1,041393	1,342423	1,041393	1,342423	1,653213
0	1,041393	0,90309	1,041393	1,041393	1,342423	1,50515	1,50515
0,60206	1,342423	1,041393	1,041393	1,20412	1,342423	1,342423	1,041393
0	0,90309	1,20412	1,041393	1,041393	1,20412	1,041393	1,041393
0,60206	0,90309	0,778151	1,041393	0,778151	0,778151	0,90309	0,778151
0	0,90309	0,90309	1,041393	1,041393	0,90309	0,90309	0,778151
0,60206	0,778151	0,60206	0,778151	0,778151	0,90309	0,90309	0,90309
0	0,90309	0,778151	0,778151	0,778151	0,778151	1,20412	0,778151
0,60206	1,50515	0,90309	1,041393	0,90309	1,041393	1,342423	1,20412
0	0,778151	1,342423	1,50515	1,041393	1,342423	1,342423	1,20412

0,200687	1,053797	0,941928	1,049726	1,092335	1,088373	1,2231	1,144542
0,085573	0,069416	0,056784	0,053606	0,082681	0,06392	0,080251	0,089077

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	32	90	90	392	512	392	512
1	8	45	45	192	128	90	64
1	64	32	45	90	256	256	64
1	8	16	32	64	128	64	32
11	32	45	64	128	512	512	256
1	32	32	45	392	1024	512	256
4	22	64	90	392	512	512	512
1	64	90	128	1028	512	512	512
11	16	64	64	90	90	128	90
1	22	128	128	256	392	512	512
1	16	128	192	512	512	256	362
1	2	22	32	64	90	128	64
1	32	90	64	90	128	128	256

0	1,50515	1,954243	1,954243	2,593286	2,70927	2,593286	2,70927
0	0,90309	1,653213	1,653213	2,283301	2,10721	1,954243	1,80618
0	1,80618	1,50515	1,653213	1,954243	2,40824	2,40824	1,80618
0	0,90309	1,20412	1,50515	1,80618	2,10721	1,80618	1,50515
1,041393	1,50515	1,653213	1,80618	2,10721	2,70927	2,70927	2,40824
0	1,50515	1,50515	1,653213	2,593286	3,0103	2,70927	2,40824
0,60206	1,342423	1,80618	1,954243	2,593286	2,70927	2,70927	2,70927
0	1,80618	1,954243	2,10721	3,011993	2,70927	2,70927	2,70927
1,041393	1,20412	1,80618	1,80618	1,954243	1,954243	2,10721	1,954243
0	1,342423	2,10721	2,10721	2,40824	2,593286	2,70927	2,70927
0	1,20412	2,10721	2,283301	2,70927	2,70927	2,40824	2,558709
0	0,30103	1,342423	1,50515	1,80618	1,954243	2,10721	1,80618
0	1,50515	1,954243	1,80618	1,954243	2,10721	2,10721	2,40824

0,206527	1,294866	1,734829	1,83036	2,290382	2,445253	2,387551	2,269111
0,112574	0,113664	0,079518	0,066822	0,108214	0,098583	0,092064	0,119898

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	45	90	128	256	256	192	181
1	22	90	90	90	128	32	22
4	45	22	45	45	45	32	22
8	22	22	32	45	90	32	22
1	64	64	128	392	1028	192	128
1	45	32	45	192	512	256	256
6	32	45	90	90	90	90	128
6	64	45	192	392	512	128	90
11	22	90	90	90	90	45	22
4	22	128	128	192	256	90	64
1	22	45	64	128	90	32	16
4	8	16	22	22	22	16	16
4	32	64	90	90	128	32	22

