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## INFECTIOUS CHICKEN BRONCHITIS: THE CAUSE OF RESPIRATORY SYNDROME IN BROILER CHICKENS

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### ABSTRACT

The article presents materials on an epizootological examination of a broiler poultry farm, in which signs of respiratory disease in broiler chickens were observed, accompanied by a deterioration in safety and weight gain. One of the pathological factors was identified in the chicken circulating infectious bronchitis virus (IB). As a result of studying the immunobiological properties of the isolated virus, the antigenic characteristics and virulence of the pathogen were determined for developing chicken embryos and broiler chickens. The use of molecular genetic research methods (RT-PCR-RV, genomic sequencing) made it possible to determine the identity of the isolate to the variant strain QX of the IBI virus.

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## 1. INTRODUCTION

Infectious The high efficiency of modern intensive technologies for industrial poultry farming cannot be achieved without the detailed development and flawless implementation of veterinary programs aimed at ensuring the biosecurity of poultry enterprises (Kuklenkova et al., 2018). This is especially true for large broiler poultry farms working with highly productive crosses of chickens, showing increased sensitivity to various violations in the technology of raising, feeding and keeping poultry (Balykina et al. 2018). At the same time, timely and accurate diagnosis of infectious diseases of birds, occurring associated with signs of the respiratory syndrome, is of great importance. These diseases of viral and bacterial etiology include Newcastle disease (NB), chicken infectious bronchitis (IBC), infectious laryngotracheitis (ILT), metapneumovirus infection (MPVI), hemophilia (contagious runny nose), colibacteriosis, respiratory mycoplasmosis of birds (RMP). The results of diagnostic monitoring make it possible to reliably determine the epizootic situation in the poultry industry and to develop an effective program for the specific prevention and control of respiratory

diseases of birds, among which chicken infectious bronchitis (IBS) occupies a special place (Shcherbakova et al. 2018).

The problem of chicken infectious bronchitis in industrial poultry farming as one of the pathological factors in recent years has received a new development. This is due to the identification in different regions of the world, including in the Russian Federation, new field strains of IBI virus that differ in antigenic activity and have different virulence for birds (D274, D1466, 793B, CR88, 4/91, IT02, D388) (Teryukhanov, 1976). The emergence of new variant strains of the virus is due to the genetic structure of coronavirus IBC, amenable to relatively rapid mutation and genetic recombination. Circulation in poultry farms of virulent field, incl. variant strains of the IBC virus significantly complicate the work of veterinary specialists of farms in the diagnosis and specific prevention of the disease. Widely used in previous years, live and inactivated vaccines based on the classic Massachusetts serotype virus from strains N-120, H-52, Ma5, M41, Chapaevsky currently in many cases do not provide the formation of reliable immunity in vaccinated birds (Ovchinnikova, et al, 2010). This requires new approaches to the study of the epizootic situation in each individual household. Identified epizootological features help to develop effective programs for the specific prevention of IBS. An important condition, in this case, is the conformity of the antigenic properties of the used vaccine strains of the virus and the circulating field isolates of the pathogen. In this regard, we were faced with the task of conducting a comprehensive epizootological examination of two poultry farms in the meat sector, in which there were signs of respiratory disease in chickens, accompanied by a deterioration in safety and weight gain in broiler chickens. The purpose of this work is to identify the main causes of respiratory syndrome in broiler chickens and, taking into account the results obtained, improve the scheme of prevention and control of the disease. The data obtained can be used to assess the epizootic situation in poultry farms with the manifestation of the respiratory syndrome in broiler chickens and to adjust the specific disease prevention schemes (Kuklenkova et al. 2018, Javadov et al., 2019).

## 2. MATERIALS AND METHODS

**Epizootological examination** of poultry farms was carried out in accordance with the "Methodological guidelines for epizootological examination of a poultry farm" (Birman, et al, 2004).

**Selection of material.** For microbiological, virological, and molecular biological studies, sick chickens were removed from the trachea and larynx during the incubation period of the disease or in the first hours of the manifestation of visible clinical signs of pathology. Scrapes of the trachea, the larynx of the bronchi, pieces of lungs, kidneys, air sacs, heart, spleen were taken from forcedly killed or just fallen birds. For serological testing, individual blood samples were taken from the axillary vein from suspected ill chickens (less developed by weight) and clinically healthy broiler chickens 36–40 days old, 25 samples from each group of birds. Serum was separated from blood samples by the conventional method, which was examined for the presence of humoral antibodies to various pathogens of infectious diseases of birds.

