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APPROACHES TO DESIGNING OF NEW GENERATION VACCINES AGAINST THE SHEEP POX DISEASE

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In this review the authors analyzed the sheep pox disease, which occurs outbreaks all over the world particularly in Asia and Africa causing substantial losses in trade of animal and animal products. They categorized the sheep pox disease is one of the prioritized groups of diseases against which the World Organization for Animal Health is fighting. Data concerning a sheep poxes' history, epidemiology, epizootiology, mortality and economic impact, clinical and pathological signs, features of capripoxvirus that forms the disease are given. Diagnosis treatment and vaccine have been investigated as well. The main conclusion is done according which the designing of new vaccine generation against the sheep pox disease could be as an alternative approach against sheep pox.

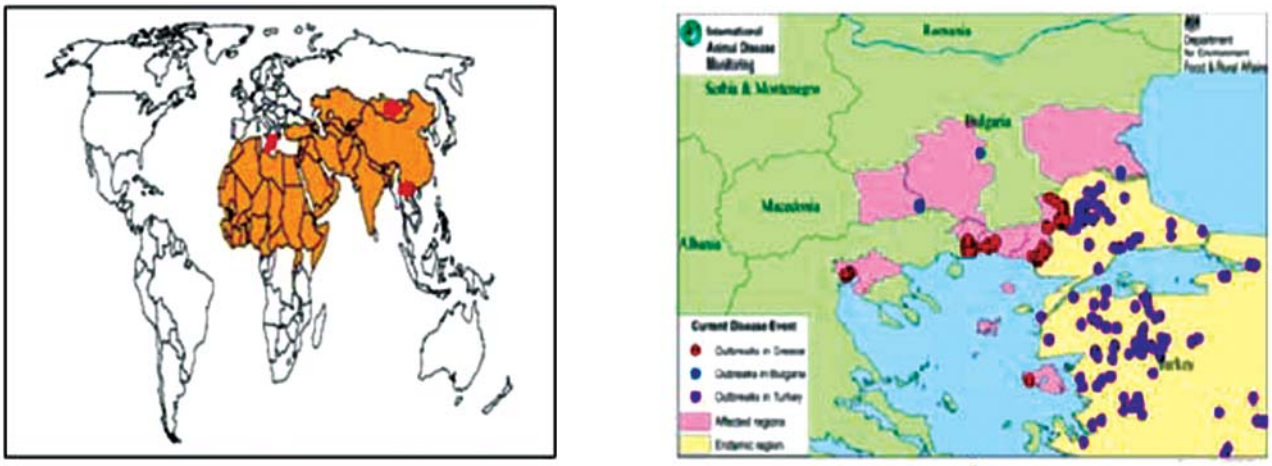
Key words: Sheep pox, synthetic peptide vaccines, Capripoxvirus, Polymeric adjuvant.

SHEEP POX DISEASE

Sheep pox disease is a highly contagious viral disease characterized by lesions of the skin. The virus of this disease causes lesions or scarring on the skin, infections of the respiratory tract, damage to skin and wool of the animal, reduction in milk production, weight gain, in wool quality, increases abortion rates [1–3]. It has been reported that the pathogen can be transmitted via air, saliva droplets, flowers strewn, milk and, natural infection transmits by droplet from animal to animal, in some cases insects may play a role as a vector and after the virus infects the organism, viremia occurs and which allows it to spread to the whole body [4]. Currently, sheep pox virus is classified as a potential bioterrorism agent because of its ability to use blood-sucking insects as vectors by United States Department of Agriculture [4, 5]. Furthermore, sheep pox disease is one of the main diseases requiring immediate solution classified as a Group A Disease by the World Organization for Animal Health (OIE). OIE limits the trade of livestock, meat and meat products in countries that have had an epidemic of this disease which can cause severe economic losses [6, 7].