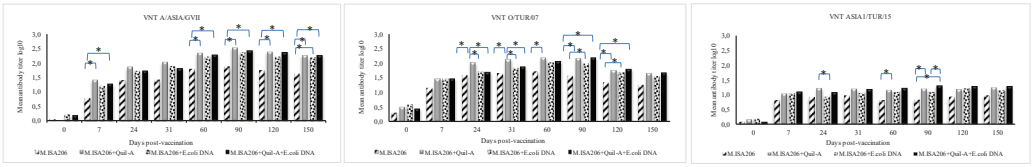
0	1,653213	1,954243	2,10721	2,40824	2,40824	2,283301	2,257679
0	1,342423	1,954243	1,954243	1,954243	2,10721	1,50515	1,342423
0,60206	1,653213	1,342423	1,653213	1,653213	1,653213	1,50515	1,342423
0,90309	1,342423	1,342423	1,50515	1,653213	1,954243	1,50515	1,342423
0	1,80618	1,80618	2,10721	2,593286	3,011993	2,283301	2,10721
0	1,653213	1,50515	1,653213	2,283301	2,70927	2,40824	2,40824
0,778151	1,50515	1,653213	1,954243	1,954243	1,954243	1,954243	2,10721
0,778151	1,80618	1,653213	2,283301	2,593286	2,70927	2,10721	1,954243
1,041393	1,342423	1,954243	1,954243	1,954243	1,954243	1,653213	1,342423
0,60206	1,342423	2,10721	2,10721	2,283301	2,40824	1,954243	1,80618
0	1,342423	1,653213	1,80618	2,10721	1,954243	1,50515	1,20412
0,60206	0,90309	1,20412	1,342423	1,342423	1,342423	1,20412	1,20412
0,60206	1,50515	1,80618	1,954243	1,954243	2,10721	1,50515	1,342423

0,45454	1,476731	1,687388	1,875545	2,056496	2,174926	1,797971	1,673932
0,109636	0,068472	0,07706	0,074984	0,103851	0,127848	0,107128	0,122538

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	22	22	22	64	32	22	90
1	8	11	16	11	11	11	22
1	16	11	11	6	22	11	6
1	22	6	6	4	8	8	8
4	16	16	45	32	32	32	16
1	8	11	11	11	16	22	22
1	22	11	16	16	22	22	32
1	16	11	22	64	22	32	32
1	6	11	22	16	11	11	8
4	8	16	11	16	32	45	64
1	11	11	16	22	32	22	16
1	8	6	6	6	16	11	8
1	11	22	22	22	32	32	16

0	1,342423	1,342423	1,342423	1,80618	1,50515	1,342423	1,954243
0	0,90309	1,041393	1,20412	1,041393	1,041393	1,041393	1,342423
0	1,20412	1,041393	1,041393	0,778151	1,342423	1,041393	0,778151
0	1,342423	0,778151	0,778151	0,60206	0,90309	0,90309	0,90309
0,60206	1,20412	1,20412	1,653213	1,50515	1,50515	1,50515	1,20412
0	0,90309	1,041393	1,041393	1,041393	1,20412	1,342423	1,342423
0	1,342423	1,041393	1,20412	1,20412	1,342423	1,342423	1,50515
0	1,20412	1,041393	1,342423	1,80618	1,342423	1,50515	1,50515
0	0,778151	1,041393	1,342423	1,20412	1,041393	1,041393	0,90309
0,60206	0,90309	1,20412	1,041393	1,20412	1,50515	1,653213	1,80618
0	1,041393	1,041393	1,20412	1,342423	1,50515	1,342423	1,20412
0	0,90309	0,778151	0,778151	0,778151	1,20412	1,041393	0,90309
0	1,041393	1,342423	1,342423	1,342423	1,50515	1,50515	1,20412

0,092625	1,08561	1,072241	1,178134	1,204297	1,303626	1,277463	1,273488
0,062707	0,054988	0,048066	0,067547	0,102287	0,058031	0,06586	0,099209



log ort

A/ASIA/GVII

	0	7	24	31	60	90	120	150	0	7	24	31	60	90	120	150
M.ISA206	0,069468	0,787209	1,400004	1,413927	1,794313	1,870661	1,750846	1,621767	0,069468	0,170188	0,189125	0,223941	0,157684	0,180786	0,183886	0,204125
M.ISA206+Qui	0,043004	1,405073	1,869289	2,031248	2,343685	2,520505	2,3693	2,246913	0,043004	0,084611	0,079096	0,093054	0,095672	0,087376	0,083154	0,106491
M.ISA206+E.coli	0,230035	1,201976	1,719693	1,893776	2,209354	2,359587	2,215235	2,19459	0,099278	0,084151	0,066996	0,075147	0,143077	0,142749	0,129122	0,12635
M.ISA206+Quil-A+E.	0,206527	1,294866	1,734829	1,83036	2,290382	2,445253	2,387551	2,269111	0,112574	0,113664	0,079518	0,066822	0,108214	0,098583	0,092064	0,119898

