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PRODUCTION TRIALS OF A VACCINE MADE FROM THE ATTENUATED *ESCHERICHIA COLI* AC-16 STRAIN

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Abstract. Swollen piglet disease is a significant intestinal infection caused by *Escherichia coli*, which poses a danger to animals and birds that act as reservoirs for the pathogen. The source of the pathogen in piglets is believed to be sick and over-infected animals or latent bacterial carriers, which form the continuous epizootic chain. Therefore, live vaccines are considered promising for avoiding of swollen piglet disease. This article aims to present the production trials of an attenuated vaccine from the *E.coli* AC-16 strain, which is currently being developed as a potential solution for preventing escherichiosis. The live attenuated vaccine demonstrated stable residual virulence and activity control when tested on laboratory animals (white mice). In total, 1364 piglets and 262 cows were vaccinated from 2020 to 2022. Piglets from the vaccinated swine were born viable and did not fall ill with escherichiosis. During this period, no cases of illness or death of calves from escherichiosis were reported.

Keywords: attenuated strain; *Escherichia coli;* CFU; escherichiosis; vaccine

Introduction

Escherichiosis is a significant intestinal infection caused by the pathogen *Escherichia coli*, which poses a danger to animals and birds that act as reservoirs for the pathogen [1]. Swollen piglet disease is most commonly caused by the pathogen *E. coli*, and the source of the pathogen in piglets is believed to be sick and over-infected animals or latent bacterial carriers, which form the continuous epizootic chain [2]. Despite efforts to control the disease, escherichiosis remains a major problem.

In terms of preventive measures, a special place is given to specific prophylaxis, for which modern, effective, easy-to-apply vaccines are necessary [3]. Live vaccines are considered promising for the prevention of swollen piglet disease. In veterinary practice, a live vaccine is used from the vaccine strain *E.coli* 042. The disadvantage of the vaccine is that suppressor revertants derived from streptomycin-dependent mutants of *E. coli* are used as vaccine strains. It has been found that streptomycin-dependent escherichia mutants revert to a virulent state in 50% of cases. The vaccine is used in high doses, and the duration of immunity is 2-4 months [4].

Specific prevention of escherichiosis and other infectious diseases in young piglets involves increasing the level of specific antibodies in colostrum and the nascent litter [6,7]. One way to achieve this is through colostral immunity, which can be created by immunizing deep-stemmed pigs and heifers to accumulate specific antibodies in colostrum and transmit them to offspring.

To develop a vaccine, agenic strains used for vaccine production must meet international standards and have at least two characterized genetic tags. They should possess stable biological properties, show moderate reactogenicity and residual virulence, create high-intensity immunity when administered once, and be epizootically safe while also being able to combine with other vaccines. Vaccine strains must be labeled to differentiate them from epizootic prototypes [5].

This article aims to present the production trials of an attenuated vaccine from *E.coli* AC-16 strain, which is currently being developed as a potential solution for preventing escherichiosis.

Materials and methods

A scientific and production experiment was conducted on three farms that were affected by swollen piglet disease: the "Aleksey" farm in Yenbekshikazakh rayon, and the "Arai" and "Talas"

farms in Koksu rayon of Almaty region. Subsequently, the vaccine was successfully tested in four other farms in Almaty Oblast that were not affected by swine fever in piglets.

To prepare the vaccine, the matrix-attenuated *E.coli* AC-16 strain was stored dried and inoculated on meat-peptone agar with a pH of 7.2-7.4 in vitro, and then dispersed on Petri dishes to avoid culture contamination and dissociation. The daily agar culture was checked for growth purity and agglutinability using total and monoreceptor sera. Afterwards, a suspension was prepared in physiological sodium chloride solution, and the required amount was inoculated into a Tartakovsky flask with MPA at pH 7.2-7.4. After 20 hours of incubation in the thermostat, the culture was flushed with physiological solution until a thick suspension was obtained. The bacterial concentration was checked by diluting 1 ml of the suspension to 10^9 CFU using an optical escherichiosis standard. The basic flush from Tartakovsky flasks, containing 10^{10} CFU in 1 ml, was packed into ampoules, and the same amount of skimmed milk was added [11,12].

