Testing morphological composition of inactivated vaccine against avian pasteurellosis

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Abstract. The article presents a study of the morphological composition of inactivated vaccine, which is necessary for checking the stability of the composition of the ingredients added after the construction of a biological preparation. This is especially important in cases of vaccine-related adverse clinicopathologic complications in the form of the damaging effect of harmful, toxic or incompatible for the body components. Vaccine studies are required not only for objective control, but also for biological standardization in their qualitative and quantitative morphological characteristics in the final product. Thus, the study of the morphological composition of biological preparations on the model of an inactivated vaccine against avian pasteurellosis with complex biochemical composition showed the presence of the safety of many microscopic structures, which gives an indication of the initial components of the insoluble particles and their derivatives, after complex biotechnological and biochemical processes in vitro.

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Introduction

Study of the morphological composition of a particular vaccine is necessary for checking the stability of the composition of the ingredients added after the construction of a biological preparation. This is especially important in cases of vaccine-related adverse clinicopathologic complications in the form of the damaging effect of harmful, toxic or incompatible for the body components. Many biological substances, especially having its starting composition may contain different quantity and quality of the components, given physico-chemical and biological properties of organic and inorganic nature, nonspecific and specific immune material. Vaccine studies are required not only for objective control, but also for biological standardization in their qualitative and quantitative morphological characteristics in the final product.

In this regard, was tasked to examine the macro-and micro-morphological qualitative and quantitative composition of the vaccine after complex physical, chemical and biotechnological processes occurring at the joint interaction and compatibility between a set of components before and after parenteral insertion of it to birds in order to identify possible manifestations synergies or anergizma of their actions among the components.

Studying morphostructural liquid elements of inactivated vaccine containing various biochemical and chemical reagents, primarily attention has been paid to protective antigen, i.e. morphological characteristics and possible changes after polyfactorial pasterell impacts which heaps, to evaluate the state and the ratio of components to characterize the epidemiological and immunological efficacy of the final product. Along with this, at the light level viewed antigen interaction with other vaccine morphological structures of the substrate, particularly paying attention to the phenomenon of adhesion of microbe depositing the substrate (alumina hydrate), and other morphological components.

In this case we were interested in the morphology of inactivated Pasteurella, variability of different shapes, sizes, degrees of manifestation of adhesion to the particles of aluminum hydroxide as an adsorbent of last Azure absorption. One aspect was the same character of the structures remained intact pasterell in morphological terms, as well as the nature of their injuries, to have an idea of biologicals; at the light level studies to determine criteria for assessing morphological manifestations of the main components of the vaccine, as well as assess and compare immune and morphlogical processes in cases of post-vaccination complications.

To study the objectives about the liquid inactivated vaccine against avian pasteurellosis, bank number 7, control number 7, expiration date July 2005, made in the laboratory of disease in birds and bees prepared smears on glass items. Smears were

later painted by well-known methods Romanovsky - Giemsa and Gram as a control smears were prepared from cultures of the vaccine strain, and also studied the structure of the particles of aluminum hydroxide and other vaccine components separately.

According to a preliminary patent on december 25, 2001 liquid inactivated vaccine against avian pasteurellosis consisted of bacteria Pasteurella multocida A 46 576 number (10 billion microbial cells in 1 cm^3) – 79,0 – 81,0 cm^3; 6% aluminum hydroxide gel - 19,0-21,0 cm^3, 180 mg % of amine nitrogen per 100 cm^3 of the drug. Besides the main components in the composition of the final product had horse serum, sucrose, NaSI, dibasic sodium phosphate, 40 % Glucose.

Morphological composition of the vaccine was studied in substrate digital microscope Leica DM -400V.