O/TUR/18

	0	7	24	31	60	90	120	150	0	7	24	31	60	90	120	150
M.ISA206	0,31851	1,152071	1,564253	1,630438	1,700183	1,556421	1,329211	1,247048	0,100803	0,140029	0,145177	0,176302	0,119391	0,136617	0,104414	0,120619
M.ISA206+Qui	0,523359	1,46644	2,01381	2,114807	2,178963	2,171318	1,755553	1,650599	0,078613	0,06028	0,087482	0,059698	0,066522	0,076988	0,068423	0,090391
M.ISA206+E.coli	0,600174	1,473549	1,715731	1,846574	2,011058	1,974747	1,693386	1,554504	0,085748	0,06984	0,095932	0,098173	0,113578	0,11187	0,099631	0,103582
M.ISA206+Quil-A+E.	0,45454	1,476731	1,687388	1,875545	2,056496	2,174926	1,797971	1,673932	0,109636	0,068472	0,07706	0,074984	0,103851	0,127848	0,107128	0,122538

ASIA1/TUR/15

	0	7	24	31	60	90	120	150	0	7	24	31	60	90	120	150
M.ISA206	0,092625	0,812521	0,922489	0,982346	0,807736	0,829013	0,92933	0,97058	0,062707	0,10369	0,119411	0,120591	0,117885	0,141643	0,112761	0,094613
M.ISA206+Qui	0,172017	1,035958	1,208765	1,18796	1,14591	1,202282	1,167412	1,232709	0,075435	0,051821	0,054108	0,054004	0,053128	0,060016	0,05897	0,070878
M.ISA206+E.coli	0,200687	1,053797	0,941928	1,049726	1,092335	1,088373	1,2231	1,144542	0,085573	0,069416	0,056784	0,053606	0,082681	0,06392	0,080251	0,089077
M.ISA206+Quil-A+E.	0,092625	1,08561	1,072241	1,178134	1,204297	1,303626	1,277463	1,273488	0,062707	0,054988	0,048066	0,067547	0,102287	0,058031	0,06586	0,099209

LPB A	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	1	1	512	712	1400	1024	512	362
	1	1	192	362	362	192	192	96
	1	1	45	96	96	96	45	45
	1	96	362	362	192	192	96	96
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	45	96	96	192	192	192	96
	1	1	1	1	1	1	1	1
	1	1	362	192	96	96	1	1
	1	1	192	362	192	362	192	96
	1	1	1	1	45	45	45	45
	1	1	192	256	192	192	32	1
	1	96	362	362	362	362	192	192

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	1	362	362	362	362	192	96
1	1	45	45	45	45	45	1
1	45	192	362	362	362	192	96
1	1	192	192	362	362	96	96
1	64	362	362	362	362	192	96
1	1	362	362	362	362	192	192
1	45	362	712	712	712	362	362
1	45	192	362	712	362	96	96
1	45	192	362	192	192	96	45
1	1	362	362	712	1400	712	712
1	1	362	712	1400	712	362	362
1	96	192	362	362	712	362	362
1	45	362	362	362	362	192	96
1	1	192	362	192	192	64	45

0	0	2,70927	2,85248	3,146128	3,0103	2,70927	2,558709
0	0	2,283301	2,558709	2,558709	2,283301	2,283301	1,982271
0	0	1,653213	1,982271	1,982271	1,982271	1,653213	1,653213
0	1,982271	2,558709	2,558709	2,283301	2,283301	1,982271	1,982271
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	1,653213	1,982271	1,982271	2,283301	2,283301	2,283301	1,982271
0	0	0	0	0	0	0	0
0	0	2,558709	2,283301	1,982271	1,982271	0	0
0	0	2,283301	2,558709	2,283301	2,558709	2,283301	1,982271
0	0	0	0	1,653213	1,653213	1,653213	1,653213
0	0	2,283301	2,40824	2,283301	2,283301	1,50515	0
0	1,982271	2,558709	2,558709	2,558709	2,558709	2,283301	2,283301

0	0	2,558709	2,558709	2,558709	2,558709	2,283301	1,982271
0	0	1,653213	1,653213	1,653213	1,653213	1,653213	0
0	1,653213	2,283301	2,558709	2,558709	2,558709	2,283301	1,982271
0	0	2,283301	2,283301	2,558709	2,558709	1,982271	1,982271
0	1,80618	2,558709	2,558709	2,558709	2,558709	2,283301	1,982271
0	0	2,558709	2,558709	2,558709	2,558709	2,283301	2,283301
0	1,653213	2,558709	2,85248	2,85248	2,85248	2,558709	2,558709
0	1,653213	2,283301	2,558709	2,85248	2,558709	1,982271	1,982271
0	1,653213	2,283301	2,558709	2,283301	2,283301	1,982271	1,653213
0	0	2,558709	2,85248	3,146128	2,85248	2,85248	2,85248
0	0	2,558709	2,85248	3,146128	2,85248	2,558709	2,558709
0	1,982271	2,283301	2,558709	2,558709	2,85248	2,558709	2,558709
0	1,653213	2,558709	2,558709	2,558709	2,558709	2,283301	1,982271
0	0	2,283301	2,558709	2,283301	2,283301	1,80618	1,653213