History and Epidemiology

History of the sheep pox disease is almost as old as history of human pox disease; it dates back to the 2nd century BC. However first pathological studies on the disease were made in the mid-18th century [8, 9]. A lot of sheep pox outbreaks were seen in the United Kingdom between 1847–1866 and the disease was eradicated from the country in 1866 [8, 10]. Sheep pox outbreaks were reported in Italy in 1983, in Greece between 1989–1990, in Norway in 1879, largely in Europe during World War I and reported many Asian and African countries in 20th century [8, 9, 11]. Although sheep pox disease has been eradicated in many European countries today, it still is prevalent in many Middle Eastern countries including Turkey. In regions where regular vaccination does not take place and where the animal mobility is high the disease



a *b*

Fig. 1. ***a* — global distribution of sheep pox disease, spread of the disease is shown by red arrows; *b* — outbreaks in Turkey, Bulgaria and Greece were shown in 2013–2014 [15]**

occurs as seasonal epidemics with high mortality rate (Fig. 1, *a*) [8, 10, 12, 13]. In recent notifications, it has been reported that sheep pox has spread to Eastern Europe from Bulgaria, Greece and Turkey in 2012–2013 (Fig. 1, *b*). According to 2015 reports, it was seen that several sheep pox cases in Kyrgyzstan whose large part of economy is based on livestock [14]. Also, the outbreaks caused by virus continue in especially south-eastern region of Turkey due to effects of climate change on abundance and distribution of mechanical vectors population [15].

Mortality and Economic Impact

Sheep pox is the most important group among other poxviruses due to its high mortality rate, serious economic consequences, trade restrictions in the countries that occurred outbreaks [11, 16]. Data obtained from the latest epidemic in Greece was used to follow the course of the disease by OIE. OIE reported that 82 sheep pox outbreaks (1,472 cases of 17,735 suspects and 250 dead sheep) were recorded in Greece between August 2013 and January 2014 and at the same time, 3 outbreaks were observed and 558 possible sheep cases in Bulgaria. Based on these data, OIE reported that the number of outbreaks is expected to double within a year resulting in 164 sheep-pox outbreaks. It was reported that the risk of a spreading of sheep pox due to lack of precaution is high because some animals may not show any symptoms according to the degree of the disease [17].

Sheep pox mortality rate varies between 5–10%, the ratio can reach to 50% in young animals. While native races have natural immunity against viruses, particularly European races are more susceptible to disease and can show high mortality as 100% [11, 16, 18, 19].

Ethology

Sheep pox virus belongs to Capripoxvirus the genus in the Poxviridae family [20]. The DNA structure of this virus is cubic and enveloped. Sheep pox viruses have common antigenic structure with goat pox and lumpy skin diseases pathogens. These viruses have identical serological structure and serologically cross-react with each other. In previous studies, it was reported that these pathogens can be distinguished from each other by size however a recent study showed that there is no significant difference in size between these viruses and that all virion have an oval shape with average size of 294×273 nm. Sheep and goat pox are considered as a single disease by the OIE [1, 4, 7, 12, 16, 19, 21]. Virion dimensions are about 300×270×200 nm, its genome consists of double-stranded DNA of 15 kb in length [19, 22]. It has an envelope and cubic structure [12]. Despite the fact that the natural host of viruses is sheep, there are two types of sheep pox viruses. One of these is just specific to the host, other can cause disease in both sheep and goats [8]. Capripoxvirus is extremely resistant to drying, freezing, thawing and can survive in lyophilized form for months [9]. It is sensitive to ether,

chloroform, heat and chemical substances. The agent inactivates in a short period of time when exposed to direct sunlight [12].

Epizootiology

Sheep pox disease is a very contagious disease. It spreads through contact with lesions or contaminated material (wool, bait, etc.). The virus may spread via nasal secretions, milk and urine. During an outbreak, the pathogen can easily spread to uninfected animals through infected droplets. Grassing of animals in open air accelerates spreading of the disease by generating thermal stimulus effect through ultraviolet rays combined with hot air. Skin damages caused by shrubs and barbed plants in grazing animals is an another parameter that accelerates the spread of the disease in pastures [8]. The virus can be easily transported mechanically through joints and formites [22].

Pathology and Clinical Findings

After a 4 to 8 days of incubation period, sheep pox disease presents itself with a few symptoms such as increase in body temperature, rates of heart beating and respiratory, oedema in the eyelids, nasal flow, anorexia, tears, cough, salivary secretion, lung inflammation and skin lesion a few days later than aforementioned symptoms [8, 23]. Formation of lesions highly increases the transmission rate. Lung lesions are considered as the most important cause of death [10]. Blood poisoning or secondary bacterial infections, occurring during the fever skin eruption, can lead to the death of the animal [18]. In addition, it is reported that severe brucella, tendovaginitis, orchitis, puppocking and peripheral paresis can occur after the sheep pox disease [8].

