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BIOCHEMICAL PROPERTIES AND INDUSTRIAL STRAINS OF THE AVIAN PASTEURELLA ISOLATES

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470 pathological materials from dead birds were collected at poultry farms in Almaty, Zhambyl, Kyzylorda, Aktobe, Mangystau, Semipalatinsk, East Kazakhstan, Kostanay regions, 11 Pasteurella isolates were collected from them and pasteurella was studied in comparison with industrial strains.

Of all the isolated and studied pasteurella strains, only Pasteurella multocida A biovar avium B-0054 No. 12, «Kazakh Scientific Research Veterinary Institute», G-5, Al-7, D-2, K-30, A-22 were classified as S-shaped.

As a result of studying the pathogenic properties of pasteurella, the maximum pathogenic activity was observed in the Pasteurella multocida strain and biovar avium B-0054 No.12 «Kazakh Scientific Research Veterinary Institute», which ranged from 4 to 10,000 colony-forming activities (CFA) and amounted to LD_{50} from 2-4 CFA.

Based on the study of the biological properties of epizootic Pasteurella strains, criteria for evaluating the biological properties of industrial Pasteurella multocida strains and the principles of their selection were studied and found.

In the process of studying the biological properties of pasteurels in industrial conditions, their pathogenicity and immunogenicity can be determined. The effectiveness of the pasteurellosis erythrocyte antigen diagnosticum also showed that these two properties are directly related.

***Key words*:** *birds, Pasteurella, colony, isolate, strain.*

ҚҰСТАРДАН ОҚШАУЛАНҒАН ПАСТЕРЕЛЛА ИЗОЛЯТЫНЫҢ БИОХИМИЯЛЫҚ ҚАСИЕТТЕРІ МЕН ӨНДІРІСТІК ШТАМДАРЫ

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Алматы, Жамбыл, Қызылорда, Ақтөбе, Маңғыстау, Семей, Шығыс Қазақстан, Қостанай облыстарының құс фабрикаларында өлім-жітімге ұшыраған құстардан 470 патологиялық материалдар жиналды, олардың ішінен 11 Pasteurella изоляттары іріктеліп алынды және оларға өнеркәсіптік пастереллалар штамдармен салыстыру арқылы зерттеу жұмыстары жүргізілді.

Барлық оқшауланған және зерттелген пастереллалардың ішінен тек Pasteurella multocida A biovar avium B-0054 № 12, «Қазақ ғылыми-зерттеу ветеринариялық институты», G-5, Al-7, D-2, K-30, A-22 штамдары S-тәрізді болып жіктелді.

Пастереллалардың патогендік қасиеттерін зерттеу нәтижесінде максималды патогендік белсенділік Pasteurella multocida A biovar avium B-0054 №12 «Қазақ ветеринариялық ғылыми-зерттеу институты» штаммында байқалды, ол 4-тен 10 000-ға дейін колония түзуші белсенділіктер (КТБ) арасында ауытқып, 2-4 КТБ-тен LD_{50} құрады.

Pasteurella эпизоотиялық штамдарының биологиялық қасиеттерін зерттеу негізінде Pasteurella multocida өнеркәсіптік штамдарының биологиялық қасиеттерін бағалау критерийлері және оларды таңдау принциптері зерттелді және табылды.

Өнеркәсіптік жағдайда пастереллалардың биологиялық қасиеттерін зерттеу барысында олардың патогенділігі мен иммуногенділігін анықтауға болады. Пастереллезді эритроциттік антигендік диагностикумның тиімділігі де екі қасиеттің бір-бірімен тікелей байланысты екенін көрсетті.

Түйінді сөздер: *құстар, Pasteurella, шоғыр, изолят, штамм.*

БИОХИМИЧЕСКИЕ СВОЙСТВА И ПРОМЫШЛЕННЫЕ ШТАММЫ ИЗОЛЯТОВ ПАСТЕРЕЛЛЫ, ВЫДЕЛЕННЫХ ОТ ПТИЦ

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На птицефабриках Алматинской, Жамбылской, Кызылординской, Актюбинской, Мангистауской, Семипалатинской, Восточно-Казахстанской, Костанайской областей были собраны 470 патологических материалов от павших птиц, из них отобрано 11 изолятов Pasteurella и проведено изучение пастерелл в сравнении с промышленными штаммами.