ort	0	0,432135	1,605445	1,672569	1,770347	1,759898	1,433563	1,236732
sem	0	0,228769	0,318018	0,32845	0,296283	0,292398	0,290212	0,289348

0	0,861037	2,375999	2,516326	2,559596	2,559596	2,23938	2,000854
0	0,239939	0,066616	0,07571	0,093043	0,093043	0,088362	0,181354

	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
LPB O	1	1	362	712	362	192	192	96
Grup-1	1	45	192	192	96	96	45	45
	1	1	96	128	192	45	45	1
	1	45	362	362	96	45	1	1
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	1	96	96	96	96	1	1	1
	1	1	1	1	1	1	1	1
	1	1	192	96	96	45	1	1
	1	1	96	192	96	96	45	45
	1	1	1	1	1	1	1	1
	1	1	96	362	1	1	1	1
	1	96	192	192	192	96	45	1

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	1	192	362	96	96	45	1
1	1	45	96	45	45	1	1
1	45	362	362	256	96	45	45
1	1	192	362	192	96	45	45
1	45	192	362	192	192	96	45
1	1	96	362	192	192	96	96
1	1	362	362	362	192	96	96
1	1	192	192	192	96	45	45
1	45	192	192	96	96	45	1
1	1	192	362	362	712	712	712
1	1	362	712	362	362	96	96
45	45	192	192	192	192	96	96
1	1	96	192	96	96	45	1
1	1	96	192	192	96	45	1

0	0	2,558709	2,85248	2,558709	2,283301	2,283301	1,982271
0	1,653213	2,283301	2,283301	1,982271	1,982271	1,653213	1,653213
0	0	1,982271	2,10721	2,283301	1,653213	1,653213	0
0	1,653213	2,558709	2,558709	1,982271	1,653213	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	1,982271	1,982271	1,982271	1,982271	0	0	0
0	0	0	0	0	0	0	0
0	0	2,283301	1,982271	1,982271	1,653213	0	0
0	0	1,982271	2,283301	1,982271	1,982271	1,653213	1,653213
0	0	0	0	0	0	0	0
0	1,982271	2,283301	2,283301	2,283301	1,982271	1,653213	0

0	0	2,283301	2,558709	1,982271	1,982271	1,653213	0
0	0	1,653213	1,982271	1,653213	1,653213	0	0
0	1,653213	2,558709	2,558709	2,40824	1,982271	1,653213	1,653213
0	0	2,283301	2,558709	2,283301	1,982271	1,653213	1,653213
0	1,653213	2,283301	2,558709	2,283301	2,283301	1,982271	1,653213
0	0	1,982271	2,558709	2,283301	2,283301	1,982271	1,982271
0	0	2,558709	2,558709	2,558709	2,283301	1,982271	1,982271
0	0	2,283301	2,283301	2,283301	1,982271	1,653213	1,653213
0	1,653213	2,283301	2,283301	1,982271	1,982271	1,653213	0
0	0	1,653213	2,283301	2,283301	2,85248	2,85248	2,85248
0	0	2,558709	2,85248	2,558709	2,558709	1,982271	1,982271
1,653213	1,653213	2,283301	2,283301	2,283301	2,283301	1,982271	1,982271
0	0	1,982271	2,283301	1,982271	1,982271	1,653213	0
0	0	1,982271	2,283301	2,283301	1,982271	1,653213	0

ort	0	0,559305	1,530493	1,607043	1,310513	1,014596	0,684319	0,406823
sem	0	0,243615	0,299579	0,316193	0,302703	0,275247	0,253919	0,215491

0,118087	0,472346	2,232804	2,440158	2,241729	2,148107	1,738309	1,242458
0,118087	0,207138	0,068769	0,057468	0,069844	0,080359	0,159177	0,269053