**Microbiological studies.** To isolate bacterial cultures, nutrient media of a wide spectrum of activity and special-purpose media were used: meat-peptone agar (MPA), meat-peptone broth (MPB), Saburo agar, Endo medium, Hottinger agar and broth, Levin medium, Simmons medium, Petroniani medium Wednesday Edward Wednesday Giss Wednesday King. Crops and cultivation of

material were carried out by the generally accepted method, taking into account recommendations for working with these nutrient media.

**Virological studies** of the material with the aim of isolating the virus were carried out on developing SPF chicken embryos (REC) of 9-10-day incubation. The method of sequential passages of the material was used. The work was guided by the methodological materials presented in GOST 25583-83 (2). In total, three passages were held. In each passage, 10 SPF embryos were used for infection, 5 embryos were left as controls. From samples of the taken material from birds of each age group, a 10% suspension in isotonic phosphate-buffer solution was prepared. Initially, the material was homogenized using a blender, after which it was centrifuged at 3000 rpm for 15 minutes. Antibiotics were added to the supernatant: penicillin (200 U / cm<sup>3</sup>) and streptomycin (1 mg / cm<sup>3</sup>). It was kept at 4 ° C for 2 hours. The suspension of the test material obtained in this way was used to infect SPF REC. The material was inoculated at a dose of 0.2 cm<sup>3</sup> into the allantoic cavity of each of 10 RECs and incubated at 37 ° C for 120 hours. Ovoscropy of the embryos was performed daily. The dead embryos were discarded during the first 24 hours after infection, considering this death non-specific, and were not taken into account in the experiment. At the end of the incubation period, the embryos were cooled at 4 ° C for 8-10 h, opened under sterile conditions and extraembryonic (allantoic) liquid and chorioallantoic membranes were collected in sterile tubes from each REC. The resulting material was checked for the absence of bacterial contamination by seeding and incubation on artificial nutrient media. The collected material of each passage was kept frozen at a temperature of -24 ° C. For the second passage, the material was thawed, homogenized, and centrifuged at 3000 rpm for 15 minutes. The supernatant was used to infect the next batch of SPF embryos and examined for hemagglutinating activity. The supernatant was monitored for the presence of a hemagglutinating component in a hemagglutination reaction (RGA) using a 1% suspension of rooster erythrocytes. The embryo bodies in each passage were examined for the presence of pathological changes. Material from the third passage embryos was used for titration and identification of the isolated virus.

**The neutralization reaction (PH)** was set to determine the titer of the infectious activity of the isolated IBC virus. The reaction was performed using the virus in serial ten-fold dilutions and with a constant dose of serum. For the formulation of the reaction, virus dilutions were prepared in the range of 10<sup>-2</sup>-10<sup>-8</sup>. Normal and type-specific hyperimmune sera were used for serological variants of the virus: Massachusetts (strain "H-120"), 793B (strain "4/91"), D388 (strain "QX"). The reaction was performed on developing chicken embryos (RECs) of 9-10-day incubation, obtained from chickens free of antibodies to the IBI virus. Used 6 embryos in each dilution of the virus. Infected RECs were incubated at 37 ° C for 7 days. Conducted daily ovoscropy. Embryos that died within 24 hours after infection were discarded and were not taken into account in the experiment. The neutralization reaction was considered positive in the case when the death of the embryos was not observed in the dilutions of the virus and the autopsy of the REC showed no pathological signs characteristic of IB. PH results were evaluated by a neutralization index. At a neutralization index of 1 lg, the reaction was considered negative, above 2 lg - positive, in the range of 1-2 lg - doubtful. The neutralization index was calculated by the method of Reed and Mench.

**Bioassay on chickens.** Used 35 goals of broilers of 15 days of age cross-country Hubbard F-15, obtained from a farm that is safe for respiratory diseases and infectious bronchitis of hens. Antibodies

to IBI virus were absent in the blood serum of chickens. The chickens were kept in a cage. Conditions of feeding and feeding (the composition of the diet, temperature regime, and illumination in the boxes of the vivarium, basically, corresponded to the hygiene standards for birds of this cross-country and age). Chickens were ringed, a blood sample was taken from each bird for serological testing. 3 groups of chickens were formed: 1 group (10 goals) - intact chickens ("clean" control); 2 group (10 goals), chickens, each of which was injected ocularly and intranasally with 0.2 cm<sup>3</sup> of the suspension of the studied material; 3 group (10 + 5 goals), in this case, 5 heads of chickens were planted for 10 intact chickens, each of which was injected ocularly and nasally with 0.2 cm<sup>3</sup> of extraembryonic fluid obtained from SPF REC third passage. The bird was monitored for 25 days. Daily safety, the clinical status of birds and pathological signs were recorded.