Biometric studies bacteria (Pasteurella) and other morphological structures in smears of the final vaccine was performed using 1 - micrometer - DOM 15x. To compare data precipitate disodium hydrogen phosphate solution (0,9 wt.%), NaCI (0,5 wt. %), A 40% glucose (0,4 wt. %), Sucrose (4,0 wt. %) study was separately in pure form after making with imitation (exposure) biotechnological parameters specified in the relevant specification.

As guidance and control during morphological studies using instruction for use, technical documentation for manufacturing vaccines and vaccine prepatents to detect possible residual structures of components in biological preparations.

Study of the structure in the liquid inactivated Pasteurella vaccine containing aluminum hydroxide together with other components, showed heterogeneity Pasteurella forms as a main component of the drug in the body causing the specific immune response of birds [1; 2]. Among them were detected as whole and modified Pasteurella not differ in morphology from freshly isolated or reference cultures. A distinction is also uncharacteristic changes to Pasteurella morphostructural with peculiar manifestations. Both types of Pasteurella painted mostly in dark blue, except for certain bacteria. According to their morphostructural characteristics was differentiated info small, medium and larger specimens.

Pasteurella smallest size of 0,3 x 0,2 mm or 0,3 x 0,3 microns met a very large number of all morphological biological structures, painted basophilic in navy blue color, often in isolation, 1-2 bacteria, and sometimes - from 10 to 32 and more polymorphic form large aggregates. Found spherical, rod-shaped, round-oval and shapeless items aggregated together as a result of interaction with other components of the vaccine during its manufacture or in the process of preparation and staining of smears. However, many of them with loss of the outer sheath and peeling it in the form of detritus and enlightened in the interpole portions with negligible eosinophilia (in Figures 1 and 2).

In a large number of aggregates of bacterial cells with a single dominant pole bipolars (Figure 3). They resemble clusters of numerous small structures granular in the field of view of the microscope. Dimensions concentrations reached lengths of 5 microns to 10 microns, and the width reached 0,5. Among disintegrated small granular formations could still see small formation with dark blue color, apparently, is a biochemical substrates vaccine. Fine and coarse aggregates met in one field of view in an amount of from 1 to 4 (Figure 3) with the manifestation of trends in accumulation of granular structures.

Moreover, judging by the size of the population and destroyed small pasterell large number of units, should indicate the greatest lability of the interaction of the various components in the vaccine manufacturing process and painting strokes than medium and large Pasteurella.

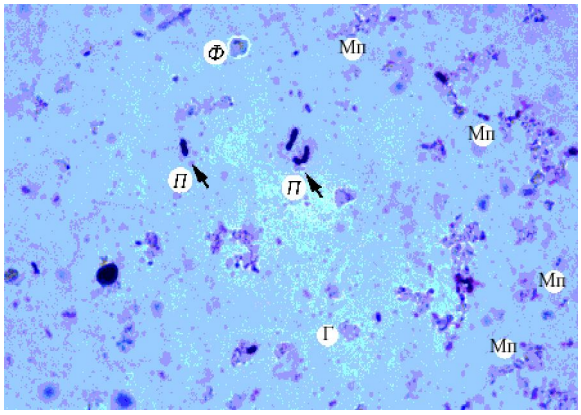

Figure 1 - Pap inactivated vaccine against Pasteurella birds.

Small ("MP") and average Pasteurella (arrow "P"), disodium hydrogen phosphate particle ("F"), globular structure of aluminum hydroxide gel ("G"). Colouring Romanovsky-Giemsa. An increase of 10 x 100, with digital microscope Swiss brand Leica DM 400 B Software "Morphology".

Medium-sized Pasteurella in whole [3; 4], relatively intact form (Figure 1) are identical to those found in cultures on nutrient media until vaccine. They similarly stained with azure blue color, resolution of 0.8 x 1.1 microns, their number in one field of view from 0 to 7 copies in the form of pairs or chains. Azure staining intensity was about the same as a small pasterell without cytopathic changes. As a rule, such instances were rather pronounced contours

without compromising the integrity of the conservation of the cytoplasm. However, single Pasteurella had no contact with the globular particles of aluminum hydroxide gel.