LPB AS	Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
	1	362	362	1400	712	712	192	192
	1	1	192	192	256	128	96	45
	1	1	96	96	128	96	96	2
	1	96	362	192	96	96	45	45
	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1
	45	45	96	45	96	192	96	96
	1	1	1	1	1	1	1	1
	1	45	192	96	45	45	1	1
	1	1	192	192	96	192	96	96
	1	45	45	45	45	45	1	1
	1	1	96	45	1	1	1	1
	45	192	192	362	192	362	192	192

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	1	192	192	96	128	45	45
1	1	1	1	1	45	1	1
1	45	192	192	192	192	96	712
1	1	192	192	192	192	96	96
1	45	96	192	192	192	96	96
1	45	128	192	362	362	128	192
1	45	256	362	362	362	96	96
1	1	192	192	192	192	96	96
1	45	192	192	96	192	96	45
1	32	96	96	45	362	192	192
1	96	362	512	192	712	192	362
1	192	362	362	362	712	712	362
1	64	192	192	96	96	96	96
1	96	192	128	96	64	45	1

0	2,558709	2,558709	3,146128	2,85248	2,85248	2,283301	2,283301
0	0	2,283301	2,283301	2,40824	2,10721	1,982271	1,653213
0	0	1,982271	1,982271	2,10721	1,982271	1,982271	0,30103
0	1,982271	2,558709	2,283301	1,982271	1,982271	1,653213	1,653213
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
1,653213	1,653213	1,982271	1,653213	1,982271	2,283301	1,982271	1,982271
0	0	0	0	0	0	0	0
0	1,653213	2,283301	1,982271	1,653213	1,653213	0	0
0	0	2,283301	2,283301	1,982271	2,283301	1,982271	1,982271
0	1,653213	1,653213	1,653213	1,653213	1,653213	0	0
0	0	1,982271	1,653213	0	0	0	0
1,653213	2,283301	2,283301	2,558709	2,283301	2,558709	2,283301	2,283301

0	0	2,283301	2,283301	1,982271	2,10721	1,653213	1,653213
0	0	0	0	0	1,653213	0	0
0	1,653213	2,283301	2,283301	2,283301	2,283301	1,982271	2,85248
0	0	2,283301	2,283301	2,283301	2,283301	1,982271	1,982271
0	1,653213	1,982271	2,283301	2,283301	2,283301	1,982271	1,982271
0	1,653213	2,10721	2,283301	2,558709	2,558709	2,10721	2,283301
0	1,653213	2,40824	2,558709	2,558709	2,558709	1,982271	1,982271
0	0	0	2,283301	2,283301	2,283301	1,982271	1,982271
0	1,653213	2,283301	2,283301	1,982271	2,283301	1,982271	1,653213
0	1,50515	1,982271	1,982271	1,653213	2,558709	2,283301	2,283301
0	1,982271	2,558709	2,70927	2,283301	2,85248	2,283301	2,558709
0	2,283301	2,558709	2,558709	2,558709	2,85248	2,85248	2,558709
0	1,80618	2,283301	2,283301	1,982271	1,982271	1,982271	1,982271
0	1,982271	2,283301	2,10721	1,982271	1,80618	1,653213	0

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	1	2	2	2	45	2	2
1	1	362	362	1400	712	362	362
1	45	192	192	1400	1400	1400	712
1	45	362	712	712	712	712	362
1	45	96	192	712	1400	712	712
1	1	192	192	256	192	192	192
1	2	362	362	362	362	192	192
1	45	96	96	96	64	96	96
1	45	192	96	96	45	45	2
1	1	96	192	96	96	45	45
1	32	96	96	96	96	45	2
1	96	96	96	96	96	96	96
1	45	362	712	712	1400	712	362

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	45	362	362	712	512	362	362
1	2	192	192	362	362	96	96
1	45	96	96	96	128	96	45
1	1	96	96	192	96	96	96
1	192	362	712	1400	1400	712	362
1	1	96	192	362	712	362	362
1	1	192	192	192	712	362	362
1	45	192	362	1400	1400	512	362
1	1	362	362	192	192	96	45
1	1	256	362	362	362	362	192
1	45	362	362	512	512	192	192
1	1	45	96	96	96	45	2
1	96	192	362	362	362	192	96