**Serological studies.** The blood serum obtained in poultry farms from broiler chickens and the blood serum from chickens of the experimental groups were examined for the presence of specific IgG antibodies to NB, IBC, ILT, MPVI, and RMP viruses. The presence of antibodies to the NB virus was determined in the hemagglutination inhibition reaction (RTGA), which was set by the standard method with 4 GAE of the NB virus antigen and 1% suspension of rooster erythrocytes. Antibodies to the viruses IBK, ILT, MPVI, and RMP were detected by enzyme-linked immunosorbent assay (ELISA, ELISA). We used IDEXX test systems and the x-Chek computer program. The presence of antibodies to the ILT virus was determined in ELISA using BioChek test systems. ELISA results were recorded on a Sunrise TECAN spectrophotometer.

**Polymerase chain reaction.** The polymerase chain reaction (RT-PCR-PB) was set in order to identify the isolated IBK virus. For this, virus RNA was isolated from pathological material by affinity sorption on silica gel using the Ribo-sorb reagent kit. The reaction was performed using the Revert-L test system with a set of exonucleases. Amplification was performed on a Tercik apparatus using the Amplisens-PCR kit. Electrophoretic analysis of amplification products was carried out in a 1.7% agarose gel containing ethidium bromide. The DNA fragment from the gel was isolated using a saturated solution of ammonium acetate. The synthesis of oligonucleotide primers was carried out at Beagle LLC (St. Petersburg). 50 ng DNA, 5 pmol primers, DYEnamic ET terminator kit were used for sequencing. The determination of the nucleotide sequence was carried out by the Sanger method using fluorescently labeled termination nucleotides on an ABI Prism3130 sequencer (Applied Biosystem, USA) according to the manufacturer's instructions. The obtained nucleotide sequences were compared with the sequences of IBK virus strains published in the NCBI international database (<http://www.ncbi.nlm.nih.gov/>) using the BioEdit program, version 7.0.5.3.

During the research, we used the materials presented in the "Guidelines for the identification of the IBI virus genome using PCR-RV (4).

**Statistical processing of results.** Statistical processing of the obtained data was carried out using the Microsoft Excel program.

### 3. RESULT AND DISCUSSION

**A brief description of the epizootic state of the economy.** The broiler poultry farm, in which an epizootological examination was carried out, and based on the materials of which this article was prepared, works with hens of the Hubbard F-15 cross. The farm has its own parent flock. The technology provides for the cellular content of broiler chickens. The planned broiler feeding period is

40 days. In accordance with the current preventive treatment regimen, broiler chickens are vaccinated against Newcastle disease (NB), infectious bursal disease (IBD), infectious laryngotracheitis (ILT) and infectious bronchitis (IBI). Chickens are vaccinated against IBI using live virus vaccines based on the classic ("H-120") and variant ("4/91") virus strains. In accordance with the approved growing technology, drug treatment of the broiler population is carried out.

The dynamics of safety and weight gain in broilers of different ages at the time of the epizootological examination of the farm is presented as follows:

- age 22 days, safety - 97.1%; the average daily weight gain is 48.6 g / goal;
- age 30 days, safety - 95.4%; the average daily weight gain is 46.1 g / goal;
- age 38 days, safety - 94.9%; the average daily weight gain is 44.3 g / goal.

In broiler chickens, the following dynamics of the development of a common pathology with a dominant manifestation of respiratory damage were recorded for several months. The first weakly expressed clinical signs of pathology in broilers are recorded at the age of 20-22 days and appear as rhinitis, the mild outflow from the nose, sneezing. Chickens become less active, feed intake is reduced. At the age of 25-28 days, the disease proceeds in a more pronounced form of a respiratory symptom complex. At the same time, in clinically sick chickens, as a rule, the profuse outflow from the eyes and nasal openings is recorded, making breathing difficult. Chickens are oppressed, inactive, craning their neck, trapping air with their open beaks, often shaking their heads. In addition, swelling of infraorbital sinuses, serous-catarrhal rhinitis, conjunctivitis, cough, wheezing in the lungs, depression (lethargy, drowsiness), poor eatability and even refusal of food are additionally noted. During pathological dissection of such chickens, mainly, clearly defined signs of respiratory disease are revealed: serous-catarrhal and / or fibrinous tracheitis and bronchitis, focal catarrhal or catarrh-fibrinous pneumonia, serous-fibrinous aerosacculitis. In many chickens, an autopsy reveals puffiness, anemia, deposition of urates in the kidneys and ureters, and nephros-nephritis. In some cases, corpses of birds revealed foci of necrosis in the liver. After 30-33 days, the clinical signs of the disease in broiler chickens gradually "fade away" and at the final stage of feeding, the condition of the chickens is almost completely normalized. The antibacterial treatment of the livestock partly contributes to this.