Из всех выделенных и изученных пастерелл только штаммы Pasteurella multocida A biovar avium B-0054 № 12, «Казахского научно-исследовательского ветеринарного института», G-5, Al-7, D-2, K-30, A-22 были отнесены к S-образным.

В результате изучения патогенных свойств пастерелл, максимальная патогенная активность наблюдалась у штамма Pasteurella multocida A biovar avium B-0054 №12 «Казахский научно-исследовательский ветеринарный институт», которая колебалась от 4 до 10 000 колониеобразующих активностей (КОА) и составляла $ЛД_{50}$ от 2-4 КОА.

На основании исследования биологических свойств эпизоотических штаммов Pasteurella были изучены и найдены критерии оценки биологических свойств промышленных штаммов Pasteurella multocida и принципы их отбора.

В процессе изучения биологических свойств пастерелл в промышленных условиях можно определить их патогенность и иммуногенность. Эффективность пастереллезного эритроцитарного антигенного диагностикума также показала, что эти два свойства напрямую связаны между собой.

Ключевые слова: *птицы, Pasteurella, колония, изолят, штамм.*

Introduction. One of the main directions of poultry farming in our sovereign country is to provide the population of Kazakhstan with the highest quality poultry products (meat, eggs and poultry wool). One of the most damaging diseases that impede the rapid development of these poultry farms is avian pasteurellosis. The economic damage caused by this disease to poultry farms is enormous. This is because a quality product is never obtained from birds infected with avian pasteurellosis. Birds exposed to Pasteurella disease, as carriers of Pasteurella throughout their lives, damage the environment. The disease proceeds in the following acute and chronic forms. Birds with an acute form of the disease are subject to mortality from several hours to one day. Therefore, an inactivated vaccine for the Prevention of Pasteurella disease [1 s.9, 2 s.5, 3 s. 11] and biological drugs have been prepared for the compilation and separation of immunological reagents of high specificity and sensitivity, which allow us to detect antibodies in the blood serum in a timely manner [4 s.3, 5 s.7, 6 s.9, 7 s.10, 8 s.12, 9 s.5, 10 s.13].

Purpose of research: To study the epizootic situation of poultry farms of Almaty, Zhambyl, Kyzylorda, Aktobe, Mangistau, Semipalatinsk, East Kazakhstan, Kostanay regions with further cultivation, isolation of the most highly immunogenic isolates of pasteurella for the manufacture of biological preparations.

For this purpose, we set the following tasks:

- isolate field isolates and selected the most highly immunogenic isolates in particular Pasteurella multocida A biovar avium B-0054 No. 12, “Kazakh Research Veterinary Institute”, G-5, Al-7, D-2, K-30, A-22, which were classified as S-type);

– study the pathogenic properties of Pasteurella multocida strain Pasteurella multocida A biovar avium B-0054 №12 “Kazakh Research Veterinary Institute”, which ranged from 4 to 10 000 colony forming activity (CFA) and which was LD50 from 2-4 CFA;

- study biological properties of epizootic strains of Pasteurella and to find criteria for evaluating biological properties of industrial strains of Pasteurella multocida;

- study the biological properties of Pasteurella in industrial conditions it is possible to determine their pathogenicity and immunogenicity. The efficacy of Pasteurella erythrocyte antigenic diagnosticum, and showed that these two properties are directly related.

Materials and methods. During the period from 2004 to 2009, 470 samples were collected from the dead birds at poultry farms in Almaty, Zhambyl, Kyzylorda, Aktobe, Mangystau, Semey, East Kazakhstan, Kostanay regions of the country, out of which 158 direct of pasteurella were isolated. Therefrom, the biological properties of 11 shoots have been studied comprehensively and in comparison, with industrial strains. It was found that all isolated and industrial Pasteurella strains have similar growth-morphological properties. The main features of the separated shoots are as follows: stable in a solid nutrient medium, because the sown shoots are distinguished by thick good growth, and the Pasteurella shoots passed through the body of white mice grew separately in the nutrient medium in the form of dew-like clusters. The shoots of Pasteurella, which were kept in meat peptone broth for a long time, grew very poorly. Straight Pasteurella stored in a solid nutrient medium Pasteurella shoots grew very well on Hottinger Agar with the addition of horse blood serum or 5% cattle blood serum. All separated Pasteurella shoots grew in Hottinger Agar in the form of small, rounded clusters of light color, and the clusters grew inside the Agar over time, turning gray in color (S – shaped).