Very rarely observed marked changes in violation of the cell membranes and strong distinct swelling of the cell with the central part of its enlightenment (Figure 3).

Large Pasteurella with identical tinctorial and preserve the integrity of their structures, as well as average, differed only in magnitude, which reached 2 x 1,1 m to 2,4 x 1,2 m.

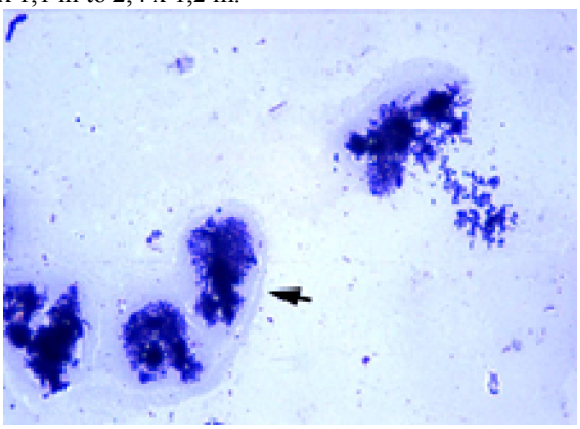

Figure 2. Granular aggregates from demolished pasterell gel (arrow).

Colouring Romanovsky-Giemsa. An increase of 10 x 100, with digital microscope Swiss brand Leica DM 400 B Software "Morphology".

They are often localized singly; in the same field of view met from 1 to 2 Pasteurella. Sometimes traced quick formed morphological changes of microbial cells and the partial nature of the total detected at the light level. In the latter case, a partial violation of all membranes, swelling, and the formation of short dissociation of radial fibrils on the surface of Pasteurella [5; 6]. It also increased the contours pasterell 1,5 times (rather than intact) as a rule, they were deprived of bipolarity, and their cytoplasm was heavily coated, with homogenization and acquisition of eosinophils (Figure 3). It should be noted that medium and larger, relatively intact, Pasteurella probably have ultrastructural changes, do not catch the light-optical microscope, although the variability in the nature of circulating and reference strains of Pasteurella in length and width, as high - within 0,4-1,2 microns length 0,3-0,4 micrometers width.

Cell wall modified Pasteurella 3-4 times increased loosened, dissociated longitudinal contour lines and bacteria perpendicular to the horizontal surface of the shell. Shell vague, sometimes or all equally distinct in that part where the destruction occurred [7; 8]. It should be noted that the adhesion to these Pasteurella aluminum hydroxide gel, which is an adsorbent, we have not detected. Consequently aluminum hydroxide gel in the globular form shows no adhesive properties to large Pasteurella (Figures 1 and 3).

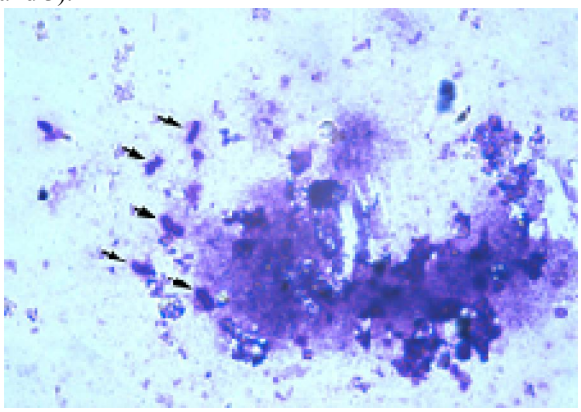

Figure 3. Units with gel. Major degradation able Pasteurella (arrows).

Colouring Romanovsky-Giemsa. An increase of 10 x 100, with digital microscope Swiss brand Leica DM 400 B Software "Morphology".