0	0	0,30103	0,30103	0,30103	1,653213	0,30103	0,30103
0	0	2,558709	2,558709	3,146128	2,85248	2,558709	2,558709
0	1,653213	2,283301	2,283301	3,146128	3,146128	3,146128	2,85248
0	1,653213	2,558709	2,85248	2,85248	2,85248	2,85248	2,558709
0	1,653213	1,982271	2,283301	2,85248	3,146128	2,85248	2,85248
0	0	2,283301	2,283301	2,40824	2,283301	2,283301	2,283301
0	0,30103	2,558709	2,558709	2,558709	2,558709	2,283301	2,283301
0	1,653213	1,982271	1,982271	1,982271	1,80618	1,982271	1,982271
0	1,653213	2,283301	1,982271	1,982271	1,653213	1,653213	0,30103
0	0	1,982271	2,283301	1,982271	1,982271	1,653213	1,653213
0	1,50515	1,982271	1,982271	1,982271	1,982271	1,653213	0,30103
0	1,982271	1,982271	1,982271	1,982271	1,982271	1,982271	1,982271
0	1,653213	2,558709	2,85248	2,85248	3,146128	2,85248	2,558709

0	1,653213	2,558709	2,558709	2,85248	2,70927	2,558709	2,558709
0	0,30103	2,283301	2,283301	2,558709	2,558709	1,982271	1,982271
0	1,653213	1,982271	1,982271	1,982271	2,10721	1,982271	1,653213
0	0	1,982271	1,982271	2,283301	1,982271	1,982271	1,982271
0	2,283301	2,558709	2,85248	3,146128	3,146128	2,85248	2,558709
0	0	1,982271	2,283301	2,558709	2,85248	2,558709	2,558709
0	0	2,283301	2,283301	2,283301	2,85248	2,558709	2,558709
0	1,653213	2,283301	2,558709	3,146128	3,146128	2,70927	2,558709
0	0	2,558709	2,558709	2,283301	2,283301	1,982271	1,653213
0	0	2,40824	2,558709	2,558709	2,558709	2,558709	2,283301
0	1,653213	2,558709	2,558709	2,70927	2,70927	2,283301	2,283301
0	0	1,653213	1,982271	1,982271	1,982271	1,653213	0,30103
0	1,982271	2,283301	2,558709	2,558709	2,558709	2,283301	1,982271

0	1,05444	2,099779	2,168131	2,309925	2,388059	2,158007	1,882195
0	0,22969	0,165018	0,177574	0,2107	0,162276	0,210156	0,267352

0	0,859958	2,259716	2,384727	2,531022	2,572841	2,303499	2,07034
0	0,26008	0,079285	0,076677	0,103813	0,108891	0,100096	0,175136

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	1	1	1	1	1	1	1
1	1	362	712	192	192	128	96
1	64	96	96	362	192	192	192
1	45	362	362	512	362	192	192
1	1	2	45	192	362	192	192
1	1	128	192	96	96	45	1
1	1	362	256	192	192	96	45
1	1	96	45	1	1	1	1
1	1	45	96	1	1	1	1
1	1	96	128	45	45	1	1
1	1	1	64	45	1	1	1
45	96	45	45	45	45	45	45
1	1	192	362	712	712	192	192

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	45	192	362	192	192	192	96
1	1	192	362	128	96	96	45
1	1	45	45	1	1	1	1
1	1	96	96	45	1	1	1
1	96	362	362	712	362	192	192
1	1	96	192	192	96	96	45
45	45	96	192	96	96	96	45
1	45	192	362	362	256	192	96
45	45	192	256	96	45	1	1
1	1	192	362	192	192	96	45
1	96	192	192	192	96	45	45
1	1	1	1	1	1	1	1
1	96	96	192	96	45	45	1

0	0	0	0	0	0	0	0
0	0	2,558709	2,85248	2,283301	2,283301	2,10721	1,982271
0	1,80618	1,982271	1,982271	2,558709	2,283301	2,283301	2,283301
0	1,653213	2,558709	2,558709	2,70927	2,558709	2,283301	2,283301
0	0	0,30103	1,653213	2,283301	2,558709	2,283301	2,283301
0	0	2,10721	2,283301	1,982271	1,982271	1,653213	0
0	0	2,558709	2,40824	2,283301	2,283301	1,982271	1,653213
0	0	1,982271	1,653213	0	0	0	0
0	0	1,653213	1,982271	0	0	0	0
0	0	1,982271	2,10721	1,653213	1,653213	0	0
0	0	0	1,80618	1,653213	0	0	0
1,653213	1,982271	1,653213	1,653213	1,653213	1,653213	1,653213	1,653213
0	0	2,283301	2,558709	2,85248	2,85248	2,283301	2,283301