Drying was performed under the following regime: freezing in a vacuum at -40 °C for 20 hours, followed by drying at 25 °C for 24 hours. The resulting dried culture was an amorphous mass that easily dissolved in physiological solution or cooled boiled water, turning into a uniform suspension. After drying, the culture was checked for purity and live bacterial content on Petri dishes [11,12].

Biochemical properties were evaluated based on the microorganisms' ability to ferment arabinose, xylose, glucose, lactose, maltose, sucrose, rhamnose, mannose, sorbitol, galactose, and xylose, and to form hydrogen sulfide and indole. The relatedness of isolated colonies was clarified using Chromocult® Coliform Agar (Merck KGaA, Darmstadt, Germany) and MUG chromogenic substrates following instructions of the manufacturer. The number of bacteria was controlled by diluting and subsequently dispersing them on Petri dishes (from 2-3 ampoules) to a density of 1000 cells according to the optical standard. The number of grown colonies was counted, and it was found to be 50-70% of the number of bacteria seeded according to the optical standard (i.e., 500-700 colonies of seeded cells). The entire vaccine preparation process was performed in the laboratory of antibacterial biotechnology at the Kazakh National Agrarian Research University, including lyophilic drying.

A stability control for residual virulence and activity was performed on 10 white mice (14-16 g). Initially, the live vaccine in the form of a suspension in physiological solution was tested on individual groups of piglets. Subsequently, the breeding stock was immunized with the dry live vaccine from the attenuated strain of *E. coli* AC-16.

Results

On Endo agar, *E. coli* colonies that are positive for lactose exhibit a characteristic metallic sheen appearance due to the presence of crystallized fuchsin on their surface (Figure 1 and 2).

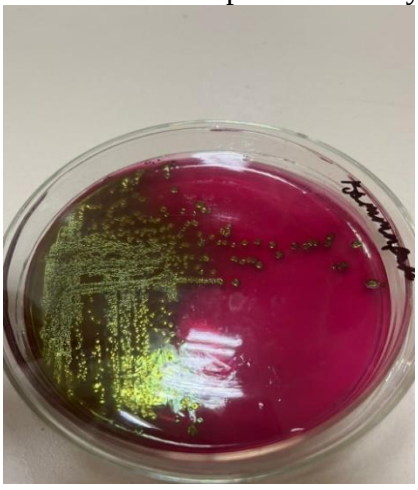

Figure 1 – Cultivation of E. coli AC-16 on Endo agar medium

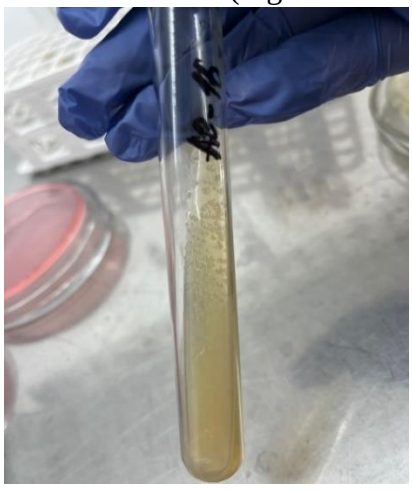

Figure 2 – Cultivation of *E. coli* AC-16on meat-peptone agar

Table 1 – Biochemical characteristics of attenuated and virulent strains of *E. coli*

Name strains	Arabinoza	Xylose	Glucose	Lactose	Maltose	Saccharose	Ramnose	Mannit	Sorbit	Dulcit	Salicin	Indol	Hydrogen sulfide	Gelatin
AC-16	-	-	+	+	+	+	-	+	+	+	+	+	-	-
39	-	-	+	+	+	+	-	+	+	+	+	+	-	-