An epizootological examination and an analysis of its results confirmed the poor state of the economy in relation to the pathology of broiler chickens, which occurs with signs of a respiratory syndrome.

**The results of microbiological studies.** As a result of a microbiological study of the material taken during the post-mortem autopsy of dead and 30-day-old broiler chickens, 6 bacterial cultures were isolated, including 3 cultures of *Escherichia coli* from the trachea and larynx, infraorbital sinuses and lungs 2 cultures of *Staphylococcus aureus* (*Staphylococcus aureus*), infraorbital sinuses and trachea 1 culture of *Pseudomonas aeruginosa* (*Pseudomonas aeruginosa*). Pathogens of bacterial diseases of birds were not isolated from the material from corpses of chickens of other age groups. The causative agents of ornithobacteriosis, pasteurellosis, and respiratory mycoplasmosis were not isolated.

**The results of a virological study.** When the developing SPF - embryos of chickens of 9-10-day incubation were infected with a suspension of material at the first passage, a partial death of the



embryos was observed 48 hours after infection. The total number of fallen RECs in the first passage was 40%. In the second passage, the number of dead embryos increased to 60%. In the third passage, 90% of RECs fell. In embryos that died 96 hours after infection, hyperemia, edema, and hemorrhage on the skin were noted. The liver and kidneys were enlarged, blood vessels were filled. In embryos that died 120 hours or more after infection, growth retardation was observed, the so-called "Dwarfism." The "dwarf" embryo was approximately two times smaller than the control; deformation of the legs was noted in such embryos. As an additional sign characteristic of IB, noted the twisting of the embryo into a "ball". In embryos with pronounced signs of "dwarfism", a densification of the contents of the yolk sac and an increase in the amount of allantoic fluid were noted. Extraembryonic fluid from dead RECs with changes characteristic of the IBI virus did not agglutinate rooster erythrocytes.

**The results of the neutralization reaction.** The titer of the infectious activity of the virus was 6.5 lg EID<sub>50</sub> / 0.1cm<sup>3</sup>

**Chicken bioassay results.** The pathogenicity of the isolated IBC virus was studied in an experiment on chickens that were infected with the ocular and nasal suspension of material and extraembryonic fluid from SPF REC third passage. The results of the experiment were positive but slightly different in groups of birds. The incubation period after infection of the chickens with a suspension of the material was 120 hours, after infection with a virus-containing extraembryonic fluid, it was 72 hours. Clinical signs of pathology in contact infection in group 3 chickens showed up after 96-144 hours. Chickens in the "clean" control group remained healthy throughout experience. Symptoms of the disease were observed in infected chickens: in the initial stage, weakness, deterioration of food intake, catarrhal rhinitis, and shortness of breath were recorded. Then wheezing, coughing was noted, chickens stretched their necks, took air with an open beak, shook their heads. Sinusitis, unilateral or bilateral conjunctivitis was noted. In group 3, in chickens, the clinical signs of a respiratory symptom complex were more pronounced. In this group, chickens additionally showed signs of renal pathology: puffiness, one- or two-sided nephros-nephritis, accumulation of urates in the ureters, uric acid diathesis. The obvious clinical form of the disease lasted until the age of 25-27 days. In the next 4-6 days, the clinical manifestation of the disease was attenuated, and by the end of the experiment, the absence of clinical signs of pathology was noted. In chickens of the 2nd group, in the course of the experiment, the death of one chicken was noted. In group 3, the number of dead chickens was 3 heads. As a result of the autopsy of dead carcasses of chickens in all 4 cases, pathological anatomical signs characteristic of infectious bronchitis of chickens were revealed. Our data are consistent with the materials Bochkova Yu.A.

From infected and control chickens during the experiment, swabs from the larynx were taken. At autopsy, pieces of the trachea, lungs, and kidneys were aseptically taken from the fallen chickens. Samples were frozen at -35 ° C and used in PCR to isolate the IBV virus genome.