0	1,653213	2,283301	2,558709	2,283301	2,283301	2,283301	1,982271
0	0	2,283301	2,558709	2,10721	1,982271	1,982271	1,653213
0	0	1,653213	1,653213	0	0	0	0
0	0	1,982271	1,982271	1,653213	0	0	0
0	1,982271	2,558709	2,283301	2,283301	2,85248	2,558709	2,283301	2,283301
0	0	1,982271	2,283301	2,283301	1,982271	1,982271	1,653213
0	0	1,653213	1,653213	1,982271	1,982271	1,982271	1,653213
0	1,653213	1,653213	1,982271	2,283301	1,982271	1,982271	1,653213
0	1,653213	2,283301	2,558709	2,558709	2,40824	2,283301	1,982271
0	1,653213	1,653213	2,283301	2,40824	1,982271	1,653213	0	0
0	0	2,283301	2,558709	2,283301	2,283301	1,982271	1,653213
0	1,982271	2,283301	2,283301	2,283301	1,982271	1,653213	1,653213
0	0	0	0	0	0	0	0
0	1,982271	1,982271	2,283301	1,982271	1,653213	1,653213	0

0,12717	0,41859	1,663147	1,961462	1,685559	1,546807	1,27147	1,109377
0,12717	0,221402	0,261665	0,195938	0,287099	0,311764	0,296059	0,302237

0,25434	0,966128	1,987755	2,151575	1,86551	1,59762	1,391186	1,116454
0,172189	0,260502	0,177469	0,193986	0,243604	0,262828	0,27344	0,259853

Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	1	1	1	1	96	1	1
1	45	192	362	362	362	192	192
45	64	96	96	362	512	362	192
45	45	362	192	362	712	362	1400
1	45	96	96	192	362	192	362
1	1	96	96	96	192	45	96
1	1	192	96	192	96	96	45
1	32	96	45	45	45	45	96
1	45	96	45	45	45	1	1
1	1	96	96	45	45	45	45
45	96	96	96	96	192	45	45
45	192	96	192	96	64	96	96
1	96	362	362	192	712	362	192

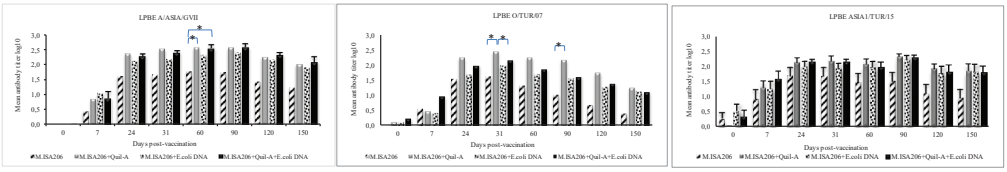
Day 0	Day 7	Day 24	Day 31	Day 60	Day 90	Day 120	Day 150
1	96	362	362	362	362	362	362
1	1	96	128	96	128	96	96
1	64	96	45	1	45	45	1
1	1	96	45	45	96	1	45
96	192	362	712	712	712	362	192
1	32	96	96	45	192	96	96
1	192	96	96	45	362	192	192
1	192	192	192	192	712	192	192
96	192	192	362	128	96	64	1
1	45	96	192	192	192	192	96
1	192	362	192	192	96	96	96
1	1	45	32	45	96	1	96
1	96	96	96	192	192	96	96

0	0	0	0	0	1,982271	0	0
0	1,653213	2,283301	2,558709	2,558709	2,558709	2,283301	2,283301
1,653213	1,80618	1,982271	1,982271	2,558709	2,70927	2,558709	2,283301
1,653213	1,653213	2,558709	2,283301	2,558709	2,85248	2,558709	3,146128
0	1,653213	1,982271	1,982271	2,283301	2,558709	2,283301	2,558709
0	0	1,982271	1,982271	1,982271	2,283301	1,653213	1,982271
0	0	2,283301	1,982271	2,283301	1,982271	1,982271	1,653213
0	1,50515	1,982271	1,653213	1,653213	1,653213	1,653213	1,982271
0	1,653213	1,982271	1,653213	1,653213	1,653213	0	0
0	0	1,982271	1,982271	1,653213	1,653213	1,653213	1,653213
1,653213	1,982271	1,982271	1,982271	1,982271	2,283301	1,653213	1,653213
1,653213	2,283301	1,982271	2,283301	1,982271	1,80618	1,982271	1,982271
0	1,982271	2,558709	2,558709	2,283301	2,85248	2,558709	2,283301