The methods of Chromocult® Coliform Agar and MUG chromogenic substrates involve the use of a substrate called Salmon-GAL, which is cleaved by the ß-D-galactosidase enzyme found in coliform bacteria. These result in the formation of colored colonies that are used to count coliform bacteria. The colonies of these bacteria are then visually identified as salmon red in color due to the reaction that takes place. The process of quantifying *E. coli* involves the utilization of two substrates, X-glucuronide and Salmon-GAL, which are cleaved by ß-D-glucuronidase and ß-D-galactosidase, respectively, both of which are specific to *E. coli*. The presence of *E. coli* leads to the cleavage of both substrates, resulting in colonies that display a dark blue to violet color (Figure 3), which can be distinguished from the salmon red color of colonies formed by other coliform bacteria. Sorbitol-positive organisms appear as yellow colonies on Mug agar. Sorbitol-positive organisms do not change their negative color and appear as green colonies in greenish-blue medium (Figure 4).

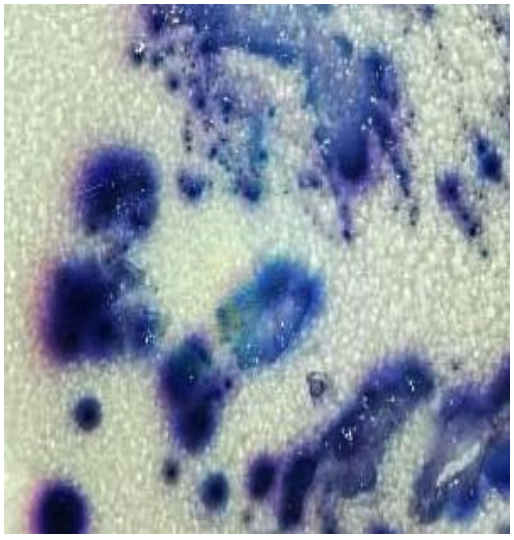

Figure 3 – Enterobacteriaceae cultures in Chromocult

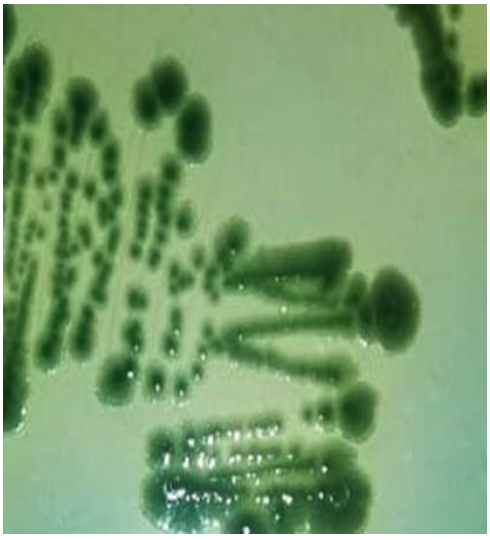

Figure 4 – Cultures of Enterobacteriaceae in MUG agar

A control for the stability of residual virulence was performed on 10 white mice (14-16 g). White mice were injected subcutaneously with 10^7 CFU (0.2 ml of a 5-10 ml suspension of the dried culture). The mice were kept alive for 15 days (Table 2).

Table 2 – Residual virulence of attenuated *E. coli* AC-16 strain in experiments on white mice

Name strain	Number of	Dose of m.c.	Method of dose	Results

	animals	injection.	admistration	Dead	Survived	Survival rate (%)
E. coli AC-16	10	10^5	Peritoneal in/peritoneal	-	10	100
	10	10^6	Peritoneal	-	10	100
	10	10^7	Peritoneal	-	10	100
	10	10^8	Peritoneal	2	8	90
	10	5-10^8	Peritoneal	10	0	-
E. coli 39 (virulent strain)	10	10^3	Peritoneal	2	8	80
	10	10^4	Peritoneal	7	3	30
	10	10^5	Peritoneal	10	-	-
	10	10^6	Peritoneal	10	-	-
	10	10^7	Peritoneal	10	-	-

An activity control was also tested on white mice. 10 mice were injected subcutaneously with 10^5 CFU (0.2 ml 10^6 suspended from the dried culture). After 15 days, 10 inoculated and 5 control mice were infected subcutaneously with a virulent culture of *E. coli* 39 at a dose of 10^6 CFU. Control mice died within 1 day, while vaccinated mice all remained alive.