At the end of the experiment, blood samples were taken from chickens of all groups, serum was separated, which was examined in RTGA for the presence of antibodies to the NB virus and in ELISA for the presence of antibodies to the IBS virus. Antihemagglutinating antibodies to the NB virus were not detected in any case. In the blood serum of infected chickens, regardless of the method of infection, antibodies to the IBI virus were detected in ELISA. The level of antibodies was in the range: 1: 3840 - 1: 5066. No statistically significant differences in the titer of antibodies depending on

the groups of birds were detected.

**Results of RT-PCR-RV.** For determination and comparative analysis of the structure of the S1 gene, 7 samples of material positive in RT-PCR were taken, incl. 2 samples based on farm material from the farm, 3 samples of extraembryonic material from infected SPF REK of the third passage, and 2 samples of material from experimental broiler chickens. A comparative analysis of the nucleotide sequences of the S1 gene fragment showed that of the studied positive samples in 1 case, the vaccine strain of the IBC virus “N-120” was detected. In 6 positive samples of the material, an isolate was identified that is identical to the variant strain “QX” of the IBC virus (serological variant D388). The results obtained are mainly consistent with the data of Dandal A.Sh. et al. and Scherbakova L.O.

**The results of serological studies.** The results of a study in RTGA of blood serum samples from clinically sick and healthy broiler chickens from a farm of 36-40 days of age for the presence of antibodies to the Newcastle disease virus are presented see Table 1.

**Table 1:** The results of the study in RTGA of the blood serum of broiler chickens for the presence of antibodies to the virus NB

Group birds	Qty samples(pcs.)	1:2	1:8	1:16	1:32	1:64	1:128	Quality immunity (%)
Clinically healthy	25	2	4	7	7	4	1	92
Clinically sick	25	4	5	6	9	1	-	84

<sup>a</sup> Broiler chickens on the farm are vaccinated against NB using virus vaccines from strain "B1" and "La Sota"

The detected immune response indicates a relatively pronounced post-vaccination immunity to Newcastle disease. In clinically sick chickens, the level of immune response is slightly lower, which allows Newcastle disease to be excluded from the list of causes that cause bird pathology with respiratory symptoms.

The results of the ELISA study of blood serum samples of broiler chickens from a farm of 36-40 days of age for the presence of antibodies to the IBC virus, MPVI, ILT, RMP, ORT is presented see Table 2.

**Table 2:** The results of the study in the ELISA of the blood serum of broiler chickens for the presence of antibodies to the virus IBC, MPVI, ORT, RMP, ILT

Group birds	No. of samples (pcs.)	Disease	The average titer of antibodies	Coeff. variations (%)	Min value caption	Max. value caption	Quality immunity (%)	Amount put on responsive birds (%)
Healthy	20	IBK	2180	78,4	548	2616	100	-
Sick	20	IBK	5946	24,6	256	9094	100	-
Healthy	20	MPVI	324	94,8	132	916	-	10
Sick	20	MPVI	872	78,6	318	2756	-	20
Healthy	20	ORT	446	77,5	130	1755	-	10
Sick	20	ORT	908	60,6	265	2076	-	20
Healthy	20	RMP	268	72,2	57	803	-	0
Sick	20	RMP	1336	54,2	382	2014	-	20
Healthy	20	ILT	970	81	136	2850	20	-
Sick	20	ILT	1228	112	9	3485	20	-

The number of positively reacting chickens and the detected level of antibodies indicates the relative well-being of the studied groups of broiler chickens by MPVI, ILT, ORT, and RMP. At the

same time, significant differences in the immune response of healthy and sick chickens with respect to chicken infectious bronchitis were revealed. This retrospectively confirms that the main cause of the disease of chickens is a field IBC infection. The causative agents of pneumovirus infection, ornithobacteriosis, and respiratory mycoplasmosis can be considered as concomitant contaminants, somewhat complicating the overall epizootic situation in the economy.

#### 4. CONCLUSION

In the course of an epidemiological examination of the farm, broiler chickens were found to have an infectious disease occurring with the respiratory syndrome. The results of comprehensive diagnostic monitoring, including microbiological, virological and serological studies, have shown that the main etiological factor in the pathology of chickens is the hepatitis B virus circulating in the household. As a result of studying the immunobiological properties of the isolated virus, its antigenic properties and virulence were determined for chicken SPF embryos and broiler chickens. According to the study of the isolate in the neutralization reaction, RT-PCR-RV and the method of genomic sequencing, the identity of the isolated pathogen to the strain QX of the chicken infectious bronchitis virus was established.

#### 5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

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