0	1,982271	2,558709	2,558709	2,558709	2,558709	2,558709	2,558709
0	0	1,982271	2,10721	1,982271	2,10721	1,982271	1,982271
0	1,80618	1,982271	1,653213	0	1,653213	1,653213	0
0	0	1,982271	1,653213	1,653213	1,982271	0	1,653213
1,982271	2,283301	2,558709	2,85248	2,85248	2,85248	2,558709	2,283301
0	1,50515	1,982271	1,982271	1,653213	2,283301	1,982271	1,982271
0	2,283301	1,982271	1,982271	1,653213	2,558709	2,283301	2,283301
0	2,283301	2,283301	2,283301	2,283301	2,85248	2,283301	2,283301
1,982271	2,283301	2,283301	2,558709	2,10721	1,982271	1,80618	0
0	1,653213	1,982271	2,283301	2,283301	2,283301	2,283301	1,982271
0	2,283301	2,558709	2,283301	2,283301	1,982271	1,982271	1,982271
0	0	1,653213	1,50515	1,653213	1,982271	0	1,982271
0	1,982271	1,982271	1,982271	2,283301	2,283301	1,982271	1,982271

0,508681	1,244002	1,964784	1,914159	1,956345	2,217585	1,755394	1,804707
0,220265	0,24566	0,174885	0,177797	0,188009	0,12722	0,23752	0,249775

0,304965	1,565045	2,136295	2,129646	1,942056	2,258599	1,7966	1,765804
0,206462	0,257518	0,079177	0,109519	0,192971	0,100709	0,233237	0,225918



LPB A

	0	7	24	31	60	90	120	150	0	7	24	31	60	90	120	150
M.ISA206	0	0,432135	1,605445	1,672569	1,770347	1,759898	1,433563	1,236732	0	0,228769	0,318018	0,32845	0,296283	0,292398	0,290212	0,289348
M.ISA206+Quil-A	0	0,861037	2,375999	2,516326	2,559596	2,559596	2,23938	2,000854	0	0,198432	0,066616	0,07571	0,093043	0,093043	0,088362	0,163501
M.ISA206+E.coli DNA	0	1,05444	2,099779	2,168131	2,309925	2,388059	2,158007	1,882195	0	0,195162	0,165018	0,177574	0,2107	0,162276	0,210156	0,267352
M.ISA206+Quil-A+E.coli DNA	0	0,859958	2,259716	2,384727	2,531022	2,572841	2,303499	2,07034	0	0,222722	0,079285	0,076677	0,103813	0,108891	0,100096	0,175136

LPB O

	0	7	24	31	60	90	120	150	0	7	24	31	60	90	120	150
M.ISA206	0	0,559305	1,530493	1,607043	1,310513	1,014596	0,684319	0,406823	0	0,243615	0,299579	0,316193	0,302703	0,275247	0,253919	0,215491
M.ISA206+Quil-A	0,118087	0,472346	2,232804	2,440158	2,241729	2,148107	1,738309	1,242458	0,118087	0,207138	0,068769	0,057468	0,069844	0,080359	0,159177	0,269053
M.ISA206+E.coli DNA	0,12717	0,41859	1,663147	1,961462	1,685559	1,546807	1,27147	1,109377	0,12717	0,221402	0,261665	0,195938	0,287099	0,311764	0,296059	0,302237
M.ISA206+Quil-A+E.coli DNA	0,25434	0,966128	1,987755	2,151575	1,86551	1,59762	1,391186	1,116454	0,172189	0,260502	0,177469	0,193986	0,243604	0,262828	0,27344	0,259853

LPB AS

	0	7	24	31	60	90	120	150	0	7	24	31	60	90	120	150
M.ISA206	0,25434	0,906455	1,680819	1,652225	1,45419	1,488921	1,088377	0,933738	0,172189	0,290971	0,274354	0,284826	0,292577	0,300175	0,293938	0,283163
M.ISA206+Quil-A	0	1,273231	2,112894	2,155898	2,048209	2,310462	1,907758	1,839591	0	0,229308	0,168811	0,172903	0,172677	0,094096	0,166118	0,227183
M.ISA206+E.coli DNA	0,508681	1,244002	1,964784	1,914159	1,956345	2,217585	1,755394	1,804707	0,220265	0,24566	0,174885	0,177797	0,188009	0,12722	0,23752	0,249775
M.ISA206+Quil-A+E.coli DNA	0,304965	1,565045	2,136295	2,129646	1,942056	2,258599	1,7966	1,765804	0,206462	0,257518	0,079177	0,109519	0,192971	0,100709	0,233237	0,225918