Vaccination was performed to cover all young animals regardless of their body condition and development, subcutaneously, once, in the area of the lower third of the neck, at a dose of 1 ml (10^9 CFU). The vaccinated animals were clinically observed, and several hours after vaccination, piglets showed short-term depression, but appetite was maintained. The local reaction was characterized by edema formation (3x4-4x5 cm in size), which resolved by the 4^{th}-6^{th} day. Furthermore, the vaccine was also tested on swine in the last stage with a supernatant, in a dose of 2 ml (2-10^9 CFU) intramuscularly, in the area of the lower third of the neck, once. Only a local reaction to the vaccination developed in swine. Piglets born from vaccinated swine were viable and did not develop escherichiosis.

Table 3 – Animals vaccinated with dry live vaccine from attenuated strain of *E. coli* AC-16 in farms of Almaty region in 2020-2022

Name of agricultural formations	Vaccinated animals with a dry live vaccine from an attenuated strain of *E. coli* AC - 16	
	pigs	piglets
Farm "Alexey"	40	360
Farm "Aray"	16	162
Farm "Talas"	25	230
Other farms of Almaty region	49	488

Totally during 2020-2022 a total of 1364 piglets and 262 cows including 132 cows 20-25 days before polling and 124 piglets, including 40 cows and 360 piglets in Khibit farm, 16 cows and 162 piglets in Arai farm and 25 cows and 230 piglets in Talas farm and 49 cows and 488 piglets in other farms of Almaty Region were vaccinated. During this period no cases of illness and death of calves from escherichiosis were registered.

Discussion

Pig farms of these farms have been unhappy with swine sickness of piglets for a number of years. The newborn piglets in the farms were systematically immunized with concentrated formolvax vaccine according to the instructions. In spite of this, rather frequent cases of piglet swollen disease were observed on the farms. From the fallen piglets, the diagnosis of Escherichiosis caused by *E. coli* was repeatedly confirmed by bacteriological laboratories.

The damage caused by Escherichiosis is not only in the death of livestock, but also in the fact that sick animals, as carriers of the bacteria, can contaminate the environment for a prolonged period. Therefore, it is necessary to study the epizootic and epidemiological situation of this infection, identify the main factors of the infectious process, and improve the therapeutic, specific prevention, and development of veterinary and sanitary measures [13-15]. The basis of the prevention of Escherichiosis in animals is through seropreventive and vaccine prophylaxis. To date, a large number of inactivated and live vaccines have been proposed. Live vaccines are recommended for immunization of safe herds, while inactivated vaccines are recommended for the first-time vaccination of farms unprepared for these diseases to improve livestock health. Obtaining and implementing live vaccines from strains of Escherichia isolated from this type of animal is an urgent task in improving the specific prevention of the disease on farms.

The observation of vaccinated piglets and swine has shown that the vaccine against swine disease of piglets from attenuated strain of *E. coli* AC-16 does not cause any complications.

Epizootologic data of the farms where the vaccine tests were carried out. in comparison with previous years testify to efficiency and safety of the experimental live vaccine and indicate the possibility of its wide application as one of the measures against swollen piglet disease.

Economic efficiency as a result of immunization of piglets swellings disease by live vaccine from *E. coli* strain AC-16 is reached at the expense of decrease in disease incidence and mortality of piglets, labour costs and makes up 14 tenge per one spent tenge. The efficacy of immunization of swine in non-positive farms on Escherichiosis was studied by carrying out the epizootological analysis before and after its using and taking into account the decrease of percentage of morbidity and death of pigs.