When staining smears taken from blood and internal organs by the Gram method, the pathogens are small, short rod-shaped, Gram-negative (length 0.4-3.0 width 0.3-1.0 microns). When staining with Leffler blue or Romanovsky-Gimza paints, it was found that pasteurellas turned into a kind of bipolars (the last particles of bacterial cells were painted in more detail). From the painted smears, the causative agents of Pasteurella growths were visible, arranged in a coccyx stick, singly, in pairs, sometimes in series. When bacteria are viewed through a microscope, pasteurelles do not move, they form a clammy membrane, but do not form spores. In the horizontal incident light, we saw that Pasteurella pathogens were fluorescent and open S– shaped, as they formed a shell; the meat became smooth turbid in the peptone broth, forming a clammy sediment at the bottom of the glass tube, which, when we shook them, became like a braid and rose to the surface of the culture medium.

When testing the growth capacity of Pasteurella shoots isolated from Hottinger's solid and liquid food media, the clusters were of different shapes. The clusters of shoots obtained by duplicating and passing isolated Pasteurella shoots from the body of white mice were only S-shaped. The first clusters were very small (0.4-1.2 microns), transparent, giving a bluish color to the horizontally incident light. The second clusters are large (2.0-3.0 microns), which were also brightly colored.

Of all the isolated and studied shoots, only strains of Pasteurella multocida a biovar avium B-0054 «Kazakh research veterinary institute» No. 12, G-5, Al-7, D-2, K-30, A-22 was classified as S-shaped. The mentioned strains were observed in a liquid culture medium only after 18-24 hours at a temperature of 37^{o}C. After 72-96 hours (+20-22 ^{o}C) in the nutrient medium, it was clear that a clastic sediment was formed at the bottom of the glass tube.

In semi-liquid meat peptone Agar, Pasteurella shoots gave growth only where they were sown with needles. This proves that Pasteurella shoots are immobile. It has been proven that Pasteurella shoots after 2 and 5 grafting, as well as 18-hour shoots of +20 – 22^{o}C in meat peptone broth with sterile vaseline oil, retain their viability for 12 months.

Shoots stored in ordinary and other culture media, it was shown that they could retain their viability for only 2-4 months, as well as in solid Culture Media, falling into their own state, releasing indole and emitting a characteristic smell.

During repeated grafting of externally isolated Pasteurella shoots from the body of white mice, it was observed that the morphological properties of bacteria changed and their size decreased 2-3 times. When we painted the Pasteurella shoots using the Gram method and looked at them through a microscope, it looked like a very fine micrococcus.

Of all Pasteurella shoots studied, 6 Pasteurella isolates were selected and compared with industrial strains.

Results The biochemical properties of Pasteurella shoots are presented in Table 1.

The fact that pasteurelles have weak biochemical properties can be seen in Table 1. When methylene blue was added, the milk color did not change, the milk did not ferment, the litmus color remained unchanged. When 40% bile was added to meat-peptone broth (MPB), there was no growth of microorganisms. Indole was formed during the fermentation of glucose, mannitol, sorbitol, sucrose, maltose, and galactose by pasteurella.

Pasteurelles fermented glucose, mannitol, sorbitol, sucrose, maltose, galactoses, and formed indole.

From all isolated and studied strains only strains Pasteurella multocida A biovar avium B-0054 “Kazakh Research Veterinary Institute” No. 12, G-5, Al-7, D-2, K-30, A-22 were referred to S-type strains. The mentioned strains were observed in liquid culture medium only after 18-24 hours at ^{37o}C. After 72-96 hours ($^{+20-22o}$C) in the nutrient medium, a flake-like precipitate was seen to have formed at the bottom of the glass tube.

In semi-liquid meat-peptone agar, Pasteurella strains gave growth only where needles were sown. This proves that Pasteurella cultures are immobile. It was proved that Pasteurella strains after 2 and 5 inoculations, as well as 18-hour strains at $^{+20-22o}$C in meat-peptone broth with sterile vaseline oil retain viability for 12 months.