Encountered microscopy fibrils in smears of the vaccine can be divided into two groups. In the first group should be noted the ones we find the remains of loose components consisting of a muscle or perivascular connective detritus (fragments) with a rough and relatively short fibrillar structure may occur after hydrolysis occurring in the manufacture of nutrient medium Hottinger (Figure 4). In a network of these fibers can be seen solitary, sometimes arranged in groups, with different Pasteurella morpho- tinctorial characteristic that suggests the possibility of manifestation of prolonged action after parenteral inoculation of the vaccine at the injection site.

The second group of fibrous structures can include a long, fine fibers may restructure interacting with different components of the vaccine. In this fibrillar eosinophilic tinctorial resembles connective tissue, but, unlike the first group of fibrillar structures, characterized by a weak clearance structures and capacity for pulping and fragmentation. Believe that the reproduction and resynthesis of the constituent elements of fibrillar structure of the second group may also participate pluripotent composite structure consisting of many biochemicals tissue, humoral and bacterial origin [9; 10]. Usually they eosinophilic with non-uniform optical density, reach a length of up to 135 microns in thickness of the individual fibers - 1 or 2 microns (Figure 5).

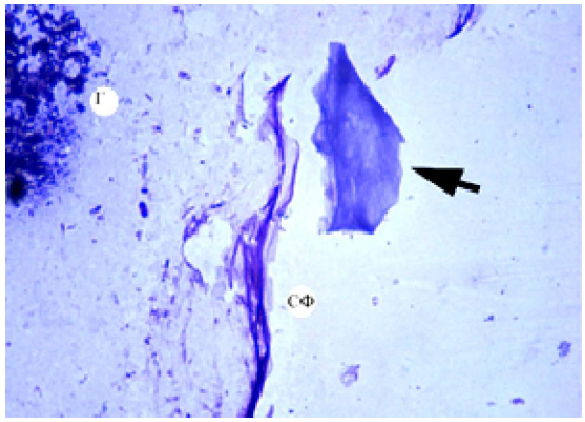

Figure 4. Connective fibrils ("SF"). Chemical particle (arrow) and the globular cluster pasterell ("G").

Colored Romanovsky-Giemsa. An increase of 10 x 100, with digital microscope Swiss brand Leica DM 400 B Software "Morphology".

In Biopreparat considerable importance is the dispersion grade aluminum hydroxide has an effect on prolongation immunogenesis together with other components. In this regard, proper selection deponatora for the constructed vaccine requires special attention.

Smears prepared from liquid inactivated vaccine, and stained with Romanovsky - Giemsa, the smallest particles of the globular forms were often among the clusters of bacterial cells and fibrillar structures. Typically, these particles were deprived tinctorial activity towards Azura. This property remained unchanged and in bottles, where a layer of hydroxide gel retains its original structure, uniformity and color on the bottom of the vial with a biologic. Measurement of the particle size of aluminum hydroxide gel showed fluctuations in their 0,8 x 0,8 microns to 2 microns.

To compare the morphological structures of aluminum hydroxide gel added as deponatora vaccine conducted in morphological analysis by microscopy native powder.

Native fine powder - aluminum hydroxide [Al (OH) 3] TU 6-09-3714-74 Donetsk plant of chemicals represented visually spotlessly white, homogeneous structure and the weight of flour -like consistency, while microscopy consisted of uniform particles with a size of 1 x 1 mu.m to 2 x 1,8 mm and smaller globular form of 0,8 x 0,8 microns. In the field of vision as they met in bunches, adhering to each other. With an increase of 10 x 100 they look with dark contour and enlightened center. However, none of them exhibit any other particles in the form of crystals, which were detected in smears of vaccine.