Diagnosis, Protection and Treatment

Sheep pox disease does not have any specific treatment. The spread of disease can be prevented by isolating of infected animals and herds, commercial restrictions, taking quarantine measures and disinfection. The disease begins to show clinical signs after a 1–2 week of incubation period in its regular course [9]. Clinical signs sometimes can be confused with other infectious or metabolic diseases such as ecthyma, blue tongue, peste des petits ruminants, photosensitization, dermatophytes, insect sting, parasitic pneumonia, caseous lymphadenitis, mange. Because this disease must be notified in many countries, laboratory diagnosis is obligatory for the termination of quarantine application [5]. Diagnosis of the disease is usually based on clinical signs, pathology, and host-specific effects. Capripox virus exhibits change in host specificity and pathogenesis. Since sheep pox virus is serologically identical, their unequivocal identification depends exclusively on molecular techniques. Several PCR methods are developed and used for the sensitive detection of sheep pox DNA in field clinical samples such as blood, ovine nasal, ocular or rectal swabs [24, 25]. Especially many studies have been performed with real time polymerase chain reaction which is advantageous in many ways [26, 27]. However, they are not deployable in less equipped rural diagnostic settings and the loop mediated isothermal amplification assay would be of choice over these assays [28]. Also in the recent years, ELISA assay has been used [12, 20].

Vaccination is the most effective protection way for sheep pox disease. First vaccination against sheep pox disease was made as ovulation which was applied by infected scab material onto the scratch formed on the bottom or subcutaneous inoculation of the suspension prepared in glycerinated saline. This application has been used in the Indian and Mid-Asian countries for centuries until commercial vaccination was possible [10]. Capstick and Coackley showed that single capripoxvirus vaccine can be used to provide protection in sheep, goats and cattle [29]. Following this study, Davies et al. developed the attenuated capripoxvirus vaccine to provide protection for sheep, goat and cattle in Kenya [30]. Kitching and colleagues developed the strain isolated during the Kenyan outbreak, which provided an effective protection [11, 18]. Over the years, there have been a lot of vaccines developed for the sheep pox disease through isolation from attenuated virulent strains [8, 10, 11]. However, European Union (EU) member states introduce restriction to use of SPPV live vaccine. It has been reported that some attenuated live vaccines induced severe reactions at the injection site or even mild disease in vaccinated animals. Poor quality live vaccines may also serve as vehicles for extraneous virus contaminants [31].

Recently, studies on new generation vaccines have gained importance in order to avoid the disadvantages of conventional vaccines. Conventional vaccines have some disadvantages such as causing local reactions due to high viscosity of aluminium hydroxide and oil adjuvants, necessity to use in large and multiple doses, using an inactivated or weakened virus which can lead to infection.

Because of these reasons, new generation vaccines produced by DNA technology and synthetic peptide opened a new page in modern medicine [21, 23, 32].

NEW GENERATION VACCINES

Vaccines have an important role in preventive medicine and in control of infectious diseases. Vaccination began with the discovery of pox vaccine by Edward Jenner in the 18th century. It is preferable using veterinary vaccines because it is strong, reliable, biologically stable, cheap and easy to apply it provides long-term immunity, it has fewer side effects. The main purpose of vaccination in livestock is to increase the population immunity more than individual immunity and effect of the vaccination is measured with herd immunity. High immunity rate in the herds reduce the spreading rate of the infection. For this reason, it is aimed that a more effective, reliable and with improved quality vaccine to be developed using sciences such as molecular biology, immunology and biotechnology in the recent years [21, 32–34]. This new approach focuses on only the antigenic region is obtained and purified by tissue purification, chemical synthesis and genetic engineering methods rather than entire microorganism. The advantages of these vaccines are that they are not infectious because the viable microorganism is not used; they are economical, easy to store and can be stored for a long time. In addition, single-dose administration can be sufficient with adjuvant agent to provide adequate immunological response [21, 33].

DNA Vaccines

DNA vaccines include the antigenic protein expressing genes from bacteria, virus and parasitic microorganisms on expressive plasmid vectors which are injected into the organism followed by expression of this genetic material within the cells [35]. The principle of any gene transfer and vaccine development related to this methodology or gene therapy is to identify the vector which will carry the desired gene into the cell. DNA vaccines stimulate both TH 1 T cell and CD8+ T cell-mediated cytotoxicity [36].

Some concerns about the safety of DNA vaccines are also discussed in the scientific community. One of the concerns is the integration of plasmid DNA into cellular DNA. However, nowadays it has been discussed that integrated DNA vaccines cause insertional mutation which can cause activation of oncogenes or inactivation of tumour suppressor genes. Another concern is; specific DNA sequences induce anti-DNA antibodies and lead to autoimmune diseases. The issue of antibiotic resistance is also brought up when discussing DNA vaccine and it was reported that the use of DNA vaccines may be limited due to possible transfer of the gene antibiotic resistance from carrier bacteria [37].