Conclusion

The studies involved immunizing all breeding stock with a dry live vaccine made from an attenuated strain of *E. coli* AC-16, which has high immunogenic properties. The live attenuated vaccine demonstrated stable residual virulence and activity control when tested on laboratory animals (white mice), followed by a control infection. In total, 1364 piglets and 262 cows were vaccinated from 2020 to 2022. Piglets from the vaccinated swine were born viable and did not fall ill with escherichiosis. During this period, no cases of illness or death of calves from escherichiosis were reported. The economic efficiency resulting from immunization of piglets against swelling disease with the live vaccine from *E. coli* strain AC-16 is achieved through decreased disease incidence and mortality of piglets and reduced labor costs, resulting in a 14 tenge return for every tenge spent.

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Conflict of interest: The authors declare the absence of a conflict of interest.

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ESCHERICHIA COLI АС – 16 АТТЕНУИРЛЕНГЕН ШТАМЫНАН АЛЫНҒАН ВАКЦИНАНЫҢ ӨНДІРІСТІК СЫНАҚТАРЫ

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Аннотация. Торайлардың ісіну ауруы қоздырғышының резервуары жануарлар мен құстарға қауіп төндіріп, ауыр ішек инфекциясын тудыратын *Escherichia coli*. Қоздырғыштың көзі ауру және қайта жұқтырған жануарлар немесе үздіксіз эпизоотиялық тізбекті құрайтын жасырын бактерия тасымалдаушылары болып саналады. Сондықтан тірі вакциналар аурудың алдын алу үшін перспективті болып саналады. Бұл мақаланың мақсаты – қазіргі уақытта аурудың алдын алудың ықтимал шешімі ретінде *E. coli* АС-16 штамы негізінде жасалған әлсіздірілген вакцинаның өндірістік сынақтарын ұсыну. Тірі әлсіздірілген вакцина зертханалық жануарларды (ақ тышқандарды) сынау кезінде тұрақты вируленттілік пен белсенділікті көрсетті. 2020 жылдан 2022 жылға дейін барлығы 1364 шошқа мен 262 сиыр вакцинацияланды. Вакцинацияланған шошқалардан туған торайлар өміршең болып эшерихиозбен ауырмады. Сонымен қатар, бұзаулардың эшерихиозбен ауруы немесе өлімі тіркелген жоқ.

Түйін сөздер: аттенуирленген штамм; *Escherichia coli;* КОЕ; эшерихиоз; вакцина

ПРОИЗВОДСТВЕННЫЕ ИСПЫТАНИЯ ВАКЦИНЫ ИЗ АТТЕНУИРОВАННОГО ШТАММА *ESCHERICHIA COLI* АС – 16

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Аннотация. Отечная болезнь поросят – это тяжелая кишечная инфекция, вызываемая *Escherichia coli*, представляющая опасность для животных и птиц, которые выступают в качестве резервуаров возбудителя. Считается, что источником возбудителя у поросят являются больные и перезараженные животные или латентные бактерионосители, которые образуют непрерывную эпизоотическую цепь. Поэтому живые вакцины считаются перспективными для профилактики болезни. Цель данной статьи – представить производственные испытания аттенуированной вакцины из штамма *E.coli* АС-16, которая в настоящее время разрабатывается как потенциальное решение для профилактики эшерихиоза. Живая аттенуированная вакцина продемонстрировала стабильную остаточную вирулентность и контроль активности при испытании на лабораторных животных (белых мышах). Всего с 2020 по 2022 год было вакцинировано 1364 поросят и 262 коровы. Поросята от вакцинированных свиней родились жизнеспособными и не заболели эшерихиозом. За этот период не было зарегистрировано случаев заболевания или гибели телят от эшерихиоза.

Ключевые слова: аттенуированный штамм; *Escherichia coli*; КОЕ; эшерихиоз; вакцина