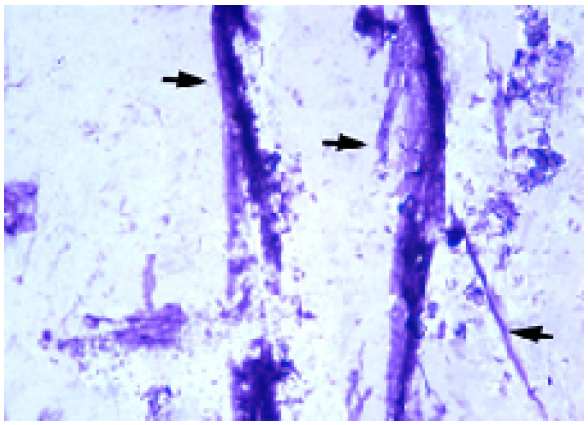

Figure 5. Structuring fibrils (arrows).

Colouring Romanovsky-Giemsa. An increase of 10 x 100, with digital microscope Swiss brand Leica DM 400 B Software "Morphology".

Adhesive activity of aluminum hydroxide gel in all probability is shown not only by the smallest pasterell varying degrees of disintegration, but also connects with many biochemical components: protein-carbohydrate, lipid (polysaccharide) and other complexes, some of which are waste products of Pasteurella. In our opinion depositing and prolong the inactivated vaccine also arises due to the optimal amount of gel by mechanical contact and the delay between the helium particles Pasteurella.

An indirect confirmation of insufficient adhesion activity hydroxide particles against Pasteurella whole can be explained by the accumulation layer and a white precipitate with slight grayish tinge on the bottom of the vaccine vial.

Among the salts and other ingredients in the vaccine possess good solubility NaCI, yeast extract, sucrose, glucose, dibasic sodium phosphate (12 aq, analytical grade, Na2HPO4 12H2O, Standard 4 (72-76)). Of these components should be noted that some of these crystals may remain in small amounts in the final vaccine, which prompted the description of the individual particles.

Preliminary check on the solubility in distilled water, as well as during the preparation of biotechnological processes inorganic components, dissolution of these drugs was not visually long.

Microscopy of powder or pellet of a native solution of dibasic sodium phosphate (0,9 wt. %) Revealed heterogeneity of the particle size, shape and brightness. Grains substances were examined in two light natural and artificial light with frosted filter. In the first case, the difference in magnitude to the dominant number of particles with a spotlessly clean and uniformly white fluffy snow to resemble fine-

grained surface with a slightly noticeable sheen. At the same time, sometimes there are as crystal, polymorphic, more transparent, enlarged, sometimes visually looked like a finely crushed ice, color and consistency that resembles a "thin transparent ice on the mountain stream".

By lamplight described micro-and macrostructure autolyuminestsensii have the property: "snow" - dark blue, and the" ice " - transparent blue and at higher light – black blue shades.

Thus, disodium hydrogen phosphate particles in the microscope with artificial lighting were darker, sometimes with a bluish tinge, and of various sizes. The denser of these may include "ice", which is difficult to dissolve in the composition of the complex vaccine. In prepared 0,5 % solution of its hot water on the bottom of dishes remained opalescent loose sediment. Derivatives of its particles within 4 hours after preparation of the solution presented in the form of crossed needles with sharp ends, as well as "ice" forms, which indicates that the same as in the original size of the native material, respectively - 1 x 110 mm and 2,5 x 6,3 um.

Of the compounds examined, and salts (NaCI, 40 % th glucose, sucrose (4,0 wt. %)) Microscopy restructuring as crystals differed only in the dry state, sodium chloride (0,5 wt. %). In this distinguished education needles, cubes and large pyramids - crystals, which could not be found in the vaccine. Morphostructure needle crystals was identical as in the solution of disodium hydrogen phosphate.