Recombinant Vaccines

In this method, the genes encoding the antigenic proteins of the microbes are determined and these genes are transferred in to a carrier cell where they are synthesized in abundant quantities [38]. Recombinant vaccine studies continue against sheep pox disease. In studies, at least 12 immunogenic proteins have been detected in sheep pox virus and evaluated immunogenicity and neutralizing activity of the virus, which are considered to use for recombinant vaccine studies [39].

Recombinant vaccines have more of a limited stimuli effect in general and local immunological response and cytotoxic T cell response because they are not capable of *in vivo* replication and colonization. Also, they have a risk of inability in providing effective protection against infectious agents showing long-lasting and antigenic variations because of a limited number of antigens contained [40, 41].

Viral Vector Vaccines

Living recombinant vector vaccines consist of non-pathogenic or genetically modified microorganisms which are capable of expressing one or more conserved genes from a different microorganism. These vectors do not have risk of turning into the original virulent subspecies and are not pathogenic even in hypersensitive animals [33].

Synthetic Peptide Vaccines

In this method, peptides are composed out of 20 to 30 amino acids representing the specific epitope sequence of the pathogenic antigen. These sequences have a highly attractive design for the construction of site specific antibodies. For the sheep pox virus, the P32 is a structural protein presented in all capripoxvirus isolates and contained a major antigenic determinant. EAKSSIAKHFSLWKSYADADIKNSENK (92–118) and FHNSNSRILFNQEN NNFMYS (156–175) residues of this protein demonstrate strong antigenic properties for B cells [20]. These sequences consist of 27 and 20

amino acid residues; molecular weights are 3068.39 g/mol and 2476.67 g/mol and contain 59% and 60% hydrophilic residues, respectively. Molecular weight and properties of sequence were calculated by using BACHEM' peptide calculator [42].

Synthetic peptide vaccines do not require any infectious agent and they can be chemically defined if some of their advantages. Because the peptide sequence is synthesized chemically it is not possible for it to mutate like those in viruses. These vaccines have no risk of contamination and with chemical modifications the stability of the peptide can be increased and its' side effects can be reduced. Vaccines produced by this method are not only safe but also cheaper; it does not contain expensive and demanding processes such as isolation, production and attenuation of microbiological organisms [43–47]. Many peptide vaccine studies such as HIV, Hepatitis C, Anti-cancer, Malaria, Influenza, Anthrax, Screed, HPV, Pancreas, Melanoma, Zika vaccines are ongoing [44, 48, 49].

Chemical synthesis of peptides is carried out by two methods; conventional solution method and solid phase method. Solid phase Peptide Synthesis method (SPPS) was developed by Bruce Merrifield in 1962. In 1984, he was awarded Nobel Chemistry Prize for his work. Merrifield also developed the first semi-automated peptide synthesis device [50]. SPPS method is based on the principle that the growing chain (peptide or other type of oligomer) is attached to a stationary and solid particle (called the solid phase, or resin) and remains attached to this resin during synthesis. Other soluble chemicals during synthesis are removed by filtration and washing. At the final stage, the desired product is separated from the solid phase and purification and characterization are performed [51]. Microwave-assisted solid phase peptide synthesis has been used frequently in recent peptide studies. Microwave assisted heating prevents the aggregation problem of chain of peptides growing on the resin [50–52].

Even though synthetic peptide vaccines, have advantages in many aspects compared to classical and recombinant vaccines, the immunogenic properties and the residence time in the body is limited due to the small molecular size of the peptides [53]. Binding these peptides to carrier molecules in order to solve these limitations and develop new generation vaccines is one of the current research areas. The key steps of creating a new generation vaccine are, detecting the antigenic sites on the surface proteins of viruses, producing them with synthetic methods and using them most effectively in the organism with the appropriate carrier molecule (Fig. 2). Mustafaev et al. showed that polyelectrolytes in linear form can be used to increase immunogenicity because it increases the production of antibodies against synthetic peptides [54–56], polyacrylic acid (PAA) [57–59], poly N-isopropylacrylamide (NIPAA) [60, 61], poly(methyl vinyl etherco-maleic anhydrate) [62, 63], polycarboxymethylcellulose (CMC), polyvinylpyridine-polysethylpyridine and poly (N-vinyl-2-pyrrolidone-acrylic acid) [46, 64–66] copolymers are commonly used as linear carriers and such polyelectrolytes exhibit both carrier and adjuvant properties for the peptide molecule. The conjugation of synthetic peptides to natural or synthetic macromolecules has great importance in a wide range of fields in medicine and biotechnology