It was found that strains stored in conventional and other culture media could retain viability for only 2-4 months, as well as in solid culture media, falling into its own state, secreting indole and emitting a characteristic odor.

When Pasteurella shoots isolated externally from the body of white mice were repeatedly inoculated, we observed a change in the morphological properties of the bacteria and a 2-3-fold decrease in their size. When we stained Pasteurella shoots by Gram's method and examined them by microscope, they looked like very small micrococci.

From all Pasteurella cultures examined, 6 Pasteurella isolates were selected and compared with industrial strains.

Of the strains studied, only Pasteurella multocida A biovar avium strain B-0054 “Kazakh Research Veterinary Institute” No. 12 is the most promising strain for the production of biological preparations.

Discussion In the poultry farms of Almaty, Zhambyl, Kyzylorda, Aktobe, Mangistau, Semey, East Kazakhstan, Kostanay regions, 6 Pasteurella isolates were selected from the shoots of Pasteurella-forming isolates isolated from 470 damaged pathological materials from deadly birds and Pasteurella was studied in comparison with industrial strains.

Table 1 – Biochemical properties of Pasteurella shoots

Name		Erythrocyte diagnostics for the determination of Pasteurella	40% of bile, BCH	Milk			Fermentation of the Hissa medium											
				ordinary	litmus	methylene blue	glucose	mannite	sorbitol	arabinosa	raffinosa	sucrose	lactose	maltose	gelatin	indole	galactose	dulcite
P.multocida	B-0054 KazRVI №12	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	G-5	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	Al-7	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	D-2	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	K-30	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	A-22	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	B-74	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	M-62	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	Kaz-8	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	Sem-1	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
	T-4	+	-	-	-	-	К	К	К	-	-	К	-	К	-	+	К	-
Note: «К» – acid-forming fermentation; «+» – positive; «-» – negative; MPS – meat peptone soup.																		

Of all the isolated and studied Pasteurella shoots, only strains of Pasteurella multocida a biovar avium B-0054 «Kazakh research veterinary institute» No. 12, G-5, Al-7, D-2, K-30, A-22 was classified as S-shaped.

Conclusions. To determine the toxicity properties of the studied Pasteurella, epidemic and industrial strains, it was determined by taking white mice weighing 18-20 G and inoculating micro-organism shoots on them.

As a result of the study of the toxicity properties of Pasteurella shoots, the maximum toxic activity was observed in Pasteurella multocida a biovar avium B-0054 strain No. 12 «Kazakh research veterinary institute», which ranged from 4 to 10,000 colony formation activity (CFA) and amounted to LD_{50} 2-4 CFA.

Based on the study of the biological properties of epidemic strains of Pasteurella, the criteria for assessing the biological properties of industrial strains of Pasteurella multocida and the principles of their selection were studied and found.

In the process of studying the biological properties of pasteurells in industrial conditions, it is possible to determine their toxicity and immunogenicity. Effective pasteurellosis erythrocytic antigenic diagnosticum also showed that these two properties are directly related to each other.

As criteria for the toxicity of the industrial strain, the degree of mortality of rabbits in 24-48 hours of diluted 1.0 cm^3 to 10^{-6} of Pasteurella shoots grown in meat peptone broth for 18-20 hours was taken. The studied strains met the indicated toxicity criteria.

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DETECTING THE PRESENCE OF ANTIMICROBIALS IN RAW MEAT IN THE AKMOLA REGION

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Antimicrobials are important drugs in animal production. However, their prolonged use has led to unexpected threats associated with the emergence and spread of bacterial resistance to antibiotics. Moreover, failure to observe the withdrawal period in animals used for food production, antimicrobial residues may enter the food chain causing direct toxicity, allergies in consumers. During the study, we monitored the content of antibiotics in meat and meat products in the Akmola region using a modern system Evidence Investigator (Randox). The study results revealed that horsemeat, mutton, pork do not contain antibiotics and are safe for consumption. However, the content of antibiotics in beef and poultry meat exceeds maximum concentration limit (MCL) in some cases by more than two-fold, which makes this type of