Among the variety of morphological structures studied vaccine met the larger and more dense particles disodium hydrogen phosphate with the versatility of their surface. This drug was added as a buffer in the culture medium in the inoculants. Rarely detectable particles in the test swabs of vaccine characterized by an uneven surface, different values - from 23 to 25 microns, and sometimes as crystal and transparent particles of up to 50 x 70 microns. Angles particles - often blunt, some of them stained with azure from light blue to dark blue, the presence on the surface in various stages of disintegration, very small with the phenomenon of Pasteurella adsorption (Figure 5). Along with the above described properties of the particles were also lighter particles hardly stained azure identical in crystal structure, hence their lack of adhesion phenomenon and Pasteurella Azura (Figure 6).

These data suggest that not all particles disodium hydrogen phosphate in a vaccine, by the action of some factors are not fully dissolved. Presumably we can say that smaller particles are more dense and highly variable structures.

As for the raw material from which it was prepared yeast extract, when it consisted of a microscope small, medium and large, homogeneous structure of whole yeast cells from light gray to pale greenish hues. Large cells of their number had double circuit shell. In smears of the vaccine, it has not been possible to detect them, as they remain on the filter after filtration of the starting material.

During the light-optical study of the morphological composition of the vaccine in sight sometimes found as the presence of dark yellow polymorphic particles resembling remnants of gelatin or agar detritus.

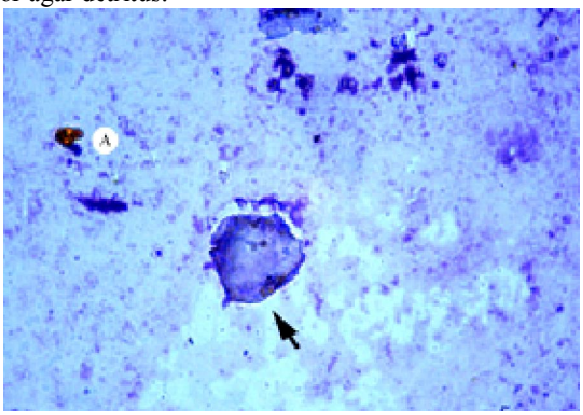

Figure 6. Particle crystal disodium hydrogen phosphate (arrow).

Detritus agar ("A") on the background of gel structures. Colored Romanovsky-Giemsa. An increase of 10 x 100, with digital microscope Swiss brand Leica DM 400 B Software "Morphology".

Furthermore, the series of control process of the preparation is necessary to check it for the presence of extraneous microflora by smear of the vaccine of the substrate, since this part of biological control may be achieved by other methods considering the fact that the final product before dispensing into vials are subjected to autoclaving at a temperature of 120 °C for 30 minutes.

The data obtained can be used for controlling a variety of living or non-living vaccines to the complex composition of ingredients comprising Korrigents stabilizers, sorbents, immunostimulants and clarifying the nature of their relationship in the composition of the final product, as well as for the complete characterization of the vaccine used in clarifying or harmful and harmless, immunogenic or reactogenic properties in vivo.

Identified residues capable of save as fragments of the original fibrillar separate biological substrate, a component of the vaccine as a bio-stimulator of nonspecific immunity factors.

Thus, the study of the morphological composition of biologicals model pasteurellosis vaccine birds with complex biochemical composition showed the presence of many preservation of

microscopic structures, which gives an indication of the initial components of the insoluble particles and their derivatives, after complex biotechnological and biochemical processes in vitro.

Currently, specific prophylaxis of pasteurellosis birds are carried out in different vaccines, in particular: vaccine against salmonellosis, colibacillosis and pasteurellosis birds [11], dry live vaccine of attenuated strains BM- 1 and LA - 25 against pasteurellosis birds [12], subunit inactivated vaccine against avian pasteurellosis [13], Polyvalent formalin-killed vaccine against pasteurellosis birds [14], Inactivated Emulsion vaccine against avian pasteurellosis [15], vaccine against avian pasteurellosis inactivated adsorbed [16], versin 424 bacterin against pasteurellosis (Cholera) bird (liquid) [17], AviPro 108 PM / FC inactivated vaccine against avian pasteurellosi [18].

Currently for all the above vaccines are no test conducted.

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