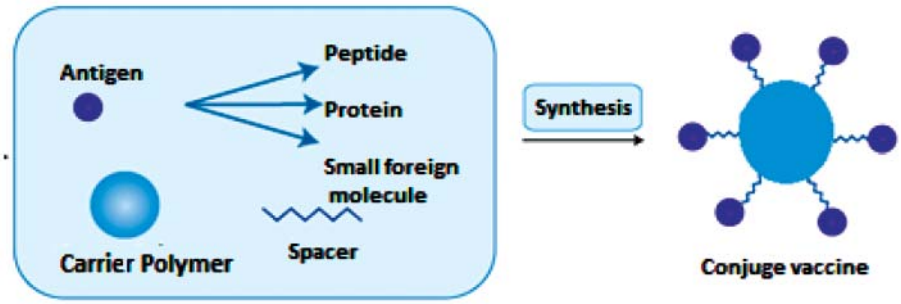


Fig. 2. **Representation of the action mechanism of new generation vaccines system**

such as drug release, immobilized enzymes, affinity-based immunoassay studies, synthetic vaccine production [54–56].

So, in this review, information about sheep pox disease which can still emerge as epidemics in many regions today and can cause animal deaths and economic losses have been given. In the light of these data and with the developments achieved in biotechnology, molecular biology, immunology and medicine one of our primary goals is designing a more efficient and cheaper new generation vaccine system in order to prevent a worldwide pandemic of sheep pox. For this purpose, our group is planning to synthesis EAKSSIAKHFSLWKSYA DADIKNSENK (92–118) and FHNSNSRILFNQENNNFMYS (156–175) amino acid residues of antigenic determinant by microwave-assisted SPPS method. Conjugate and complex formation will be done with several polymer such as polyacrylic acid, poly N-isopropylacrylamide, poly (N-vinyl-2-pyrrolidone-co-acrylic acid), in order to development new effective vaccine system after cytotoxicity and immunological studies.

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ПІДХОДИ ДО РОЗРОБЛЕННЯ ВАКЦИН НОВОГО ПОКОЛІННЯ ПРОТИ ВІСПИ ОВЕЦЬ

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В огляді авторами здійснено аналіз захворювання віспи овець, що спричинює епідемії в усьому світі, особливо в Азії та Африці, та призводить до великих втрат у торгівлі тваринами і продукцією тваринництва. Віспу овець віднесено до однієї з найбільш пріоритетних груп захворювань, проти яких проводить боротьбу Всесвітня організація охорони здоров'я тварин. Наведено дані щодо історії цього захворювання, епідеміології, епізотіології, смертності та економічних збитків, клінічних і патологічних симптомів, властивостей карпіпоксвірусу, який спричинює це захворювання, подано також схему лікування і розроблювані вакцини. На підставі наведених даних зроблено висновок щодо необхідності розроблення вакцин нового покоління проти віспи овець, що може бути альтернативним підходом до вже існуючих.

Ключові слова: захворювання віспа овець, синтетичні пептидні вакцини, карпіпоксвірус, полімерний ад'ювант.

ПОДХОДЫ К РАЗРАБОТКЕ ВАКЦИН НОВОГО ПОКОЛЕНИЯ ПРОТИВ ОСПЫ ОВЕЦ

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В обзоре авторами осуществлен анализ заболевания оспы овец, которое вызывает эпидемии во всем мире, особенно в Азии и Африке, что приводит к большим потерям в торговле животными и продукцией животноводства. Оспа овец отнесена к одной из наиболее приоритетных групп заболеваний, против которых проводит борьбу Всемирная организация по охране здоровья животных. Приведены данные относительно истории этого заболевания, эпидемиологии, эпизотиологии, смертности и экономического ущерба, клинических и патологических симптомов, свойств карпипоксвируса, который вызывает это заболевание, представлены также схема лечения и разрабатываемые вакцины. На основании приведенных данных сделан вывод о необходимости разработки вакцин нового поколения против оспы овец, что может быть альтернативным подходом к уже существующим.

Ключевые слова: заболевание оспа овец, синтетические пептидные вакцины, карпипоксвирус, полимерный адъювант.